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Review

Oxidative stress, cellular senescence and ageing

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Abstract: Almost a half century ago, the free radical theory of ageing proposed that the reactive oxygen species (ROS) is a key component which contributes to the pathophysiology of ageing in mammalian cells. Over the years, numerous studies have documented the role of oxidative stress caused by ROS in the ageing process of higher organisms. In particular, several age-associated disease models suggest that ROS and oxidative stress modulate the incidence of age-related pathologies, and that it can strongly influence the ageing process and possibly lifespan. The exact mechanism of ROS and oxidative stress-induced age-related pathologies is not yet very clear. Damage to biological macromolecules caused by ROS is thought to result in many age-related chronic diseases. At the cellular level, increased ROS leads to cellular senescence among other cellular fates including apoptosis, necrosis and autophagy. Cellular senescence is a stable growth arrest phase of cells characterized by the secretion of senescence-associated secretory phenotype (SASP) factors. Recent evidence suggests that cellular senescence via its growth arrest phenotype and SASP factors is a strong contributing factor in the development of age-associated diseases. In addition, we suggest that SASP factors play an important role in the maintenance of age-associated pathologies via a positive feedback mechanism. This review aims to provide an overview of ROS mechanics and its possible role in the ageing process via induction of cellular senescence.

Keywords: oxidative Stress; ROS; cellular senescence; cancer; ageing

1. Introduction

Ageing is a natural process that all living beings experience over the course of their lifetimes. One of the first renowned theories on the topic is known as the free radical theory of ageing (FRTA)

proposed by Denham Harman, which postulates that damage to cellular milieu due to the accumulation of free radicals is a key factor in the ageing process and that it may serve as the deciding factor of lifespan [1]. The theory was later refined by Harman himself to emphasize the role of mitochondrial ROS, as the majority of free radical oxygen species (ROS) production originates in the mitochondria of mammalian cells, and was termed as the mitochondrial theory of ageing [2]. Recent findings suggest that the theory provides a somewhat simplified approach to the biological causes of ageing [3], and that the programmed theories are equally important in the ageing process [4].

Harman's FRTA is one of the theories grouped under the Damage or Error concept of Ageing, which also include Wear and Tear, Rate of Living, Cross Linking and Somatic DNA Damage theories [4]. The causative agent in these theories is always some sort of a stress. There are many variants of stress that can affect the dynamics of cellular functionality i.e., thermal, baric, ionizing radiation etc. However, for the purposes of this review, the primary focus will be on oxidant related or oxidative stress (OS) which is described as a disturbance in the balance of ROS in a cell as well as its defense mechanisms [5]. ROS are generally short-lived highly reactive molecules that are derived from the partial reduction of oxygen. Although there are multiple sources of ROS (described below), a majority is produced in mitochondria from a leakage of electrons in the electron transport chain (ETC). The reaction of free moving electrons with molecular oxygen produces unstable molecules such as superoxide anions ($O_2\cdot^-$), hydroxyl radicals ($OH\cdot$), as well as hydrogen peroxide (H_2O_2). The body has particular defense mechanisms in place to protect against excess ROS in the form of enzymes like superoxide dismutase (SOD), catalase, thyroiodin as well as some small molecule antioxidants (Vitamin C, Vitamin E, and glutathione) [6]. Eventually, the body's ability to combat excess ROS becomes increasingly difficult over time and hence, induces the beginning of the ageing process.

The excess ROS in a cell can lead to four different cellular fates or phenotypes- apoptosis, necrosis, autophagy and senescence. These cellular fates, in general have negative consequences to a normal cellular and tissue homeostasis, which eventually lead to the development of age-associated pathologies at the organismic level and can adversely impact life span of an organism. Although the exact effect of ROS and oxidative stress on life span is still a matter of debate, understanding how ROS and antioxidant enzymes modulate age-related pathologies in the various diseases models can still provide a greater insight into the potential therapeutic mechanisms to ameliorate such maladies. In this review, we briefly discuss the different sources and defenses of ROS, and examine ROS-induced cellular fates, in particular cellular senescence, which can accelerate various age-related pathologies and diseases such as cancer, cardiovascular and neurodegenerative diseases.

2. Sources of ROS and antioxidants

An organism, and its tissues, cells and macromolecules encounter ROS primarily from within its cells and also from the outside environment. These sources of ROS are defined as endogenous and exogenous sources respectively (Figure 1).

