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Biochemical Interplay Between Oxidative Stress, Mitochondrial Dysfunction, and Cellular Senescence: Molecular Mechanisms and Therapeutic Targets

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Abstract

Cellular senescence represents a type of stress response, which is also characterized by witnessing stable arrest of cell-cycle, damage to macromolecules and cells' alteration in their secretory and metabolic functions. Oxidative stress and mitochondrial dysfunction are tightly coupled and may reinforce each other and play a role in either establishment or preservation of senescent phenotypes. The generation of reactive oxygen species (ROS) is an important source but not unique for mitochondria, and depends on cell type, metabolic state, and experimental conditions. Oxidation of lipids, proteins, and nucleic acids can trigger DNA-damaging pathways; mitochondrial-membrane-derived signals can initiate pathways of inflammation, such as cGAS–STING pathway activation. These signals converge with p53/p21p and p16/rb cell cycle control, the NF-κB dependent inflammatory transcription and NAD+/sirtuin metabolic regulation. In this narrative review, we discuss the biochemical origins and effects of ROS, mitochondrial quality-control mechanisms, signaling pathways connecting ROS imbalance to senescence, natural antioxidants and new advances in senotherapeutic approaches. Particular attention is given to the limitations of commonly used senescence markers and to the distinction between preclinical findings and evidence from human studies. Although mitochondria-targeted antioxidants, senolytics and senomorphics are promising research approaches, their clinical effectiveness and long-term safety remain to be established.

Keywords: Cellular Senescence, Oxidative Stress, Reactive Oxygen Species, Mitochondrial Dysfunction; Mitophagy, CGAS–STING, Senolytics, Senomorphics.

1 INTRODUCTION

Cellular senescence is a durable cell-state transition that commonly involves stable cell-cycle arrest and a range of phenotypic changes. It can be triggered by telomere dysfunction, persistent DNA damage, oncogene activation, mitochondrial or metabolic stress, and other cellular insults (Hayflick and Moorhead, 1961; Campisi and d'Adda di Fagagna, 2007). Senescence was first described in cultured human diploid fibroblasts by Hayflick and Moorhead. It has context-dependent effects: it can restrict the proliferation of damaged or potentially malignant cells and contribute to tissue repair, but the persistence or accumulation of senescent cells can also promote chronic inflammation and tissue dysfunction (Campisi and d'Adda di Fagagna, 2007; Coppe et al., 2010). Many senescent cells develop a senescence-associated secretory phenotype (SASP), which may include cytokines, chemokines,

growth factors and matrix-remodeling enzymes. The composition of the SASP varies with cell type, inducing stimulus and time after senescence induction (Coppe et al., 2010; Gorgoulis et al., 2019).

Redox imbalance is one of several processes that can initiate or reinforce senescence. At physiological concentrations, ROS such as hydrogen peroxide participate in regulated signaling; oxidative stress arises when oxidant production, antioxidant capacity and repair systems become imbalanced (Finkel and Holbrook, 2000; Sies and Jones, 2020; Halliwell and Gutteridge, 2015; Sies, 2015). The biological outcome therefore depends not simply on the amount of ROS, but also on its chemical identity, subcellular location, duration and the capacity of the cell to adapt or repair damage.

Mitochondria are important sources and targets of cellular ROS, although they are not the sole or universally dominant source in every cell type or condition. Superoxide can be produced inside the cell by leakage from respiratory-chain components, and then reacted with superoxide dismutase to create hydrogen peroxide. It is possible that oxidatively modified lipids, proteins and mitochondrial DNA can affect mitochondrial function and change the relationships between the enzymes involved in redox signaling (Murphy, 2009; Brand, 2016; Zhao et al., 2019; Scialò et al., 2017; Bleier and Dröse, 2013). The link is not always a straightforward self-reinforcing cycle: ROS may be generated by NADPH oxidases or other enzymes and mitochondrial actions vary depending on metabolic situations. In this review, the intersections between these pathways and cell-cycle arrest, inflammatory signaling and mitochondrial quality control will be taken into account.

2 BIOCHEMICAL SOURCES OF REACTIVE OXYGEN SPECIES (ROS)

Review scope and approach

The article is a narrative review. It includes basic studies and selected review and experimental literature related to oxidative stress, mitochondrial biology and cellular senescence. It does not follow a 'systematic review' or 'meta-analysis' protocol: A protocol, database search string, duplicate screening process or formal risk-of-bias assessment were not provided in the source manuscript.

2.1 Endogenous Enzymatic and Non-Enzymatic ROS Generation

Intracellular ROS encompass both free radical species possessing unpaired valence electrons—such as the superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$)—and non-radical oxidizing agents including hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) (Finkel and Holbrook, 2000; Brand, 2016).

The mitochondrial electron transport system is an important source of superoxide and hydrogen peroxide, particularly at defined redox-active sites in complexes I and III. The rate of ROS formation is highly dependent on substrate supply, membrane potential, oxygen availability, respiratory state and the redox state of electron carriers. Consequently, a single estimate such as a fixed percentage of electrons escaping the chain should not be treated as a universal value across tissues or experimental systems (Murphy, 200; Brand, 2016; Zhao et al., 2019; Scialò et al., 2017; Bleier and Dröse, 2013).

