



Review article

Redox control of senescence and age-related disease

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ABSTRACT

The signaling networks that drive the aging process, associated functional deterioration, and pathologies has captured the scientific community's attention for decades. While many theories exist to explain the aging process, the production of reactive oxygen species (ROS) provides a signaling link between engagement of cellular senescence and several age-associated pathologies. Cellular senescence has evolved to restrict tumor progression but the accompanying senescence-associated secretory phenotype (SASP) promotes pathogenic pathways. Here, we review known biological theories of aging and how ROS mechanistically control senescence and the aging process. We also describe the redox-regulated signaling networks controlling the SASP and its important role in driving age-related diseases. Finally, we discuss progress in designing therapeutic strategies that manipulate the cellular redox environment to restrict age-associated pathology.

1. Biological theories of aging

Why do we age? The answer to this perennial question has encouraged the scientific community to pursue hypotheses that can delineate the mechanisms behind the human body's functional decline with age. However, despite medical advances there remain challenges with respect to delineating controls that regulate the span of human life and health. Elucidation of these control mechanisms may lead to better-targeted interventions for the gamut of diseases propagated by age. Ultimately, amelioration of age-associated pathologies and comorbidities would limit age-related frailty and improve the well-being of our aging population.

Biological aging is the progressive manifestation of accumulated cellular damage with age, and is determined by environmental or genetic factors [1–4]. The process of aging is complex and can be explained by a number of complimentary theories. The wear and tear theory, proposed by August Weismann in 1882, compares organisms to machines, suggesting that cells 'wear out' with time and accumulate damage through excessive consumption of fat, sugar, or exposure to UV radiation, leading to declines in functional efficiency of organs. However, this theory disregards the ability of cells to repair damage. The stochastic theory which is a by-product of the wear and tear theory, postulates that aging is due to the accumulation of damage in cells resulting from failure of wound repairing mechanisms, infections, UV radiation, or environmental stress [5]. Evolutionary theories of aging support the concept that specific genes implicated in longevity are involved in the stability and maintenance of somatic cells, and are

susceptible to mutations with age. Medawar's mutation accumulation theory links aging to natural selection, hypothesizing that there is no selection pressure on the aging population, as there is no known evolutionary mechanism to eliminate a population of mutations that cause deleterious effects only in aged species [6]. This suggests that detrimental late-acting alleles may accumulate with age and exert adverse effects on survival. The antagonistic pleiotropy model suggests that a pleiotropic gene that selectively increases fitness and survival during the early stages of life can still induce adverse late-acting effects in the population [7]. In the context of cellular aging, this could apply to replicative senescence that suppresses tumor formation early in life, but may promote cancer in later stages. The disposable soma theory, put forth as an extension of the antagonistic pleiotropy model, highlights the balance between somatic maintenance and repair versus reproduction and suggests that with age extensive maintenance would be necessary as cells can accumulate multiple lesions in parallel [8,9]. The rate of living theory puts across the idea that organisms that metabolize oxygen more rapidly have a higher energy expenditure and typically have shorter lifespans that can be extended by caloric restriction [10–13]. According to the cross-linking (or glycosylation) theory of aging, cross-linked proteins or sugar moieties can bind to DNA causing replicative damage or an age-related decline in protein turnover that can be linked to the loss of functional proteins, further promoting age-associated pathologies [14].

While all of the aforementioned theories have been examined and laboriously tested, the mitochondrial free radical theory of aging, later termed the oxidative stress theory (OST), is currently one of the most

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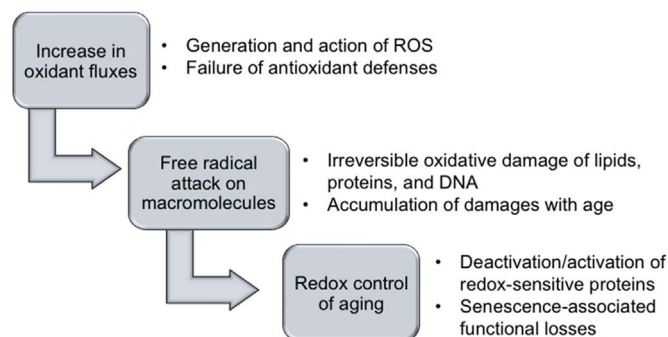


Fig. 1. Schematic representation of the oxidative stress theory of aging. The key features of this hypothesis are that increase in oxidants and concomitant failure of antioxidant mechanisms cause structural damage to macromolecules which accumulates with age leading to corresponding decline or loss in function.

popular correlative theories of the aging process. OST explains aging at the molecular level and results from failure to maintain oxidative defenses, mitochondrial integrity, proteostasis, barrier structures, DNA repair, telomeres, immune function, metabolic regulation, and regenerative capacity (Fig. 1). More than half a century ago, Gerschman introduced that oxygen free radicals, commonly considered to be too reactive to exist in biological systems, are generated in situ in response to radiation and oxygen poisoning and are responsible for the associated toxicities [15,16]. Later, Denham Harman proposed that hydroxyl and hydroperoxyl radicals generated endogenously from oxygen-consuming metabolic processes play crucial roles in the aging process [17]. This hypothesis coalesced from a number of observations: (i) indications that free radicals are produced in vivo; (ii) free radicals cause pervasive damage to macromolecules in vitro; and (iii) the deleterious effects of exposure to irradiation and hyperoxia are alleviated by antioxidant administration. Free radicals are highly reactive atoms or molecules with one or more unpaired electron(s) in their outermost shell and can be formed when oxygen interacts with certain molecules. They can be formed endogenously by natural biological processes, or generated upon exposure to external stimuli. Reactive oxygen species (ROS) collectively describes a number of reactive molecules, such as superoxide ($O_2^{\cdot-}$), hydroxyl radical (OH^{\cdot}), hydroperoxyl radical (HO_2^{\cdot}), nitric oxide (NO^{\cdot}), nitrogen dioxide (NO_2^{\cdot}), and peroxy (ROO^{\cdot}), produced as by-products during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes. Also, hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen (1O_2), hypochlorous acid ($HOCl$), nitrous acid (HNO_2), peroxy-nitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), and lipid peroxide ($LOOH$), are oxidants that can easily lead to free radical reactions in cellular systems [18]. Expanding knowledge of the mechanisms of ROS generation, intracellular sources, antioxidant defenses, and the chemistry of ROS-induced oxidative damage, was critical to improve understanding of the homeostasis between pro-oxidants and anti-oxidants, thereby evolving the free radical theory into the OST of aging [19,20]. The “structural damage-based” hypothesis driving this theory is that age-associated functional losses are due to the accrual of oxidative damage to macromolecules such as lipids, DNA, and proteins by free radicals, and that the progressive oxidant/anti-oxidant imbalance leads to the consequent disruption of redox-regulated signaling mechanisms. Beckman and Ames divided this hypothesis into “strong” and “weak” versions: The strong version proposed that oxidative damage determines life span, while the weak version correlated oxidative damage to age-associated pathologies [21].