2.1. Exogenous sources

Exogenous sources such as UV and ionizing radiations, and manmade sources such as smoke exposure, chemotherapeutics, environmental toxins and other pollutants can induce various DNA mutations and cause increased ROS in mitochondria and cytosol [7,8]. Exposure to exogenous ROS-

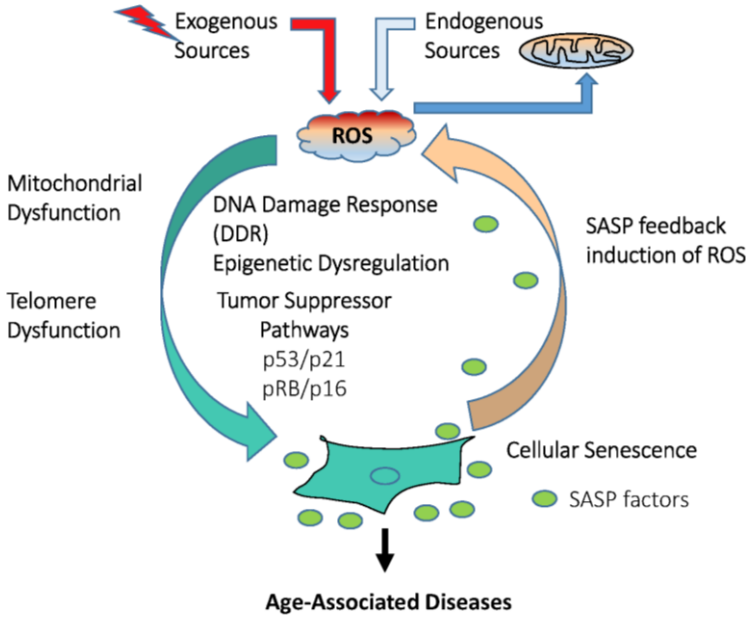

Figure 1. ROS produced by exogenous sources such as radiation and smoke, and endogenous sources, principally by mitochondria induce cellular senescence via a DNA damage-response pathway. DNA damage response mediated by ATM and CHK kinases, together with epigenetic dysregulation such as downregulation of polycomb group proteins and their histone posttranslational modification (PTM) activities upregulates tumor suppressor pathways comprising of mainly p53/p21 and pRB/p16 pathways. The p53/p21 pathway establishes the growth arrest phenotype. The pRB/p16 pathway reinforces the irreversibility of senescent cells by inhibiting cell cycle progression. These pathways as well as ongoing DNA damage response can promote a ROS positive feedback loop. Once the cells are growth arrested, they start expressing senescence-associated secretory phenotype (SASP) factors. The SASP factors can further feedback and generate more mitochondrial ROS, and mitochondrial and nuclear DNA damage, which feed forward into more cycles of senescence induction. While a fraction of senescent cell population could get cleared by immune function, a majority of cells are likely to survive and metabolically active generating more of the SASP factors, ultimately resulting into tissue degeneration and tissue dysfunction. ROS can also induce mitochondrial dysfunction and telomere dysfunction, which will further feed into senescence induction pathways. Thus, ROS can initiate and maintain senescence via multiple pathways.

producing sources is especially prevalent in skin cells as they are constantly exposed to the environment. Radiation can react with oxygen and form $O_2^{\cdot -}$, OH^- (hydroxide anion) and $OH\cdot$ radicals that are able to undermine the structural integrity of DNA via breakdown of nitrogen base-pairing and phosphodiester bonds [9]. In addition, chronic exposure to cigarette smoke promotes lipid peroxidation and has downstream effects like increased risk for cardiac and respiratory dysfunction [10]. Some xenobiotics appear to interfere with mitochondrial bioenergetics and promote superoxide production [11].

2.2. Endogenous sources

The majority of ROS is produced intracellularly in different cell compartments by a variety of enzymes (Figure 2). Peroxisomes exhibit superoxide production from internal enzymes like xanthine oxidase as well as the production of hydrogen peroxide from beta-oxidation of fatty acids [12]. Conversely, leaky transfer of electrons from NADPH to cytochrome P450 can cause ROS formation in the endoplasmic reticulum [13]. NAPDH oxidases, also known as NOXs, are a group of enzymes with oxidase activity that have been observed to be an important player in free radical production associated with ageing [14]. Initially, NADPH oxidases were known to be natural producers of ROS only in phagocytes as a mechanism for microbial killing. The discovery of other members of the NOX family demonstrated that the ROS generating function is not limited to just phagosomes but is found in virtually every tissue. Thus, these isozymes have the propensity to play a prominent role in age-related pathologies through possible redox signaling pathways [15]. There are a total of seven members of the NOX family identified thus far (NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2) and each one serves a particular function as specific regulatory mechanisms, and downstream targets in the cellular and tissue levels [16]. NOX proteins function as transmembrane enzymes that transfer electrons from the cytoplasmic NADPH to molecular oxygen after responding to a specific upstream stimuli, resulting in $O_2\cdot^-$ or H_2O_2 radicals [17].