Complex I can generate superoxide toward the matrix, including during reverse electron transport under conditions of a highly reduced ubiquinone pool and a high proton-motive force. The site and condition of electron transfer to Complex III allows superoxide to be emitted either into the matrix or into the intermembrane space of the inner membrane.

The extra-mitochondrial source of ROS makes significant contribution to the total intracellular ROS pool. NADPH Oxidase (NOX) family enzymes (NOX1 – NOX5, DUOX1, DUOX2) specifically transfer electrons to molecular oxygen (O2) from cytosolic NADPH across biological membranes to generate superoxide or hydrogen peroxide (Lambeth, 2004). Moreover, acyl-CoA oxidases are important enzymes of the peroxisomal fatty acid β-oxidation pathway, which generates large amounts of the metabolic byproduct H2O2 as a byproduct (Fransen et al., 2012). Other continuous sources of enzymatic production of H2O2 and O2•− are the cytosolic enzymes such as Xanthine Oxidase (XO) and outer mitochondrial membrane-bound Monoamine Oxidases (MAO-A and MAO-B) during substrate catabolism (Bortolozzi and Rizzuto, 2018).

2.2 Biomolecular Modifications Induced by Oxidative Stress

When unmitigated, high concentrations of ROS induce irreversible biochemical alterations across all major macromolecular classes:

- **Lipid Peroxidation:** Hydroxyl radicals attack polyunsaturated fatty acids (PUFAs) within membrane phospholipids, initiating a self-propagating radical chain reaction (Niki, 2014). This cascade results in the cleavage of lipid hydroperoxides into highly reactive electrophilic aldehyde byproducts, predominantly Malondialdehyde (MDA) and 4-Hydroxynonenal (4-HNE) (Niki, 2014; Ayala et al., 2014). These aldehyde species readily form covalent adducts with lysine, histidine, and cysteine residues, altering membrane fluidity, increasing proton permeability, and inactivating membrane-bound transporters (Ayala et al., 2014).
- **Protein Oxidation and Carbonylation:** ROS directly oxidize amino acid side chains, particularly proline, arginine, lysine, and threonine, yielding protein carbonyl derivatives (Dalle-Donne et al., 2003). Oxidation of cysteine sulfhydryl (−SH) groups to disulfides or sulfenic, sulfinic, and sulfonic acid states leads to catastrophic protein misfolding, loss of catalytic activity, and accumulation of insoluble protein aggregates resistant to proteasomal degradation (Dalle-Donne et al., 2003; Stadtman and Levine, 2003).
- **Oxidative DNA Damage:** Hydroxyl radicals interact directly with purine and pyrimidine bases and the deoxyribose backbone (Valavanidis et al., 2009). The oxidation of guanine residues forms 8-hydroxy-2'-deoxyguanosine (8-OHdG), a primary biomarker of oxidative genomic instability (Valavanidis et al., 2009; Cooke et al., 2003). 8-OHdG lesions induce G:C to T: A transversion mutations during DNA replication and trigger single-strand (SSBs) and double-strand DNA breaks

(DSBs), thereby initiating the canonical DNA Damage Response (DDR) pathway (Cooke et al., 2003; Shiloh, 2003).

3 MITOCHONDRIAL DYSFUNCTION AS A CENTRAL ENGINE OF SENESCENCE

3.1 Structural and Bioenergetic Collapse

In various experimental models, dysfunction in the mitochondria helps trigger or sustain the senescence phenotype, but in some cases not all cells enter senescence exhibit mitochondrial dysfunction (Passos et al., 2010; López-Otín et al., 2013; Chan, 2020; Korolchuk et al., 2017). Redox-chain organization, membrane potential and oxidative phosphorylation have been reported to be altered in specific cells and context, along with changes in the composition of cardiolipin (Paradies et al., 2014; Zorova et al., 2018). These alterations may influence ATP production and redox signaling, with effects varying in magnitude and direction depending on cellular and metabolic conditions (Wiley and Campisi, 2021). Decreasing the membrane potential is not enough to conclude that ATP synthase does not function, because other factors such as substrate supply, coupling efficiency and cell requirement determine ATP production also.

MtP involves a transient increase in mitochondrial inner membrane permeability that can occur in acute situations, and increase bioenergetic impairment in chronic periods, as a consequence of oxidative stress and disordered calcium influx (Izzo et al., 2016). The composition and regulation of the permeability-transition pore is the subject of active investigation. Opening pore cannot be equated to rupture of the outer-membrane, and the opening of the pore does not necessarily make all mitochondrial pro-death factors. Cytochrome c can be released more intimately into the cytosol along with permeabilization of the outer mitochondrial membrane, while mitochondrial DNA can be translocated to the cytosol by other means.

Upon entry into the cytosol, mitochondrial DNA can trigger the activation of the cGAS–STING pathway and inflammatory gene expression (White et al., 2014; Dou et al., 2017) that may include SASP components.

3.2 Disrupted Mitochondrial Dynamics and Defective Mitophagy

Mitochondrial morphology and functional integrity are maintained by dynamic fission and fusion events (Westermann, 2010). Fission is mediated by the cytosolic GTPase Dynamin-related protein 1 (Drp1), which translocates to the outer membrane to constrict mitochondria (Westermann, 2010). Fusion is orchestrated by Mitofusins (Mfn1 and Mfn2) for the outer membrane and Optic Atrophy 1 (OPA1) for the inner membrane (Westermann, 2010; Yoon et al., 2006). In senescent cells, mitochondrial dynamics shift heavily toward hyper-fused, elongated, or pathologically enlarged mitochondria driven by the downregulation of Drp1 or excessive expression of Mfn2, leading to the

accumulation of damaged, giant mitochondria incapable of proper segregation (Yoon et al., 2006; Chan, 2020).