Although widely accepted, the OST has been severely refuted in recent years; most criticisms try to discredit the theory pointing at failure to increase longevity by increasing antioxidants and the production of fewer free radicals in certain long-living species [22–26]. Recently, a critical idea has emerged that ROS at non-toxic levels function as signaling molecules that induce protective defenses against

age-dependent damage, and that peroxisomes may be implicated in the regulation of aging [27]. Currently, the two major traits correlating organismal aging and longevity to the OST of aging are considered to focus primarily on the species-specific low mitochondrial ROS generation rates at complex I of the electron transport chain (ETC) in long-lived animals, and low levels of fatty acid unsaturation on cellular and mitochondrial membranes [28–30].

2. Cellular senescence

Cellular senescence, also termed replicative senescence, was first proposed four decades ago [31,32] and has since been proven to be a significant tumor constraining mechanism that halts the proliferation of primary mammalian cells after a finite number of population doublings [33–37] allowing the organism to antagonize the potentially detrimental effects of uncontrolled growth. Proliferation is checked by persistently arresting cell growth at the G1- [38–42], G2- [43–45], G2/M- [46] or S-phase of the cell cycle leading to failure of DNA replication. So far, senescence-associated growth arrest has been shown to depend functionally on the tumor-suppressor pathways controlled by p16^{INK4a} and pRB (retinoblastoma protein), as well as by p53. Progressive telomere attrition due to persistent DNA damage responses [47] has been attributed to be one of the clocking mechanisms that keeps track of the number of times a cell divides, providing an appropriate trigger for the onset of telomere-induced senescence through the p53 pathway [34,48–52]. ROS can induce a senescent growth arrest in vitro, by triggering the DNA damage response (DDR) pathway, with ATM or ATR kinases blocking progression of the cell cycle through stabilization of p53 and transcriptional activation of the cyclin-dependent kinase (CDK) inhibitor p21 [53]. However, in response to persistent DNA damage, p16^{INK4a} is activated via p38-MAPK-mediated mitochondrial dysfunction and ROS production. Nuclear lamin B1 downregulation triggers modifications in chromatin methylation and induces senescence progression with the formation of highly condensed regions of chromatin called senescence-associated heterochromatin foci [54]. These foci are enriched in chromatin modifications that associate with genes required for maintenance of senescence-associated growth arrest. Senescence can also be induced by cell-type specific stress independent of telomere shortening, resulting in increased expression of the tumor suppressor p16, in which case it is termed stress-induced senescence [42,55–57]. This includes chronic signaling by anti-proliferative cytokines that increase cellular oxidative stress and potentiate senescence [58–60]. Oncogene-induced senescence (OIS), characterized by aberrant DNA replication and the associated DNA damage response, is affected by activated oncogenes or the loss of tumor-suppressor genes in healthy cells [45,61–64] and upregulation of the CDK inhibitors p15^{INK4b}, p16^{INK4a}, and p21^{CIP1} [65]. Therapy-induced senescence (TIS) has been described in tumor cell lines in response to induction with selective preoperative neoadjuvant chemotherapeutics or radiation agents, and is considered crucial to mitigate toxicity-related side effects, thereby presenting a novel therapeutic target for cancer [66,67].

3. Senescence-Associated Secretory Phenotype (SASP)

Cellular senescence is a significant tumor constraining mechanism that halts cellular proliferation in response to damage that occurs during replication. However, senescent cells acquire a deleterious, irreversible senescence-associated secretory phenotype (SASP) involving secretion of soluble factors (interleukins, chemokines, and growth factors), degradative enzymes like matrix metalloproteases (MMPs), and insoluble proteins/extracellular matrix (ECM) components [33,68] that can alter tissue microenvironments and affect neighboring epithelial cells in a paracrine fashion to promote tumor progression [65,69,70]. Also termed the senescence-messaging secretome (SMS) [71], these factors promote shedding of membrane-associated proteins,

degradation of signaling molecules, and/or ECM processing, thereby disrupting normal tissue framework and function [72]. Morphologically, senescence is characterized by cellular enlargement and flattening with an accompanying increase in cell volume, increased size of organelles including Golgi, lysosomes and nucleus, appearance of vacuoles in the cytoplasm and ER and an increase in the number of cytoplasmic microfilaments.

The senescent phenotype has been long associated with driving organismal aging, and has recently been linked to development and tissue repair. The SASP is primarily a persistent DDR coupled as a positive feedback loop with the senescent stimuli ROS, prompting telomere-dependent or -independent signaling mechanisms to facilitate growth arrest. The non-cell-autonomous activities mediated by SASP serve to deplete stem and progenitor cells and demonstrate that the heterogeneous functional networks of senescence are formed by multiple effector pathways involving highly dynamic signal amplification [73]. The SASP can also be uncoupled from the senescence-associated cell-cycle arrest, as it exemplifies a perpetual state of damage in comparison to growth arrest. SASP has been characterized by analysis and conservation of factors between human and mouse cells cultured under physiological oxygen conditions [74] and its occurrence has been seen in vivo in mice and humans [75,76] and amongst diverse proliferative cell types (fibroblasts, epithelial cells, endothelial cells, astrocytes, etc.) [76–78].

The SASP components that modulate neighboring cells can lead to the activation of signaling cascades involved in several disease processes. It is sufficient for a given tissue to be comprised of less than 20% of senescent cells to exert SASP-mediated systemic effects [79]. The gamut of downstream effects initiated by SASP include tumorigenesis (paracrine), immunomodulation (paracrine), senescence amplification (paracrine and autocrine), transformation of the tissue micro-environment (paracrine), and perturbation of stem cell niche [80]. Continued exposure to senescent cells has been shown to potentially promote senescence in healthy bystander fibroblasts via gap junction-mediated cell-cell contacts and processes implicating ROS [81]. While a deluge of evidence points out to the role of ROS in inducing senescence, Lawless et al. employed an empirical stochastic step model of replicative senescence to suggest that increased mitochondrial ROS generation in senescent cells is a repercussion of SASP rather than the reverse [82].

The proteins of the SASP component are broadly conserved, although differences exist among cell types [76]. The contribution of non-protein factors such as nucleotides, bradykines, prostenoids, or ceramides to SASP's effects is also relatively unexplored. The composition of the SMS may vary dynamically with time after the initiation of senescence and partly depends on the senescence-inducing mechanism. This 'late-induced' senescence phenomenon propagates by forming cytoplasmic chromatin fragments with DNA lesions that eventually undergo lysosome-mediated proteolysis causing overall histone loss, thereby illustrating perpetual genomic and epigenomic remodeling in senescence [76].