In addition, when transition metals like that of iron are available, H_2O_2 may undergo the Fenton reaction producing the highly reactive $OH\cdot$ and OH^- radicals or get converted into these radicals via Haber Weiss Reaction in the presence of $O_2\cdot^-$ radicals (Figure 2). These highly reactive ROS molecules have the capability to further disrupt cellular functioning by inducing cell death and/or cellular senescence (Figure 2) [18]. The environment in which ROS is produced from NOXs is generally in either the extracellular space or interstitial fluid. NOX enzymes seem to display a crucial role in age-associated endothelial dysfunction as inhibition of various NADPH oxidase subunits demonstrated improved endothelial vasodilation, lowering the risk of cardiac complications in aged humans [19]. NOX4 has been found to be the isoform in greatest abundance, but recent studies point to NOX2 as the primary producer of ROS [20]. These NOX enzymes have also been observed to be involved in transducing mechano-signals of stress through the alteration of the redox balance [21]. Although the response elicited is dependent on the type and degree of stress induced, NOX enzymes activation can either have a negative or positive affect on the cell. When exposed to a more moderate mechano-stress, the ROS formation from NOX induced proteins are at times able to support a greater level of cellular adaptation and protection. The higher levels of stretching and sheer force has been associated with greater ROS production due to increased activation of NOX enzymes, potentially leading to oxidative damage to cellular DNA and organelles [22].

About 90% of the intracellular ROS is generated in the Mitochondria through the mitochondrial electron transport chain (ETC) (Figure 2). The ETC is located in the inner membrane of the mitochondria and is responsible for the extraction of energy from electrons that are deposited by the electron carriers (NADH & $FADH_2$) through formation of a proton gradient creating ATP. The NADH and $FADH_2$ feed the electrons in to complex I and complex II respectively and eventually transferred to complex III & IV. After complex IV, the electrons are finally picked up by molecular oxygen and go on to form water. However, the electrons have a tendency to leak prematurely at complex I and III which leads to the formation of oxidants like $O_2\cdot^-$ [23]. The exact details of that occurrence remains still remains obscure, especially regarding what propels complex III to form and release $O_2\cdot^-$ to both

regions of the inner membrane [24]. Superoxide produced in the mitochondria is usually converted to H_2O_2 by the antioxidant superoxide dismutase 1 or 2 (SOD1 or SOD2) (Figure 2). As described earlier, H_2O_2 further gets converted into highly reactive OH· and OH^- radicals by Fenton and Haber Weiss reactions (Figure 2), which lead to disruption of cellular homeostasis by inducing cell death and cellular senescence and ultimately development of the age-associated diseases (Figure 2). The $O_2{\cdot}^-$ can also undergo another type of radical reaction with nitric oxide (NO·) to form the peroxynitrite ($ONOO^-$) (Figure 2). When produced in the mitochondria, this radical molecule has the capability to disrupt mitochondrial integrity as well as to cause an irreversible damage to both DNA and protein molecules [25]. The increased mitochondrial ROS production may cause mitochondrial DNA (mt DNA) mutations to occur, which eventually lead to a positive feedback loop of more ROS and more mt DNA mutations [26]. Hence, it is proposed that enhanced mitochondrial ROS and mt DNA mutations are important contributors to the ageing process [27].