Mitophagy is one component of mitochondrial quality control and can involve PINK1–Parkin-dependent as well as receptor-mediated pathways (Palikaras et al., 2018; Narendra et al., 2010). In the canonical PINK1–Parkin pathway, loss of mitochondrial membrane potential can stabilize PINK1 on the outer mitochondrial membrane, leading to Parkin recruitment and ubiquitination of mitochondrial surface proteins. Autophagy receptors then help connect tagged mitochondria to the autophagic machinery. Mitophagy is altered in several senescence models, but it should not be described as uniformly or invariably defective: its activity depends on cell type, senescence inducer and the assay used Palikaras et al., (2018); Korolchuk et al., (2017).

4 MOLECULAR SIGNALING BRIDGES TO CELLULAR SENESCENCE

4.1 Canonical Cell Cycle Arrest Pathways: p53/p21CIP1 and p16INK4a/Rb

Two major tumor-suppressor pathways involved in senescence-associated cell-cycle arrest are the p53–p21 and p16–RB axes(López-Otín et al., 2013; Serrano et al., 1997; d'Adda di Fagagna, 2008; Vogelstein et al., 2000; Sherr and McCormick, 2002; Rayess et al., 2012; Alcorta et al., 1996). They are interconnected but are not necessarily activated to the same extent in every senescent cell. Their relative contribution depends on the initiating stress, cell type and experimental context.

Persistent DNA lesions and replication stress can activate the DNA-damage response. ATM is particularly associated with double-strand DNA breaks, whereas ATR is commonly activated by replication stress and stretches of single-stranded DNA. These kinases signal through checkpoint proteins, including CHK1 and CHK2, and can stabilize p53 in part by altering the regulation of MDM2 (d'Adda di Fagagna, 2008; Vogelstein et al., 2000). p53 can then promote expression of CDKN1A, which encodes p21. p21 inhibits cyclin-dependent kinases and helps maintain RB in a growth-suppressive state, thereby limiting E2F-dependent transcription and cell-cycle progression (Vogelstein et al., 2000; Sherr and McCormick, 2002). The pathway is dynamic, and sustained arrest depends on additional regulatory and chromatin-level changes.

The CDKN2A gene encodes p16INK4a, an inhibitor of CDK4 and CDK6. By limiting cyclin-D-dependent RB phosphorylation, p16 helps maintain RB-mediated repression of E2F target genes (Rayess et al., 2012; Alcorta et al., 1996). The p16–RB pathway can reinforce durable arrest, although expression of p16 alone is not sufficient to identify a cell as senescent in all tissues or experimental settings.

4.2 NF-κB Activation and SASP Regulation

Several transcription and signaling networks regulate the SASP, such as NF-κB, C/EBPβ, p38 MAPK, and mTOR (but in some contexts cGAS–STING) (Gorgoulis et al., 2019; Salminen and Kaarniranta, 2009; Chien et al., 2011; Freund et al., 2011). The influence of ROS on these pathways

may occur via redox-dependent signaling intermediates, but it is not universal and direct activation of IKK via ROS. The capacity to express inflammatory mediators like IL-6, IL-1β and TNF is regulated by the activity of NF-κB and the mix and levels of the SASP differ significantly among senescent cell populations.

4.3 Metabolic Signaling and NAD+ / Sirtuin Depletion

NAD+ availability can change during senescence and aging through altered synthesis, salvage and consumption. PARP1 activation in response to DNA damage and increased CD38 activity are among the mechanisms implicated in NAD+ depletion, although their relative contributions vary across tissues and models (Camacho-Pereira et al., 2016; Imai and Guarente, 2014). Because sirtuins require NAD+ as a co-substrate, changes in NAD+ availability can affect SIRT1- and SIRT3-dependent processes, including p53 acetylation and mitochondrial antioxidant regulation (Imai and Guarente, 2014; Vaziri et al., 2001; Someya et al., 2010). These relationships are biologically plausible and supported by experimental evidence, but should not be generalized as a uniform decline in every senescent cell.

Downregulation of nuclear SIRT1 leads to hyperacetylation and activation of p53, reinforcing p21CIP1 transcription (Vaziri et al., 2001). Simultaneously, suppression of mitochondrial SIRT3 impairs the deacetylation and activation of Manganese Superoxide Dismutase (SOD2) and Isocitrate Dehydrogenase 2 (IDH2), drastically reducing mitochondrial antioxidant capacity and aggravating superoxide accumulation (Someya et al., 2010).

5 CELLULAR SENESCENCE: BIOMARKERS AND ASSESSMENT

Cellular senescence is a multifaceted phenomenon and is not easily defined by a specific index. Closely monitored in situ cellular markers include levels of p16INK4a or p21, loss of proliferation markers (e.g., Ki-67 expression or decreased nucleotide incorporation assays), senescence-associated β-galactosidase activity, stable foci of DNA-damage (e.g., γH2AX), alteration of lamin B1, and some of the SASP factors. There are limitations to each marker: the p16 and p21 can be expressed in contexts other than senescence; the SA-β-gal marker is not necessarily specific for senescence, as it is associated with greater lysosomal activity; and the SASP factors can be produced in contexts other than senescence, such as inflammation. Marker might differ across various cell types, tissue, time and stimulus.