3.1. Beneficial effects of SASP

The SASP is fundamentally a response to genomic damage that allows damaged cells to communicate their compromised state to neighboring cells and stimulates tissue repair and/or regeneration after insult by attracting immune cells and inducing local inflammation. However, it has been indicated that senescence is not consistently followed by immune cell infiltration and inflammation [83] as observed in highly persistent senescent cells in human melanocytic nevi. The SASP also includes several chemokines and cytokines that activate immune cells to specifically target and eventually clear senescent cells from tissues [84,85]. SASP factors such as IL-6, IL-8, protease inhibitor plasminogen activator inhibitor-1 (PAI-1), and pleiotropic protein insulin-like growth factor binding protein-7 (IGFBP-7) are also im-

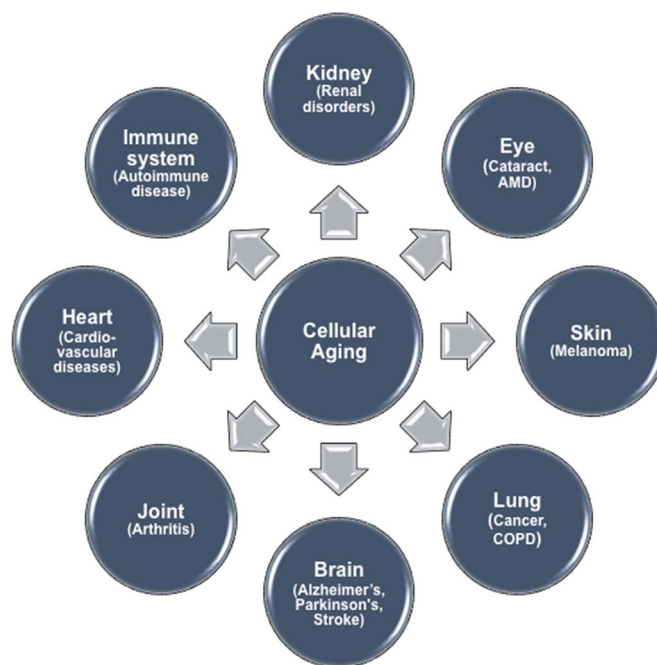


Fig. 2. Schematic representation of diseases propagated by age. Cellular senescence and the associated secretory phenotype have been implicated in initiation and progress of several acute and chronic pathological maladies driven by age-associated inflammation (inflammaging) and oxidative stress-mediated damage. AMD – Age-related macular degeneration; COPD – Chronic obstructive pulmonary disease).

plicated in tumor suppressive growth arrest of cells [75,86,87]. The SASP-produced MMPs also limit fibrosis during wound healing [88] or following liver injury [89,90]. Additionally, developmentally programmed and transiently-regulated senescence is established to be vital in promoting tissue remodeling, embryogenesis and patterning [91–93]. Finally, the deterioration of the immune system with age coupled with the kinetics and efficiency of senescent-cell clearance determines the switch of senescence from a temporal programmed process to a persistent stochastic response. The persistent stochastic response involves a multitude of concurrent signals that are paramount to the progression of chronic age-related pathologies.

3.2. SASP in disease

Cellular senescence and consequently SASP factors are involved in several acute and chronic pathological processes (Fig. 2). In a murine model, this association was supported by the delayed onset of age-related diseases when senescent cells were genetically ablated [94]. Considering that the SASP components differ in character from cytokines to growth factors and proteases the effects they can convey individually or more importantly collaboratively in different tissues may vary as well. Byun et al. have proposed that senescent cells have a definitive role in age-associated pathologies including their ability to modulate young cells through 1) release of inflammatory SASP factors i.e. IL-1; 2) secretion of autocrine or paracrine factors that modulate the activity of other senescent cells or disease-prone young cells (EMT activation by IL-6 and IL-8); 3) expression of SASP receptors (IL-17); and 4) release of anti-inflammatory molecules such as IL-10 [95]. The SASP can promote distinct disease pathologies by perpetuating a pro-inflammatory state and promoting cell transdifferentiation. For example, various cardiovascular risk factors including obesity, hypertension, diabetes and atherosclerosis that compromise metabolism are associated with an inflammatory state and increased cellular senescence and SASP [96]. Vascular calcification, another risk factor for cardiovascular disease, is linked to a SASP-driven osteoblastic transdifferentiation of senescent smooth muscle cells [97], while atherogenesis

has been shown to be in part mediated by the pro-inflammatory IL-1 α [98]. Obese individuals with type-2 diabetes show increased levels of SASP factors IL-6, IL-8, and the chemokine MCP-1 [96]. Moreover, both IL-6 and IL-1 β are independent predictors of diabetes [96]. In many prevalent neurodegenerative conditions including Alzheimer's Disease, brain tissue biopsies are present with increased levels of p16^{INK4a}- positive astrocytes, MMP-1 and IL-6 [99,100]. Other prevalent pathologies which share several damaging SASP profiles including IL-6, IL-8, GM-CSF, MMP-1 [101] are chronic obstructive pulmonary disease (COPD), biliary cirrhosis and cholangitis [102], and osteoarthritis [103].

Although cellular senescence can serve as a tumorigenic restrictive mechanism, it can also promote tumor growth and tumor invasiveness, through SASP-mediated regulatory effects [104,105]. A known pathway for SASP-promoted cancer metastasis is the induction of epithelial to mesenchymal transition (EMT); a process that can have both beneficial wound healing effects, and pathological fibrotic and invasiveness-promoting ones [106]. EMT is also a crucial process that can elicit fibrosis in Crohn's disease and graft loss after kidney transplantation [107,108]. Moreover, decreased renal function due to both acute and chronic kidney injury has also been associated with cellular senescence and SASP [109,110].

Though the SASP signature of specific pathological processes still remains to be elucidated, great attention is increasingly being paid to the effects of both biological and stress-induced senescence in different tissues. Both the beneficial and deleterious effects of SASP suggest a possible use of the implicated factors as therapeutic targets for disease control. In addition, the oxidative stress-mediated regulation of biological aging and age-associated pathologies has become a promising target for therapeutic strategies against age-associated maladies. Here, we review the recent progress in understanding the role of the oxidative stress and the cellular redox environment in senescence, with a focus on redox-control of senescence-regulatory factors. The effects of oxidative stress on cellular senescence at a cellular and nuclear level are depicted in Figs. 3 and 4 respectively (Figs. 3 and 4).