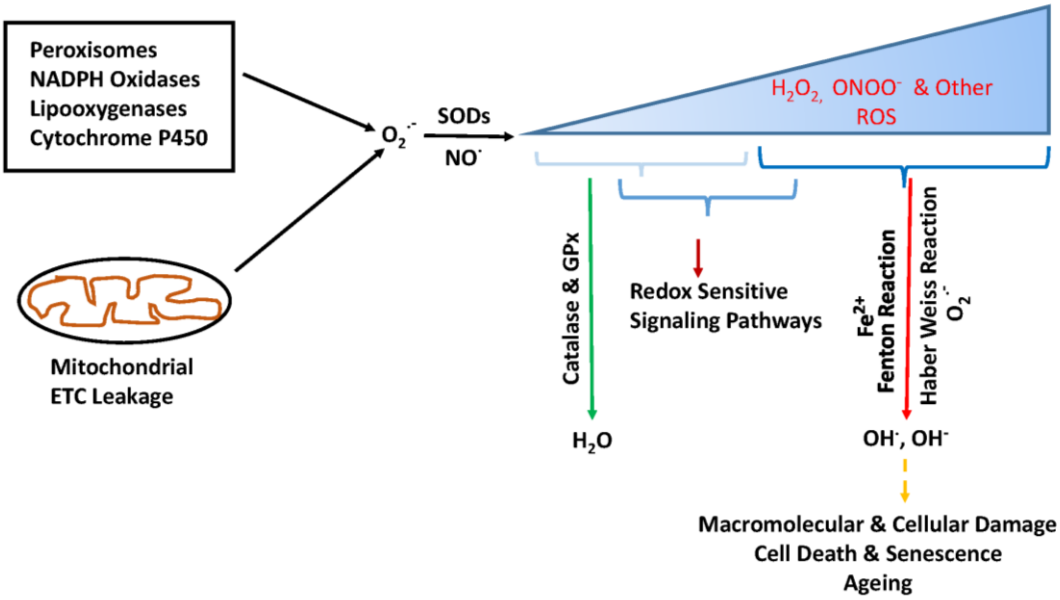

Figure 2. A simplified cartoon of the mechanisms generating endogenous ROS and downstream consequences. The endogenous/intracellular sources of ROS include cellular organelles such as mitochondria and peroxisomes, and a variety of cytosolic enzymes such as NADPH oxidases (NOXs), lipoxygenases, and cytochrome P450 systems. Electron leakage from the mitochondrial Electron Transport Chain (ETC), oxidation of NADPH by NOXs, and the activity of lipoxygenases, cytochrome P450 family members, cyclooxygenase and other cellular oxidases primarily generate $O_2{\cdot}^-$ (superoxide) radicals. The $O_2{\cdot}^-$ radicals are converted into H_2O_2 by SOD1 (superoxide dismutase 1), 2 and 3 enzymes. It can also be converted into more powerful oxidant peroxynitrite ($ONOO^-$ or $O{=}NOO^-$) by nitric oxide (NO^-). The H_2O_2 is usually converted into H_2O with the help of the antioxidant enzymes such as catalase and glutathione peroxidase (GPx), or it can initiate a specific redox sensitive stress signaling. Invariably, the excess H_2O_2 gets converted into OH· (hydroxyl) radicals via Haber Weiss Reaction in the presence of $O_2{\cdot}^-$ radicals or into OH· and OH^- (hydroxide anion) radicals via Fenton Reaction in the presence of metal cations (Fe^{2+}). The OH· and OH^- radicals cause irreversible damage to macromolecules such as DNA, proteins and lipids, and ultimately results in cell death and/or cellular senescence, which accelerate the ageing process.

2.3. Antioxidants

Superoxide dismutase enzymes are known to catalyze the dismutation of two superoxide anions into the form of oxygen and hydrogen peroxide, and play a crucial role in regulating oxidative stress and redox signaling, in the different regions of the cell. Currently, three types of SODs exist within cells: CuZn-SOD (SOD1) located in the cytosol, Mn-SOD (SOD2) in the mitochondrial region, and EC-SOD (SOD3) found extracellularly [28]. Despite an abundant number of studies towards identifying the changes in SOD levels over the course of the ageing process, great deals of controversy remain as some findings appear totally contrasting. Although most animal and human subjects showed a general decrease in SOD1 activity in most of the tissues as ageing progressed, there were no significant changes in levels in plasma, muscles, and red blood cells amongst humans [29,30]. On the contrary, animal tissues such as rat brain and skeletal muscles actually experienced an increase in SOD1 activity with ageing [31]. The other major phenomenon lies in the fact that some areas of the body experience an initial increase of SOD2 activity but eventually begins to decline after a possible threshold is reached. An example of this is evident in human skin where Mn-SOD levels experienced an increase and began to slowly decrease in the early sixties [32]. Knockdown of either form of the enzyme resulted in dramatic phenotypic alterations in the skin as well [33]. The main reason for a difference in age related SOD1 and SOD2 activity remains to be established but can be deduced that sharp tissue dependent disparities exist.