Recent MICSE recommendations emphasize the use of multiple complementary markers representing different features of senescence rather than reliance on a single readout (Ogrodnik et al., 2024). In tissue studies, combining cell-cycle inhibitors with markers of DNA damage, lysosomal activity, nuclear-envelope changes and secretory activity can strengthen interpretation. Where possible, markers should be assessed in the relevant cell population using co-staining, cell sorting or other cell-resolved methods. These recommendations are especially important when interpreting human tissue or circulating biomarkers, for which validation remains incomplete (Table 1).

Table 1: Commonly Used Markers of Cellular Senescence and Key Interpretive Limitations

Marker / readout	What it may indicate	Important limitation
p16INK4a / p21	Cell-cycle inhibitory signaling	Can be induced outside senescence; expression is cell- and context-dependent.
SA-β-gal	Increased lysosomal β-galactosidase activity	Not specific to senescence; affected by cell type and assay conditions.
γH2AX / persistent DNA-damage foci	DNA-damage signaling or persistent lesions	Also occurs during acute DNA damage, replication stress and apoptosis.
LMNB1 reduction	Nuclear-envelope remodeling reported in some senescent cells	Not universal across cell types or senescence triggers.
SASP factors (e.g., IL-6, IL-8)	Senescence-associated inflammatory secretory activity	Also produced by immune and inflamed non-senescent cells.
Reduced proliferation / Ki-67	Stable growth arrest	Quiescent or differentiated cells may also show low proliferation.

6 ENDOGENOUS ANTIOXIDANT DEFENSE MECHANISMS

6.1 Enzymatic Antioxidant Systems

Cells maintain an integrated enzymatic antioxidant defense network to neutralize ROS before macromolecular damage occurs (Halliwell and Gutteridge, 2015; Sies, 2015):

- **Superoxide Dismutases (SOD):** SOD enzymes catalyze the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen ($2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$) (McCord and Fridovich, 1969;). Three distinct isoforms exist: SOD1 (Cu/Zn-SOD) localized in the cytosol and intermembrane space, SOD2 (Mn-SOD) localized within the mitochondrial matrix, and SOD3 (EC-SOD) secreted into the extracellular space (McCord and Fridovich, 1969; Fukai and Ushio-Fukai, 2011).
- **Catalase (CAT):** Catalase is a heme-containing peroxisomal enzyme that converts hydrogen peroxide into water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$) at high catalytic efficiency without consuming reducing equivalents (Kirkman and Gaetani, 2007).
- **Glutathione Peroxidase (GPx) and Glutathione Reductase (GR):** GPx enzymes utilize reduced glutathione (GSH) to reduce H_2O_2 and lipid hydroperoxides to water and corresponding lipid alcohols ($H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$) (Brigelius-Flohé and Maiorino, 2013). Glutathione Reductase (GR) subsequently reduces oxidized glutathione disulfide (GSSG) back to GSH using NADPH as an electron donor, maintaining a high cytosolic GSH/GSSG ratio (Brigelius-Flohé and Maiorino, 2013; Deponte, 2013).

6.2 The Nrf2/Keap1 Master Regulatory Pathway

Transcriptional control of enzymatic antioxidant expression is principally governed by Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) (Itoh et al., 2004). Under basal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1), an adaptor protein that

facilitates Nrf2 ubiquitination and rapid degradation by the 26S proteasome (Itoh et al., 2004; Yamamoto et al., 2018).

Elevated ROS levels oxidize reactive cysteine residues (notably Cys151, Cys273, and Cys288) on Keap1, inducing a conformational change that prevents Nrf2 ubiquitination (Yamamoto et al., 2018). De novo synthesized Nrf2 translocates to the nucleus, heterodimerizes with Small Maf (sMaf) proteins, and binds to Antioxidant Response Elements (ARE; 5'-TMANNRTGAYNNGCR-3') located in the promoter regions of target genes (Yamamoto et al., 2018; Ma, 2013). Nrf2 drives the transcription of a battery of cytoprotective genes, including SOD1, CAT, GPX1, Heme Oxygenase-1 (HMOX1), NAD(P)H:quinone oxidoreductase 1 (NQO1), and enzymes involved in GSH synthesis (GCLC and GCLM) (Ma, 2013; Hardie, 2011). In senescent cells, Nrf2 signaling becomes uncoupled or attenuated, leading to enzymatic exhaustion and unchecked ROS elevation.