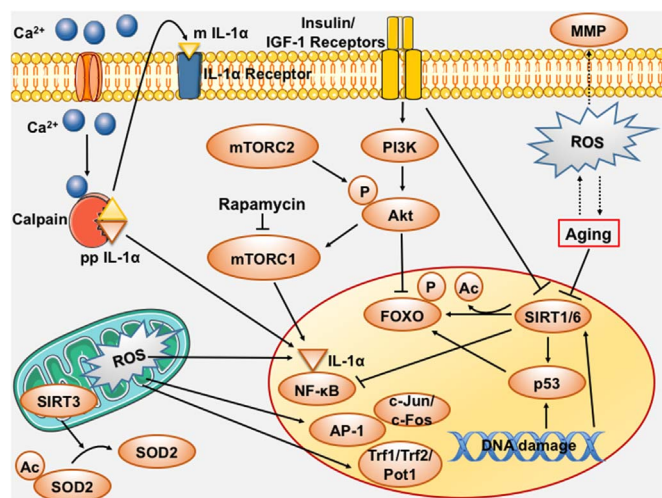


Fig. 3. Schematic representation of the redox-control of pro-aging mechanisms. Oxidative stress-mediated regulation of several factors and mechanistic pathways plays a critical role in senescence regulation. Several of these components can interact with each other to induce effects of the senescent phenotype. AP-1 – Activator protein 1; Ca²⁺ – Calcium; FOXO – Forkhead box O; IGF-1 – Insulin-like growth factor-1; IL – Interleukin; MMP – Matrix metalloproteinase; mTORC – Mammalian target of rapamycin complex; NF- κ B – Nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K – Phosphoinositide 3-kinase; Pot-1 – Protection of telomeres protein 1; pRb – Retinoblastoma protein; ROS – Reactive oxygen species; SIRT – Sirtuin; SOD2 – Superoxide dismutase 2, mitochondrial; Trf – Telomeric repeat binding factor.

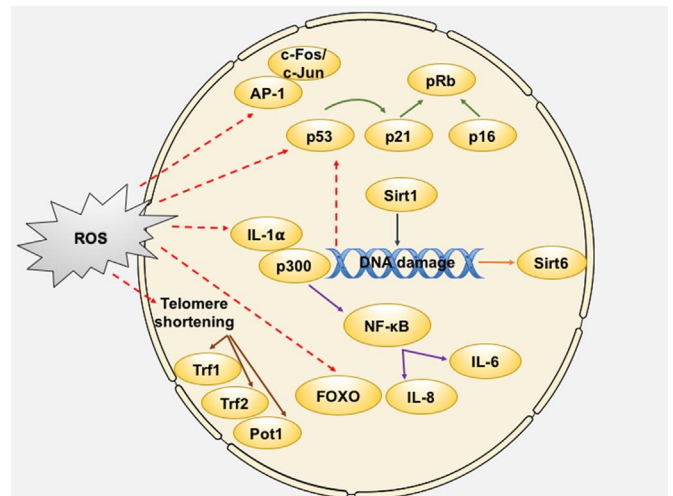


Fig. 4. Schematic representation of the redox-control of pro-aging mechanisms in the nucleus. Oxidative stress-mediated regulation of nuclear proteins can happen via expression of factors induced by DNA damage-mediated responses or at the transcriptional/translational level via modulation of protein activity, thereby allowing for precise control over senescence effector mechanisms. AP-1 – Activator protein 1; FOXO – Forkhead box O; IL – Interleukin; NF- κ B – Nuclear factor kappa-light-chain-enhancer of activated B cells; Pot-1 – Protection of telomeres protein 1; pRb – Retinoblastoma protein; SIRT – Sirtuin; Trf – Telomeric repeat binding factor.

4. Redox Control of SASP and Age-Related Pathologies

4.1. Mammalian target of rapamycin pathway

The mammalian target of rapamycin (mTOR) protein is a highly conserved large protein kinase of the phosphatidylinositol 3 kinase-related kinase (PI3KK) family, and an intracellular target of rapamycin, a pharmacological immunosuppressant approved by FDA (Food and Drug Administration, USA) in therapies against various types of cancers [111,112]. The conserved TOR complexes, mTORC1 and mTORC2, can be differentiated by their distinct associated proteins, Raptor and Rictor, respectively. mTOR complexes play crucial roles in mediating nutrient signaling, autophagy and growth regulation (mTORC1) and also regulate cell survival and spatial organization of the cytoskeleton (mTORC2) [113,114].

Inhibition of mTORC1 by rapamycin may both counter damage-inducing senescence mechanisms and enhance repair pathways, and as a consequence, result in extended lifespan in model organisms [115]. Over the past decade, the impact of mTOR signaling on cellular aging has gained traction with several studies performed in multiple cellular systems that argue for a pivotal role of mTOR in the senescent program [112,116]; however, little is known with respect to how cellular redox state contributes to mTOR regulatory function in senescence.

Cellular stresses, such as hypoxia, or low energy status, which can be sensed by the AMP-sensitive kinase (AMPK) inhibit mTOR signaling [111]. Conversely, elevated mTOR activity in response to excess nutrients leads to increased oxidative stress by enhancing mitochondrial oxygen consumption. Cysteine oxidation enhances phosphorylation of S6K, which is a direct readout of mTORC1 activation [117]. Conversely, treatment with reducing agents effectively suppresses mTORC1 activation via inhibition of S6K phosphorylation. Furthermore, oxidants destabilize the mTOR-Raptor interaction but not the mTOR-Rictor interaction suggesting that a redox-sensitive mechanism may underlie amino acid-dependent mTORC1, but not mTORC2 regulation [118]. These studies also indicate that the phosphorylation of tuberous sclerosis complex (TSC2) tumor suppressor protein regulates Rheb GTPase activity which plays an essential role in mTORC1 regulation in response to cellular redox potential. GRp58 (also known as ERp57), a widely expressed protein disulfide isomerase

regulating protein-protein interactions through a redox-based mechanism acts via its two thioredoxin-like domains and has shown to be an mTOR-interacting protein [119]. These findings suggest that a redox-sensitive mechanism regulates the activity of mTORC1, and the interaction between Raptor and proteins with redox-sensing abilities are potentially involved in the assembly and regulation of mTOR complexes. In contrast, Liu et al. demonstrated that mTOR regulation under hypoxic conditions is independent of cellular redox change [120] as H₂O₂-scavenging by catalase failed to attenuate the hypoxic hypophosphorylation of p70S6K and 4EBP1. More recently, regulation of the mTORC2 complex formation and stability has been demonstrated to be redox sensitive [121], as knockdown of p22phox inhibits NADPH-dependent superoxide generation and decreases downstream Rictor-associated mTORC2 complex activity without affecting Raptor-associated mTORC1 complex. This likely occurs through redox-control of Rictor expression and its subsequent interaction with mTORC2. mTORC1 also regulates mRNA translation and protein synthesis under conditions that favor growth, and there is evidence to suggest that regulation of mRNA translation can modulate longevity in several model organisms [122]. The cellular redox status may likely play a role in global reduction in mRNA translation through TOR signaling and would be advantageous to the aging process allowing for better maintenance of protein homeostasis. Taken together, these observations indicate that approaches that exploit the redox-sensitive nature of both mTOR complexes might prove useful in the design of therapeutics that target age-associated disorders.