Another major antioxidant that plays an important role in combating and protecting against oxidative stress is Glutathione (GSH) which is considered the most abundant. GSH serves to function in counteracting hydrogen peroxide, lipid hydroperoxides, or xenobiotics, as a cofactor of enzymes such as glutathione peroxidase or glutathione-*S*-transferase (GST) [34]. Recent findings suggest that GSH concentrations in the brain as well as erythrocytes of individuals around the age of 70 displayed a 30 to 50% reduction in activity compared to the much younger subjects around the age of 20 [35]. As observed with SODs, there were some tissues or cells where the concentration of GSH remained relatively constant, like that of human plasma. Thus, it can be evidently seen that GSH homeostasis is well maintained through the enzyme inductions of GSH synthesis. As ageing occurs, the general rise in oxidative stress increases both GSH usage and degradation to sustain the cellular redox balance. In addition, an accumulating amount of evidence exists suggesting that an overall decrease in the adaptive response of GSH synthesis possibly due to the process becoming impaired or insufficient, causing a buildup of oxidants and potential cellular damage [27]. The external environment of cigarette smoke also seems to affect GSH activity as seen in the extracellular lining of fluid in lungs. The findings of the study reported that smoked exposed lungs of older mice exhibited a significant decline in responsiveness of GSH levels compared to younger mice [36].

Catalase is also a prominent enzyme that functions to detoxify hydrogen peroxide into water and is localized in the peroxisome. Just as observed with the other antioxidants, catalase activity also seems to be tissue dependent with regards to ageing. However, there also seems to be stark contrasts in the results between human and rat models as summarized in [37], suggesting that further studies must be conducted in order to determine what role catalase may play in the ageing process. Other antioxidants include Vitamin C, E, A, minerals, as well as some flavonoids. Intake of these antioxidants from various sources of food is essential since we possess the inability to synthesize them independently. However, a few studies have found that direct supplementation does not exhibit a stark difference in the prevention of various diseases like cardiovascular disease (CVD) or cancer [38,39]. Thus the

sources and methods of absorption of these exogenous antioxidants is an essential component to maintaining redox homeostasis.

The antioxidant enzymes, in particular Mn-SOD (SOD-2) and catalase, are transcriptionally regulated by the forkhead box class O (FOXO) family of winged helix transcription factors [40]. These transcription factors, which includes FOXO1a, FOXO3a, FOXO4, and FOXO6 are the human homologues of DAF-16 in *Caenorhabditis elegans* [41]. Inactivation of DAF-2, which acts upstream of AGE1/AKT, and is an orthologue of the mammalian IGF1-R (insulin-like growth factor 1 receptor) requires DAF-16 for life span extension [42,43] and resistance to oxidative stress [44], suggesting that the DAF-16 regulates oxidative stress and longevity in *C. elegans.* Similar to DAF-16, FOXO3a mediates resistance to oxidative stress via transcriptional regulation of Mn-SOD and catalase in mammalian cells [40,45]. The FOXO transcription factors including FOXO3a act downstream of the InsR (Insulin Receptor)/IGF1-R-PI3K-AKT signaling pathway, which is activated by insulin and other growth factors and is analogous to the DAF-2-AGE-1-AKT pathway of *C. elegans* [40,46]. FOXO transcription factors can also regulate other antioxidant proteins such as SOD-1, mitochondrial peroxiredoxin-3 (Prx-3), Prx-5, glutathione peroxidase-1 (GPx-1), mitochondrial thioredoxin 2 (Trx2) and thioredoxin reductase 2 (TrxR2) [40,46]. Interestingly, oxidative stress and ROS also cross regulate expression of FOXO proteins via multiple mechanisms including posttranslational modifications [40,46], indicating that an interplay between ROS and FOXO proteins may determine the expression of antioxidant proteins, and cellular as well as the organismic response to oxidative stress.

3. ROS, oxidative stress and organismic life span

The role of ROS and OS in organismic life span is controversial. Studies using various longevity-related mutant in model organisms such as *C. elegans*, *Drosophila* and mice have yielded mixed results [47-49]. The extensive studies on both yeast and rat glomerular cells demonstrated that a short-term increase in ROS production exhibited a reaction from various antioxidants causing a greater enhanced adaptive response through increased oxidative resistance [50,51]. There has also been a study conducted in *C. elegans,* where inhibition of respiration led to an increased release of mitochondria ROS, which exhibited a significant increase in lifespan [52]. When levels of oxidative stress are low, either through the induction of exercise, caloric restriction, or any other stimuli, there has been evidence that shows an extension in lifespan through induction of mitochondrial metabolism. For example, when *C. elegans* is placed under dietary restriction, especially with lack of glucose, increased mitochondrial respiration and ROS levels induce a hermetic response which may have increased resistance to oxidative stress [53]. A separate study with glucose restriction was conducted but pre-treatment with the antioxidant N-acetylcysteine (NAC) showed no evidence of an increase in either ROS levels or longevity [54]. During moderate exercise, ROS levels has also been shown to increase gradually and possibly to promote activation of anti-ageing pathways [55]. However, exhaustive exercise is harmful, and can raise oxidants to a level of toxicity and activate apoptotic signals. Thus, a general idea can be put forth that ROS levels below a certain threshold have some beneficiary affects while beyond a certain point, are damaging for cellular components. The theory of mitohormesis is still being fully investigated and could have a decisive role in anti-ageing therapy as a possible mechanism to target only excess disease causing ROS without interfering with oxidants needed in cellular signaling.