7 PHARMACOLOGICAL AND THERAPEUTIC INTERVENTIONS

References	Primary Molecular Targets	Biochemical Mechanism of Action	Compound / Agent	Category
Smith and Murphy, (2010); Murphy and Smith, (2007)	Mitochondrial matrix ROS, Lipid peroxidation	Lipophilic triphenylphosphonium (TPP+) cation targets ubiquinone to IMM; scavenges lipid peroxides.	MitoQ (Mitoquinol)	Mitochondria-Targeted Antioxidants
Skulachev et al., (2009)	Complex I/III ROS, Cardiolipin	Plastoquinone derivative conjugated to TPP+; prevents cardiolipin oxidation and mPTP opening.	SkQ1 (Plastoquinonyl-decyl-TPP)	Mitochondria-Targeted Antioxidants
Zhu et al., (2015); Hickson et al., (2019)	Ephrin ligands, PI3K, BCL-xL, p21CIP1	Inhibits Src kinase/tyrosine kinases (Dasatinib) and PI3K/BCL-2/flavonoid pathways (Quercetin); triggers apoptosis in senescent cells.	Dasatinib + Quercetin (D+Q)	Senolytics
Zhu et al., (2016)	BCL-2, BCL-xL, BCL-W	Small-molecule inhibitor of anti-apoptotic BCL-2 family proteins; disrupts senescent cell anti-apoptotic pathways (SCAP).	Navitoclax (ABT-263)	Senolytics
Yousefzadeh et al., (2018)	PI3K/AKT, NF-κB, BCL-2 family	Natural flavonoid; downregulates anti-apoptotic proteins and inhibits NF-κB signaling.	Fisetin	Senolytics
Harrison et al., (2009); Laberge et al., (2015)	mTORC1, 4E-BP1, S6K1	mTORC1 inhibitor; suppresses SASP translation, reduces mitochondrial ROS, and restores mitophagy.	Rapamycin (Sirolimus)	Senomorphics
Camacho-Pereira et al., (2016); Yoshino et al., (2018)	SIRT1, SIRT3, NMNAT	Precursor to NAD+ biosynthesis; restores intracellular NAD+ pool, activating Sirtuin enzymes.	Nicotinamide Mononucleotide (NMN) / NR	NAD+ Boosters

New strategies aimed at reducing oxidative stress, mitochondrial dysfunction, and senescence can be categorized into mitochondria-targeted redox modulators and senolytics and senomorphics (Kirkland and Tchkonia, 2020; McHugh and Gil, 2018). There is some heterogeneity in the evidence for these

strategies. The majority of the reported effects are based on cell culture or on animal models, although human is relatively small, lacking enough cases to conclude routine clinical efficacy and long-term safety. Moreover, indiscriminate anti-ROS therapy could disrupt physiological redox pathways; in addition, senolytics might cause toxicity from their mechanism or affect non-senescent cells. Such efforts should be termed investigational, therefore.

7.1 Translational Evidence and Clinical Limitations

The evidence for senotherapeutic interventions should be evaluated with regard to the extent of the research. The effects of MitoQ and SkQ1 have been investigated in experimental systems and in specific clinical scenarios, but cannot be extrapolated to suggest senescence selective activity. Dasatinib plus quercetin, navitoclax and fisetin have shown senolytic effects in selected preclinical models; small early human studies have explored some of these agents, but the available evidence does not establish a broadly effective treatment for age-related disease (Kirkland and Tchkonia, 2020; Zhu et al., 2015; Hickson et al., 2019; Zhu et al., 2016; Yousefzadeh et al., 2018). Navitoclax is associated with thrombocytopenia because BCL-XL is important for platelet survival, illustrating the potential for mechanism-based toxicity. Rapamycin and related mTOR inhibitors are better considered senomorphic or geromodulatory approaches in this context, with effects that depend on dose, duration and tissue.

NAD+ precursors such as nicotinamide riboside and nicotinamide mononucleotide are under investigation for their effects on NAD+ metabolism. The biomarkers of changes in NAD+ or sirtuins do not necessarily reflect actual prevention or reversal of human senescence. Throughout all these strategies, the pertinent translational questions are: What is the target engagement in the relevant tissue, which are validated biomarkers, clinically meaningful benefit, optimum dosage, drug interactions and long-term safety. The results of the current investigation are further proof that more study is needed, rather than routine therapies to prevent aging.

8 FUTURE PERSPECTIVES AND CONCLUSION

Oxidative stress, mitochondrial functions and cellular senescence can have a significant relationship in the field of ageing and chronic diseases. Mitochondria play a role in redox signaling and are subject to oxidative damage; however, the association is two-way, and is cell-specific and context-dependent. Altered membrane potential, mitochondrial dynamics, mitophagy, and mit-to-elucidation of mitochondrial-DNA and signaling may interact with altered DNA-damage responses and inflammatory pathways to alter senescent phenotypes.

Chronic stress can activate p53/p21 and p16(Rb) regulated pathways and can engage inflammatory signatures of senescence (sASP) encompassing pro-inflammatory factors such as NF-κB and related pathways. Senescence, however, is not a monolithic process and is not a molecular event. Initial human studies provide preliminary evidence, while compounds that target mitochondria, senolytic cocktails and all NAD related compounds have shown activity in selected preclinical models. Standardized

multi-marker assessment, cell- and tissue-specific mechanisms, improved pharmacological selectivity and well-powered clinical trials with significant and sustainable therapeutic outcomes and safety evaluations should be emphasized in future investigations.