4.2. IL-1 α

Pro-inflammatory members of the interleukin-1 (IL-1) family of cytokines (IL-1 α and β) are important mediators of the host defense response to infection, but can also aggravate inflammation that is central to the progression of many disease processes [123]. IL-1 α , one of the upstream regulators of SASP, is a dual-function cytokine which is rapidly expressed not only upon stimulation but is present constitutively in healthy cells. It is synthesized as a precursor protein that is subsequently cleaved into two functional fragments that have distinct subcellular localizations by proteases such as calpain [124], CTL/NK-granzyme-B, mast cell chymase, or neutrophil elastase [125]. However, IL-1 α cleavage by calpain is rare and only serves to increase the potency of the mature form of the protein in activating pro-inflammatory mediators of SASP.

Basal and tumor necrosis factor (TNF)-induced expression of IL-1 α has been shown to be regulated by superoxide via modulation of mitochondrial superoxide dismutase (SOD2) that in turn influences the cellular redox homeostasis [126]. Their findings indicate a critical role for a superoxide-reactive intermediate of mitochondrial origin in regulating IL-1 α mRNA stability both constitutively and in response to TNF. The IL-1 α proximal promoter contains binding sites for the transcription factor AP-1 [127], that has previously been established to be activated by an H₂O₂-dependent mechanism [128], again suggesting the redox-dependency of IL-1 α induction. In exploration of the mechanistic drivers of IL-1 α , redox-engineered fibrosarcoma cells were used to demonstrate that the cellular redox state mediates IL-1 α expression and processing through the Ca²⁺ dependent protease calpain and alterations in calcium (Ca²⁺) homeostasis [129]. Steady state levels of intracellular hydrogen peroxide (H₂O₂) significantly promoted IL-1 α expression and nuclear translocation, where it serves as a pro-inflammatory mediator by increasing NF- κ B activity, thereby further delineating the role of IL-1 α in ROS-mediated inflammation. Similarly, in serially cultured senescent human fetal lung fibroblasts, the redox-dependent processing of IL-1 α by calpain has been shown to be critical to the regulation of SASP [70]. Senescence-associated upregulation of the mature form of IL-1 α promoted metastatic progression of neighboring healthy epithelial cells, triggering an epithelial-mesenchymal transition and was reversed by antioxidant

treatment, implying a redox-driven control mechanism. More recently, Laberge et al. have shown that mTOR drives the SASP and that treatment with the mTOR inhibitor rapamycin partly suppresses the secretory phenotype and tumor-promoting ability via translational inhibition of IL-1 α [130]. Despite its role in the regulation and amplification of SASP, there exist few studies that describe aberrant activation mechanisms for IL-1 α , thus understanding these mechanistic controls is necessary to allow successful therapeutic intervention to reduce the deleterious effects of the normal aging process.

4.3. Matrix metalloproteinase regulation

Matrix metalloproteinases (MMPs) are a group of endopeptidases with important roles in wound healing, tissue remodeling and cellular growth [131,132]. Abnormally high MMP expression is associated with age-related and chronic diseases such as cancer, Alzheimer's, atherosclerosis, osteoarthritis, and lung emphysema [132]. The mitochondrial redox control of MMPs has been previously reported, where ROS are shown as key regulators of MMPs including MMP-1, -2, -7, and -9, [133–135] while also being upregulated by high levels of MMP-2 [136]. In particular, the association between MMP-1 and ROS and the significance of these interactions in degenerative disease etiology is well established. MMP-1, cleaves the structural connective tissue components collagen type I, II and III, and is detected only at low levels under physiological conditions, but can be abnormally high in several age-associated pathologies [132,137]. The increased expression of MMP-1 can be both age- and ROS-dependent [128,138,132]. ROS and age associated MMP-1 expression involves the recruitment of the primary transcription factors Ets-1/AP-1 (c-Jun/c-Fos) to the distal promoter region of MMP-1, along with the differential recruitment of chromatin-modifying proteins P/CAF and HDAC2. These coordinated events mediated by ROS are responsible for induction of an active MMP-1 promoter complex. Multiple studies also show that while oxidants such as H₂O₂, and antioxidant inhibitors such as aminotriazole can enhance MMP expression [139], treatment with antioxidant agents such as catalase, Vitamin A, Vitamin E, trans retinoic acid, resveratrol, and N-acetyl cysteine can block or decrease MMP expression in different cell lines [140–146]. More recently, ROS-activated MMPs in the wall of cerebral vessels has also been shown to be exacerbated with age in a mouse model that recapitulates cerebrovascular alterations present in elderly humans [147], adding to the growing body of literature supporting age-associated redox-driven MMP induction. This redox sensitive regulation of MMPs provides a definitive rationale for the use of antioxidant-based therapies in the treatment of degenerative disorders associated with aberrant matrix destruction propagated by age.

4.4. IGF Signaling and FOXO regulation

The insulin/insulin-like growth factor-1 (IGF-1) signal transduction pathway controls various proliferative, survival and metabolic signaling networks. IGF-1 in association with its downstream effectors was the first evolutionarily conserved longevity regulatory network identified from yeast to humans [148,149]. Impaired insulin/IGF-1 signaling lowers the oxidative burden and the associated oxidative damage in cells which contributes to increased longevity [150–152].

Forkhead box O (FOXO) transcription factor family members (FOXO1, FOXO3a, FOXO4, and FOXO6 in mammals; DAF-16 in *C. elegans*; DFOXO in *D. melanogaster*) are evolutionarily conserved AKT substrates that regulate genes involved in cell cycle, apoptosis, DDR, metabolism, oxidative stress defense mechanisms, and aging [153]. The activity of FOXO proteins is primarily regulated by signaling in response to IGF receptor engagement in a redox-dependent fashion [154]. FOXO family members also confer protection from oxidative stress through transcription of antioxidant genes, SOD2, catalase, peroxiredoxin 3, and members of the sestrin family [155–157]. The ROS-induced formation of cysteine-thiol disulfide-dependent com-

plexes of FOXO with the p300/CBP (CREB-binding protein) acetyltransferase has also been implicated in the modulation of biological activity of FOXO4 [158]. Overall, FOXOs serve as stress sensors and appear to influence both pro- (inducing senescence and apoptosis) and anti-aging (ROS scavenging) mechanisms.

Senescent endothelial, epithelial, and fibroblast cells demonstrate high expression levels of IGF-binding proteins (IGFBPs) including IGFBP-2, -3, -4, -5, and -6 [72,159–161], and their regulating proteins (IGFBP-rPs) IGFBP-rP1, and 2 [162,163]. IGFBP-rP1 promotes senescence in part through p21 and independent of p53 [164]. Recently, IGFBP7, a potential tumor suppressor, has been identified as a marker of oncogenic BRAF-induced senescence, via negative feedback loops [87,165]. The initial response to the insulin receptor activation is the NADPH oxidase-dependent generation of H₂O₂ [166] that plays both positive and negative autoregulatory roles in the regulation of insulin/IGF-1 signaling. The endogenous production of H₂O₂ is implicated in the autophosphorylation of the insulin receptor β -subunit (IR β) tyrosine motif [167,168]. H₂O₂-dependent priming of IR β and associated proteins, coupled with reversible oxidative inhibition of protein tyrosine phosphatases, enables IR β phosphorylation and sustains insulin/IGF-1 signaling.

The insulin/IGF-1 pathway turns from a growth-promoting regulator in early stages and into a senescence-inducing pathway during the later stages of life [169,170]. A detailed characterization of the regulatory role of ROS in insulin/IGF-1-mediated signaling at all stages in life is crucial to design therapeutics targeting age-related disorders.

4.5. Calcium (Ca²⁺)

Calcium is a ubiquitous secondary signaling molecule regulating a disparate range of physiological process, including muscle contraction, gene transcription, and cell proliferation [171]. Comprehensive reviews by Ureshino et al., and Farfariello et al. summarize the Ca²⁺ signaling networks that may mediate senescence via multiple regulatory pathways including proliferation and cell cycle arrest, apoptosis, autophagy, alterations in membrane potential, altered telomerase activity, and SASP [172,173]. The role of Ca²⁺ as a key signaling intermediate involved in SASP regulation has only recently emerged [70].

Ca²⁺ signaling is central to driving morphological changes that are the hallmarks of senescence. Senescence-associated increase in cell volume is normally countered by regulatory volume decrease (RVD) which preserves the structural integrity of the cell [174]. Senescence-associated cell volume changes lead to membrane stretching or osmotic swelling-based activation of TRP vanilloid 4 (TRPV4) or TRP melastatin 7 (TRPM7) channels that mediate Ca²⁺ influx from the extracellular space, subsequently activating RVD counter-regulatory signals [175,176]. Mitochondrial Ca²⁺ importers maintain homeostatic regulation of the electron transport and increased Ca²⁺ influx decreases overall ATP production, elevating cytosolic NADH, decreasing sirtuin activity leading to senescence [177]. Similarly, recent work by Wiel et al., identified two calcium channels, inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) localized in the ER and the mitochondrial calcium uniporter (MCU), as novel regulators of senescence program by increasing Ca²⁺ uptake [178].

In aged human fetal lung fibroblasts, senescence-associated increases in basal intracellular Ca²⁺ is implicated in downstream activation of the Ca²⁺-dependent protease calpain, and the subsequent regulation of IL-1 α [70]. Treatment of senescent fibroblasts with BAPTA-AM, a Ca²⁺ chelator or calpain inhibition reduces the senescence-associated expression of IL-8 and IL-6, respectively. These observations suggest there exists both a redox and Ca²⁺-dependent process in the regulation of the prominent SASP factors IL-6 and IL-8. During OIS and replicative senescence, ITPR2 triggers Ca²⁺ release from the endoplasmic reticulum (ER), with subsequent mitochondrial Ca²⁺ accumulation via MCU channel, leading to reduced mitochondrial membrane potential, ROS accumulation, and senescence. Conversely,

intracellular Ca²⁺ reservoirs in the mitochondria are released in response to stress, and facilitate activation of cAMP-responsive element binding protein 1 (CREB) that upregulates p21^{CIP1/WAF1} expression, thereby inhibiting cell proliferation [179]. Sharov et al. have shown that age-dependent loss in approximately 2.8 mol of cysteine/mol of sarcoplasmic reticulum (SR) Ca²⁺ ATPase (SERCA) as a result of oxidation, may in part be responsible for the age-dependent decline in SERCA activity by about 40% [180]. In a similar model employing young (5 month old) and aged (21 month old) transgenic mice, myocyte-specific catalase overexpression limited oxidative modification of SERCA and preserved SR Ca²⁺, SERCA activity and myocyte relaxation [181]. The ability of catalase overexpression to preserve SERCA activity strongly suggests that the post-translational modifications of SERCA in the senescent heart are H₂O₂-dependent. Though the role of Ca²⁺ dysregulation in physiological and organ-specific aging processes has been explored, further work is needed to decipher the connection between Ca²⁺ dynamics and oxidative damage-induced senescence.

4.6. Sirtuins

Silent information regulator (Sir2) genes and their functional orthologs are conserved from bacteria to humans and constitute a family of NAD⁺-dependent protein histone deacetylases (HDACs) called sirtuins (SIRT). Initially described as gene silencers, sirtuins are implicated in a wide variety of physiological processes including apoptosis, lipid metabolism, cellular response to stress, mitochondrial biogenesis, fatty acid oxidation, insulin production, and inflammation. SIRT regulates the lifespan-extending effects of caloric restriction improved glucose tolerance, inhibit of degenerative disorders, improve endothelial function, promote regression of atherosclerotic plaques and prevent cancer [182]. The sirtuins class III family is comprised of seven human sirtuins (SIRT1-7) that exhibit deacetylation, demyristoylase, ADP-ribosylation, lipoamidase, demalonylation, desuccinylation, or deglutarylation activity depending on the substrate. SIRT 1–7 are localized in various sub-cellular compartments in mammalian cells: SIRT1, 6, and 7 are localized mainly in the nucleus [183–185], SIRT2 in the cytoplasm [186], SIRT3, 4, and 5 are active in the mitochondria [187]. As sirtuin activity requires NAD⁺ as a cofactor, cellular redox status-based NAD⁺/NADH ratio can further affect sirtuins [188] at the transcriptional or post-transcriptional level of activity [189,190]. Recent work has established a role for some sirtuins in cellular senescence.

SIRT1 is the most widely studied and well-characterized sirtuin family member and is involved in the regulation of DNA repair and apoptosis, mitochondrial biogenesis, glucose and insulin homeostasis, and cell stress responses. However, our knowledge pertaining to the molecular redox control of SIRT1 is limited. A pro-oxidative shift in the cellular environment has been shown to induce SIRT1 expression in neural progenitors both in vitro and in vivo [191]. Additionally, the SIRT1 promoter is directly trans-activated by hypoxia-inducible factor (HIF) transcription factors in response to a hypoxic environment [192]. SIRT1-mediated protection against oxidative stress-induced cellular damage involves induction of SOD2 expression via deacetylation and modulation of FOXOs, p53, p21, and proteins involved in DNA damage and repair in distinct cell types [193–195]. This fits well with the observation that oxidative stress promotes acetylation of p53 by removing the inhibitory effect of SIRT1 on p53, activating the downstream target p21, and inducing premature senescence followed by pro-tumorigenic IL-6 overexpression [196]. A similar trend is observed in the interaction of SIRT1 with RelA/p65 protein in the NF- κ B complex which deacetylates lysine 310 and potentiates the transactivation capacity of the NF- κ B complex [197]. Exposure to cigarette smoke oxidatively decreases SIRT1 levels and concomitantly increases NF- κ B-dependent release of pro-inflammatory mediators in MonoMac6 cells and in mouse lungs [198,199]. The role of SIRT1-