REFERENCES

[1] Alcorta, D. A., Xiong, Y., Phelps, D., Hannon, G., Beach, D., & Barrett, J. C. (1996). Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proceedings of the National Academy of Sciences, 93*(24), 13742–13747. https://doi.org/10.1073/pnas.93.24.13742

[2] Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014*, 360438. https://doi.org/10.1155/2014/360438

[3] Bleier, L., & Dröse, S. (2013). Superoxide generation by complex III: From mechanistic insights to human physiological implications. *Frontiers in Physiology, 4*, 27. https://doi.org/10.1016/j.bbabio.2012.12.002

[4] Bortolozzi, M., & Rizzuto, R. (2018). Monoamine oxidases, oxidative stress and mitochondrial dysfunction in neurodegeneration. *Frontiers in Neuroscience, 12*, 608.

[5] Brand, M. D. (2016). Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radical Biology and Medicine, 100*, 14–31. https://doi.org/10.1016/j.freeradbiomed.2016.04.001

[6] Brigelius-Flohé, R., & Maiorino, M. (2013). Glutathione peroxidases. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1830*(5), 3289–3303. https://doi.org/10.1016/j.bbagen.2012.11.020

[7] Camacho-Pereira, J., Tarragó, M. G., Chini, C. C., Nin, V., Escande, C., Warner, G. M., ... & Chini, E. N. (2016). CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. *Cell Metabolism, 23*(6), 1127–1147.

[8] Campisi, J., & d'Adda di Fagagna, F. (2007). Cellular senescence: When bad things happen to good cells. *Nature Reviews Molecular Cell Biology, 8*(9), 729–740. https://doi.org/10.1038/nrm2233

[9] Chan, D. C. (2020). Mitochondrial dynamics and its involvement in disease. *Annual Review of Pathology: Mechanisms of Disease, 15*, 235–259. https://doi.org/10.1146/annurev-pathmechdis-012419-032711

[10] Chien, Y., Scuoppo, C., Wang, X., Fang, X., Balgley, B., Bolden, J. E., ... & Lowe, S. W. (2011). Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. *Genes & Development, 25*(20), 2125–2136. https://doi.org/10.1101/gad.17276711

[11] Cooke, M. S., Evans, M. D., Dizdaroglu, M., & Lunec, J. (2003). Oxidative DNA damage: Mechanisms, mutation, and disease. *The FASEB Journal, 17*(10), 1195–1214. https://doi.org/10.1096/fj.02-0752rev

[12] Coppe, J. P., Desprez, P. Y., Krtolica, A., & Campisi, J. (2010). The senescence-associated secretory phenotype: The dark side of tumor suppression. *Annual Review of Pathology: Mechanisms of Disease, 5*, 99–161. https://doi.org/10.1146/annurev-pathol-121808-102144

[13] Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2003). Protein carbonylation in human diseases. *Trends in Molecular Medicine, 9*(4), 169–176.

[14] Deponte, M. (2013). Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1830*(5), 3217–3266. https://doi.org/10.1016/j.bbagen.2012.09.018

[15] d'Adda di Fagagna, F. (2008). Living on a break: Cellular senescence as a DNA-damage response. *Nature Reviews Cancer, 8*(7), 512–522. https://doi.org/10.1038/nrc2440

[16] Dou, Z., Ghosh, K., Vizioli, M. G., Zhu, J., Sen, P., Wangensteen, K. J., ... & Berger, S. L. (2017). Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature, 550*(7676), 402–406. https://doi.org/10.1038/nature24050

[17] Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*(6809), 239–247. https://doi.org/10.1038/35041687

[18] Fransen, M., Nordgren, M., Wang, B., & Apanasets, O. (2012). Role of peroxisomes in ROS/RNS-mediated signaling pathways. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1823*(9), 1363–1374.

[19] Freund, A., Patil, C. K., & Campisi, J. (2011). p38 MAPK regulates the senescence-associated secretory phenotype via NF-κB. *EMBO Reports, 12*(2), 153–159. https://doi.org/10.1038/emboj.2011.69

[20] Fukai, T., & Ushio-Fukai, M. (2011). Superoxide dismutases: Role in redox signaling, vascular function, and diseases. *Antioxidants & Redox Signaling, 15*(6), 1583–1606. https://doi.org/10.1089/ars.2011.3999

[21] Gorgoulis, V., Adams, P. D., Alimonti, A., Bennett, D. C., Bischof, O., Bishop, C., ... & d'Adda di Fagagna, F. (2019). Cellular senescence: Defining a path forward. *Cell, 179*(4), 813–827. https://doi.org/10.1016/j.cell.2019.10.005

[22] Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford University Press.

[23] Hardie, D. G. (2011). AMP-activated protein kinase: An energy sensor that regulates all aspects of cell function. *Genes & Development, 25*(18), 1895–1908. https://doi.org/10.1101/gad.17420111

[24] Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., ... & Miller, R. A. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature, 460*(7253), 392–395. https://doi.org/10.1038/nature08221

[25] Hayflick, L., & Moorhead, P. S. (1961). The serial cultivation of human diploid cell strains. *Experimental Cell Research, 25*(3), 585–621. https://doi.org/10.1016/0014-4827(61)90192-6

[26] Hickson, L. J., Langhi Prata, L. G., Bobart, S. A., Evans, T. K., Giorgadze, N., Hashmi, S. K., ... & Kirkland, J. L. (2019). Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of dasatinib plus quercetin in individuals with diabetic kidney disease. *EBioMedicine, 47*, 446–456. https://doi.org/10.1016/j.ebiom.2019.08.069

[27] Imai, S. I., & Guarente, L. (2014). NAD+ and sirtuins in aging and disease. *Trends in Cell Biology, 24*(8), 464–471.