FOXO3 axis in cellular adaptation to hypoxia-associated oxidative stress has also been elucidated by Kume et al. who demonstrate that hypoxia inhibits SIRT1 activity and prevents nuclear translocation of FOXO3, subsequent autophagy, and cell cycle arrest leading to an increase in mitochondrial oxidative damage in kidneys of aged mice [200]. Interestingly, there are ambiguities in the effect of SIRT1 on FOXO activity with variations according to gene or function; SIRT1 increases transcription of genes involved in cell cycle arrest and resistance to oxidative stress and represses genes promoting FOXO-dependent cell death [201]. Similar to the studies described above, ionizing radiation-induced ROS also promotes cellular senescence in articular chondrocytes by negative post-translational regulation of SIRT1 through the ROS-dependent activation of p38 [202]. In contrast, SIRT1 has been implicated in promoting oxidative stress through IRS-2/Ras/ERK signaling downstream of insulin/IGF-1 receptors [203]. There also is a strong link between the insulin/IGF-1 longevity pathway and SIRT1 activity in senescence. The IGF-1/SIRT1/p53 interplay involves prolonged IGF-1 treatment which inhibits SIRT1 deacetylase activity, resulting in increased p53 acetylation, stabilization, and activation leading to premature cellular senescence [204]. Ectopic expression of SIRT1 effectively abrogates IGF-1-induced cellular senescence, thereby linking IGF-1-SIRT1-p53 to cellular senescence. Recently, SIRT1 has also been shown to dissociate chromatin structure and epigenetically repress the expression of SASP factors IL-6 and IL-8 [205]. However, oxidative stress or DNA damage-mediated can dissociate SIRT1 from the promoter regions of IL-6 and IL-8 increasing their levels systemically and further expanding senescent cell populations. The RNA-binding protein HuR has been shown to stabilize SIRT1 mRNA by binding its 3' untranslated region in a manner that is inhibited by redox-dependent post-translational modifications. Overall, alterations in SIRT1 activity in response to oxidative stress appear to promote genomic instability, enhance inflammation and senescence and offers an additional target for therapeutics that limit the process of senescence.

SIRT3 acts as a tumor suppressor gene, owing to its deacetylation role which enhances the activity of two significant targets, SOD2 and isocitrate dehydrogenase 2 (IDH2), the latter of which generates NADPH for the glutathione pathway of ROS detoxification [206–208]. SIRT3 is also essential for the caloric restriction-mediated mitigation of oxidative damage by regulating the glutathione antioxidant system, thereby regulating senescence-associated pathologies propagated by oxidative stress [209]. Lack of SIRT3 leads to activation of Akt signaling suggesting a potential role in increasing cellular ROS levels and as a driver of senescence, by affecting mitochondrial function and ATP generation, ROS detoxification, and the mitochondrial unfolded protein response (UPR). Both SIRT3 transcript and protein levels are induced by the generation of mitochondrial ROS [210]. In mice fed with high fat diets for a prolonged 12-week period, evidence of hepatic redox stress, and an associated reduction in SIRT3 transcript and protein levels has been established [211]. SIRT3 is involved in cancer progression, neurodegenerative and cardiovascular disease as well as metabolic syndromes [212], and is likely a critical participant in many age-related disease processes.

SIRT6 is another key member of the sirtuin family with varied functions in DNA damage repair, maintenance of genomic stability, inflammation, tumor suppression and participates in longevity regulation [213]. The promise of employing targeting strategies against SIRT6 for limiting age-associated disease processes by impeding cellular senescence and delaying premature aging [214] or through apoptosis engagement has been established [215]. Recently, Pan et al. demonstrated that SIRT6 deficiency in human adult stem cells promotes premature and accelerated cellular senescence and is accompanied by elevated ROS, disrupted redox homeostasis, and increased sensitivity to oxidative stress. SIRT6 complexes with and transcriptionally coactivates nuclear factor erythroid 2-related factor 2 (NRF2), a critical regulator of antioxidant responses, thereby modulating stem

cell redox homeostasis, providing a unique target for constraining this senescence-associated oxidative-stress based stem cell attrition [216]. The best-known actions of SIRT6 as a tumor-suppressing protein include its inhibitory effects on c-Jun by blocking IGF-Akt signaling, and Myc, while suppression of chronic inflammation is achieved by repressing TNF- α and NF- κ B activity [217], thus enabling varying levels of senescence enforcement.

4.7. Telomere maintenance

The ends of linear eukaryotic chromosomes are protected from damage by the inclusion of tandemly repeated DNA sequences and specialized proteins, called telomeres. In mammalian species, the telomeric sequence TTAGGG extends in a 5' to 3' direction with a T-loop structure at the end comprised of a G-rich 3' overhang and associated proteins that offers telomeric protection. The repeats and the bound proteins form a complex structure called shelterin, that camouflage chromosomal ends from being recognized as damaged subsequently preventing DNA end-joining, recombination, and repair processes. Over the course of cell division, chromosomal attrition occurs, as the 5' to 3' nature of the DNA replication machinery results in incomplete replication of the lagging parent strand. In eukaryotes, this is resolved by the DNA polymerase enzyme telomerase, which adds telomeric repeat sequences to the ends of chromosomes de novo and compensates for their erosion. Telomere-associated proteins may also localize to other sub-nuclear or sub-cellular sites, and interact with other associated proteins to regulate telomere length [218].

Senescence is usually triggered when the terminal restriction fragment of telomeres shorten from an average length of 15–20 kb to 4–7 kb, by contributing to a persistent DDR that propagates and maintains senescence-associated proliferation arrest, with dysfunctional telomeres, and damaged nuclear foci termed DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) [219]. While telomere attrition has mostly been attributed to senescence induction, certain studies suggest that DNA damage, and the ensuing damage to telomere structure may be sufficient to trigger senescence independent of telomere shortening [220,221]. Also, the complex molecular T-loop structure at the tips of telomeres that are modulated differently in different cell populations, may also actively determine the cellular proliferative capacity, independent of telomere length.