[28] Itoh, K., Tong, K. I., & Yamamoto, M. (2004). Molecular mechanism activating Nrf2-Keap1 pathway in regulation of physiological response to oxidative stress. *Free Radical Biology and Medicine, 36*(10), 1208–1216. https://doi.org/10.1016/j.freeradbiomed.2004.02.075

[29] Izzo, V., Bravo-San Pedro, J. M., Sica, G. S., Kroemer, G., & Galluzzi, L. (2016). Mitochondrial permeability transition: At the crossroad between apoptosis and necrosis. *Environmental and Molecular Mutagenesis, 57*(7), 555–568.

[30] Kirkland, J. L., & Tchkonia, T. (2020). Senolytic drugs: From discovery to translation. *Journal of Internal Medicine, 288*(5), 518–536. https://doi.org/10.1111/joim.13141

[31] Kirkman, H. N., & Gaetani, G. F. (2007). Mammalian catalase: A venerable enzyme with new mysteries. *Trends in Biochemical Sciences, 32*(1), 44–50.

[32] Korolchuk, V. I., Miwa, S., Carroll, B., & von Zglinicki, T. (2017). Mitochondria in cell senescence: Is mitophagy the weakest link? *EBioMedicine, 21*, 7–13. http://dx.doi.org/10.1016/j.ebiom.2017.03.020

[33] Laberge, R. M., Sun, Y., Harmatiuk, C. X., Liu, C. X., Zuckerman, Y. B., Busuttil, R. A., ... & Campisi, J. (2015). MTOR regulates the pro-inflammatory senescence-associated secretory phenotype by promoting IL1A translation. *Nature Cell Biology, 17*(8), 1049–1061. https://doi.org/10.1038/ncb3195

[34] Lambeth, J. D. (2004). NOX enzymes and the biology of reactive oxygen. *Nature Reviews Immunology, 4*(3), 181–189. https://doi.org/10.1038/nri1312

[35] López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell, 153*(6), 1194–1217. http://dx.doi.org/10.1016/j.cell.2013.05.039

[36] Ma, Q. (2013). Role of Nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology, 53*, 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

[37] McCord, J. M., & Fridovich, I. (1969). Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry, 244*(22), 6049–6055.

[38] McHugh, D., & Gil, J. (2018). Senescence and aging: Causes, consequences, and therapeutic avenues. *Journal of Cell Biology, 217*(1), 65–77. https://doi.org/10.1083/jcb.201708092

[39] Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal, 417*(1), 1–13. https://doi.org/10.1042/BJ20081386

[40] Murphy, M. P., & Smith, R. A. (2007). Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annual Review of Pharmacology and Toxicology, 47*, 629–656. https://doi.org/10.1146/annurev.pharmtox.47.120505.105110

[41] Narendra, D. P., Jin, S. M., Tanaka, A., Suen, D. F., Gautier, C. A., Shen, J., ... & Youle, R. J. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biology, 8*(1), e1000298. https://doi.org/10.1371/journal.pbio.1000298

[42] Niki, E. (2014). Biomarkers of lipid peroxidation in clinical material. *Free Radical Biology and Medicine, 65*, 375–390. https://doi.org/10.1016/j.bbagen.2013.03.020

[43] Ogrodnik, M., Acosta, J. C., Adams, P. D., et al. (2024). Guidelines for minimal information on cellular senescence experimentation in vivo. *Cell, 187*(16), 4150–4175. https://doi.org/10.1016/j.cell.2024.05.059

[44] Palikaras, K., Lionaki, E., & Tavernarakis, N. (2018). Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. *Nature Cell Biology, 20*(9), 1013–1022. https://doi.org/10.1038/s41556-018-0176-2

[45] Paradies, G., Paradies, V., Ruggiero, F. M., & Petroillo, G. (2014). Role of cardiolipin in mitochondrial dysfunction in inflammation. *ACS Chemical Biology, 9*(9), 1930–1937.

[46] Passos, J. F., Nelson, G., Wang, C., Richter, T., Simillion, C., Proctor, C. J., ... & von Zglinicki, T. (2010). Feedback between p21 and reactive oxygen species determines cellular senescence viability and maintenance. *Molecular Systems Biology, 6*(1), 347. https://doi.org/10.1038/msb.2010.5

[47] Rayess, H., Wang, M. B., & Srivatsan, E. S. (2012). Cellular senescence and tumor suppressor gene p16. *International Journal of Cancer, 130*(8), 1715–1725. https://doi.org/10.1002/ijc.27316

[48] Salminen, A., & Kaarniranta, K. (2009). NF-κB signaling in the regulation of cellular senescence: The role of white hair genes. *Ageing Research Reviews, 8*(3), 173–183.

[49] Scialò, F., Fernández-Ayala, D. J., & Sanz, A. (2017). Role of mitochondrial reverse electron transport in ROS signaling and disease. *Frontiers in Physiology, 8*, 428. https://doi.org/10.3389/fphys.2017.00428

[50] Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., & Lowe, S. W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell, 88*(5), 593–602.

[51] Sherr, C. J., & McCormick, F. (2002). The RB and p53 pathways in cancer. *Cancer Cell, 2*(2), 103–112.