Comprehensive evidence establishes the theory that progressive telomere loss contributes to replicative cellular senescence and is an indicator of age-related disease. Telomere maintenance is regulated by genetic factors and is additively determined by telomerase activity, cell turnover rate, and stress-inducing non-genetic environmental elements. The G-rich composition of telomeres renders them highly susceptible to oxidative damage with the formation of oxidative damage lesions 8-oxo-2'-deoxyguanosine (8-oxo dG) [47,222] which can induce telomere uncapping in a cell cycle-dependent manner by titrating out essential factors such as protection of telomeres protein 1 (POT1). POT1 protects against cellular senescence by promoting telomere elongation, 3' overhang protection and inhibits chromosomal aberrations [223]. Telomeric DNA is defective in the repair of oxidant-induced single-strand breaks, therefore telomere shortening resulting from unrepaired or residual oxidative damage determines the occurrence of mutations, and unrepaired nucleotides that interfere with the replication fork [53]. This persistent stress-induced telomeric damage is characteristically independent of telomerase activity and telomere length. The repair deficiency in telomeres can be attributed to the binding of telomeric repeat binding factor 2 (TRF2) that makes the strand breaks inaccessible to DNA repair enzymes [224], inhibits ATM kinase phosphorylation [225], and interacts with polymerase β [226], thereby negatively impacting DNA repair. This also suggests that telomere-driven senescence occurs as a potential tumor-suppression mechanism in response to accumulating genomic damage, and not

after a set number of cellular divisions as clocked by telomeres. Mechanistically, telomeric foci contain multiple DDR factors signaling through ATM to p53, up-regulating p21 and causing cell cycle arrest at the G1 phase. Telomere- and DNA damage-independent up-regulation of p16 was also observed, suggesting that distinctive senescence effector pathways are not mutually exclusive and can develop in parallel, resulting in a mixed cell population, or an individual cell responding to collective signals [42].

Recently, analysis of 3D chromosomal architecture using combined biochemical, cytological, and computational techniques has revealed significant decreases in volume, and increases in chromosome compaction during cellular senescence [227]. This unique senescent chromosomal signature is attributed to an increase in short-range chromatin contacts and genome-wide contraction of chromosome arms. It is hypothesized that the structural 3D organization of chromosomes may regulate senescence-associated cell cycle arrest. Mathematical models have been employed to quantitatively predict the role of telomere shortening in senescence. One such model assessed rates of telomere loss occurring from oxidative damage and activity of C-strand specific exonuclease, and the possible interactions between these two mechanisms [228]. The model predicts that under lower oxidative burden the end-replication problem and C-strand processing are major contributors to telomere loss; however under normoxic or hyperoxic conditions, it is predicted that single strand breaks induce shortening. Another model incorporates a shortening factor and autonomous telomere loss and assumes that senescence incidence is proportional to telomere length [229]. A simple negative feedback regulation model of telomere reduction is proposed, suggesting that telomere erosion is dependent on telomere length.

Ectopic expression of telomerase alone [230], or in combination with p16^{INK4a}/pRb inactivation [231] can also prevent telomeric loss-dependent senescence in primary fibroblasts and extend replicative lifespan. Likewise, studies suggests that loss of telomeres correlates with depletion of antioxidant enzyme levels and decreases in oxidative burden by antioxidant treatment might limit the telomere shortening rates and delay cellular senescence. However, a critical review and meta-analysis on human data performed by Simons reveals no causal involvement of telomere length in the aging process, suggesting that thorough investigation of the mechanistic *in vivo* role of telomeres is crucial to assess their function in senescence [232].

4.8. Tumor suppressors p53, p21, p16

The G1 phase of the cell cycle is the main checkpoint at which DDR pathways activate cellular senescence as a protective mechanism to induce cell cycle arrest. [38,233]. Both the p16^{INK4a}/pRb and p53/p21 tumor suppressor pathways induce cell growth arrest through senescence activation [234,235]. Both p16 and p53 display compensatory interactions [236,237] and both have a role in senescence initiation and maintenance [79,238,239].

p21 and p16 are cyclin-dependent kinase inhibitors involved in preventing cell cycle progression by preventing pRB phosphorylation [37,240]. In general terms, DNA damage activates DDR, a p53/p21-dependent response that is originally transient but which can become persistent if the damage is severe enough, and which can also be reversed through the p16/pRB pathways [219,241]. After DDR activation, the p53/p21 pathway deactivates the cyclin E-CDK2 complex responsible for pRB phosphorylation [242], while the p16/pRB pathway inhibits pRB phosphorylation by deactivation of kinase CDK4/6 [239]. Preventing pRB phosphorylation allows pRB to retain its association with the transcription factor E2F1, preventing the expression of E2F1 target genes that are necessary for the cell transition from G1 to the S phase [239].

p53 has been shown to both promote and inhibit cellular senescence [243,244] partly through its up and down regulation of ROS, and its effects on mTOR. Also, a positive feedback loop between p53 and

ROS has been shown to promote senescence in human fibroblasts – a loop that can be counteracted by the cell cycle mediator cullin-4B (CUL4B) via p53 ubiquitination in stressed cells [245]. Moreover, ROS promotes p53 acetylation and induction of premature senescence by inhibition of the deacetylase SIRT1 in fibroblasts [246]. In epithelial cancer cells, the transcription factor FOXp3 accelerates p53-mediated senescence while also increasing ROS levels and p21 expression, demonstrating the intricate link between ROS and these pathways [247]. It has also been suggested that ROS can induce senescence through activation of the p16^{INK4a}/pRB pathway by activating p38 MAPK and extracellular regulated protein kinase (Erk) [248], although in extrinsically induced senescence models, the same pathway was engaged [249].

While there is evidence of the regulatory role of ROS in the p16^{INK4a}/pRB and p53/p21 pathways that lead to senescence and certain pathologies, much remains to be elucidated to fully understand the implication of oxidative stress as both an effector and affected agent in cellular senescence.

5. Summary

The complexity of the aging process and the drive to comprehend its molecular underpinnings, steers focus towards approaches to improve organismal healthspan and lifespan. Despite the transition from cell-culture curiosity to a potential regulator of cancer and aging, cellular senescence remains enigmatic and continues to raise a variety of complex questions. Oxidative stress plays a major role in several pathways that lead to cellular senescence and the associated secretory phenotype. Although initially intended to be a protective response to oncogenic insult, cellular senescence induced by DNA-damaging agents and ROS, can potentially elicit harmful effects and can drive many age-related diseases. While the ROS-driven SASP has proven to be beneficial in certain conditions, it is also associated with perpetuating a pathological inflammatory state. The ability to genetically ablate senescent cells has clearly established a causal role for senescence in many degenerative disease processes, and while there is much unknown with respect to the role of oxidative stress in such processes, the evidence compiled here suggests that precise ROS-mitigation may limit senescence and senescence-associated pathologies. However, translation of these findings into relevant human interventions is at present restricted due to our incomplete understanding of both the basic molecular biology of senescent cells *in vivo* and the etiology of senescence-associated pathologies. While the long-standing notions of the free radical theory of aging postulating a causal role of ROS in the aging process (Harman, 1956) seem to fit with several studies, there are a number of observations that suggest that the cellular effects of ROS, with regard to inducing senescence, do not unequivocally translate into organismal aging. Therefore, efforts should be made to escalate research directed at resolving the basic biology of cellular aging and the redox-control of these mechanisms to generate integrated therapeutic strategies to improve human health and lifespan.

Conflict of interest

The authors declare that there are no conflicts of interest.

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