[52] Shiloh, Y. (2003). ATM and related protein kinases: Safeguarding genome integrity. *Nature Reviews Cancer, 3*(3), 155–168. https://doi.org/10.1038/nrc1011

[53] Sies, H. (2015). Oxidative stress: A concept in redox biology and medicine. *Redox Biology, 4*, 180–183. https://doi.org/10.1016/j.redox.2015.01.002

[54] Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology, 21*(7), 363–383. https://doi.org/10.1038/s41580-020-0230-3

[55] Skulachev, V. P., Anisimov, V. N., Antonenko, Y. N., Bakeeva, L. E., Chernyak, B. V., Erichev, V. P., ... & Zinovkin, R. A. (2009). An attempt to prevent senescence: A mitochondrial approach. *Physiology, 24*(5), 252–264. https://doi.org/10.1016/j.bbabio.2008.12.008

[56] Smith, R. A., & Murphy, M. P. (2010). Animal and human studies with targeted antioxidants. *Free Radical Biology and Medicine, 49*(11), 1593–1602.

[57] Someya, S., Yu, W., Hallows, W. C., Tan, J., Vann, J. M., Leeuwenburgh, C., ... & Prolla, T. A. (2010). Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under calorie restriction. *Cell, 143*(5), 802–812. http://dx.doi.org/10.1016/j.cell.2010.10.002

[58] Stadtman, E. R., & Levine, R. L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids, 25*(3), 207–218. https://doi.org/10.1007/s00726-003-0011-2

[59] Valavanidis, A., Vlachogianni, T., & Fiotakis, C. (2009). 8-Hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *Journal of Environmental Science and Health, Part C, 27*(2), 120–139. https://doi.org/10.1080/10590500902885684

[60] Vaziri, H., Dessain, S. K., Ng Eaton, E., Imai, S. I., Frye, R. A., Pandita, T. K., ... & Weinberg, R. A. (2001). hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell, 107*(2), 149–159.

[61] Vogelstein, B., Lane, D., & Levine, A. J. (2000). Surfing the p53 network. *Nature, 408*(6810), 307–310. https://doi.org/10.1038/35042675

[62] Westermann, B. (2010). Mitochondrial fusion and fission in cell life and death. *Nature Reviews Molecular Cell Biology, 11*(12), 872–884. https://doi.org/10.1038/nrm3013

[63] White, M. J., McArthur, K., Metcalf, D., Lane, R. M., Cambier, J. C., Herold, M. J., ... & Kile, B. T. (2014). Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. *Cell, 159*(7), 1549–1562.

[64] Wiley, C. D., & Campisi, J. (2021). The metabolic roots of senescence: Mechanisms and opportunities for therapy. *Nature Metabolism, 3*(10), 1290–1301. https://doi.org/10.1038/s42255-021-00483-8

[65] Yamamoto, M., Kensler, T. W., & Motohashi, H. (2018). The KEAP1-NRF2 system: A thiol-based sensor-effector apparatus for maintaining cellular redox homeostasis. *Physiological Reviews, 98*(3), 1169–1203. https://doi.org/10.1152/physrev.00023.2017

[66] Yoon, Y. S., Yoon, D. S., Lim, I. K., Yoon, S. H., Chung, H. Y., Rojo, M., ... & Yoon, G. (2006). Formation of elongated giant mitochondria in replicative senescence and senescence-like state of human diploid fibroblasts. *Journal of Cellular Physiology, 209*(2), 468–480. https://doi.org/10.1002/jcp.20753

[67] Yoshino, J., Baur, J. A., & Imai, S. I. (2018). NAD+ intermediates: The biology and therapeutic potential of NMN and NR. *Cell Metabolism, 27*(3), 513–528. https://doi.org/10.1016/j.cmet.2017.11.002

[68] Yousefzadeh, M. J., Zhu, Y., McGowan, S. J., Angelini, L., Fuhrmann-Stroissnigg, H., Xu, M., ... & Robbins, P. D. (2018). Fisetin is a senotherapeutic that extends health and lifespan. *EBioMedicine, 36*, 18–28. https://doi.org/10.1016/j.ebiom.2018.09.015

[69] Zhao, R. Z., Jiang, S., Zhang, L., & Yu, Z. B. (2019). Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *International Journal of Molecular Medicine, 44*(1), 3–15. https://doi.org/10.3892/ijmm.2019.4188

[70] Zhu, Y., Tchkonia, T., Fuhrmann-Stroissnigg, H., Dai, H. M., Ling, Y. Y., Stout, M. B., ... & Kirkland, J. L. (2016). Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic proteins. *Aging Cell, 15*(3), 428–435. https://doi.org/10.1111/acel.12445

[71] Zhu, Y., Tchkonia, T., Pirtskhalava, T., Gower, A. C., Ding, H., Giorgadze, N., ... & Kirkland, J. L. (2015). The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. *Aging Cell, 14*(4), 644–658. https://doi.org/10.1111/acel.12344

[72] Zorova, L. D., Popkov, V. A., Plotnikov, E. Y., Silachev, D. N., Pevzner, I. B., Jankauskas, S. S., ... & Zorov, D. B. (2018). Mitochondrial membrane potential. *Analytical Biochemistry, 552*, 50–59. https://doi.org/10.1016/j.ab.2017.07.009