The next logical question is whether or not alterations in antioxidant expression affect ageing. In mice, knockout of various antioxidant genes do not show significant changes in lifespan with the exception of $SOD1^{-/-}$ that had a 30% reduction of life [3]. However, even that particular sample set seemed to be vulnerable to alternative genetic mutations as they exhibited signs of liver carcinogenesis, questioning the justification of those findings [56]. On the contrary, the genetic up-regulation of the antioxidants also does not support the free radical theory with regards to longevity. Overexpression of CuZn-SOD, Mn-SOD, catalase, or Gpx4, in independent and combination studies, had similar lifespans compared to their wild-types [3]. However, 20% increase in lifespan was observed when up-regulation of catalase expression is targeted to the mitochondria specifically [57].

Although it may be easy to assume that insignificant changes in lifespan in model organisms from manipulating antioxidant genes demonstrates ROS as a nonparticipant in the ageing process, it must be reemphasized that longevity is not the only means of measurement of a healthy lifespan. Ageing also entitles the physical and chemical changes that occur from the foundation of cells to the organism itself as well as everything in between [58]. These changes are the root cause of the decline in physiological functioning that is observed and increases our predisposition to develop various age-related diseases. Despite knockdown of various antioxidant genes showing minor changes in lifespan, they do exhibit increased incidences of accelerated cardiac and neurological disorders as seen in mice with reduced Mn-SOD or Gpx1 expression [59,60]. On the other hand, increased antioxidant expression delayed the progression of several age-related diseases while keeping longevity as a constant. An example of this is seen in overexpressing theroxidin (Trx1) mice that displayed a better ability to maintain glucose metabolism in high lipid environments as well as reduced cardiac dysfunction [61,62]. Thus, understanding how antioxidants may lead towards the development of age related ailments is certainly a direction of further studies to promote a longer time span of healthy living.

4. Oxidative stress at the macromolecular and cellular level

Exposure to ROS levels above the homeostatic threshold have been identified as a major cause of damage to cellular macromolecules. The most sensitive to ROS damage is DNA, more specifically that of mitochondria, which serves as the primary source of intracellular oxidants. The impairment to DNA molecules from oxidative stress is closely evident in the breaks in the double stranded helix as well as an increase in alteration of nitrogen bases [5]. The most studied DNA lesion is the formation of 8-OH-G [9]. Generally, for a proliferating young cell, the damage is repaired more efficiently through the use of base or nucleotide excision repair pathways as well as homologous recombination [63]. However, in cells derived from aged individuals, these repair pathways are less efficient leading to the first step in increased incidence of carcinogenesis and mutagenesis.

Proteins are also affected by increased ROS levels both in the structural and functional aspect. The most susceptible to oxidation are the cysteine and methionine residues of protein side chains due to the possible formation of disulfides amongst thiol-groups between proteins [64]. The concentration of carbonyl groups has found to provide a good measure of ROS-mediated protein oxidation. The levels of protein carbonyls have been observed to rise as the ageing process progresses though the exact damage inherited from oxidants appear to be more specific to the particular tissue [3]. Normally, proteasomes are responsible for breaking down oxidized proteins, however, as the cell begins to age, the levels of proteasomal activity is reduced dramatically causing an accumulation of these proteins, possibly leading to activation of pro-death pathways.

Another aspect of the cell that is particularly oxidant sensitive is lipids, particularly fatty acid residues of phospholipids. The process of lipid peroxidation involves free radical species that target various carbon-to-carbon bonds and result in products like hydroperoxides, which has been identified as a potential indicator of oxidative stress in various tissues [65]. Knowing that, the oxidant radical has the ability to cause damage to both the membrane and integrity of the cell. On a macroscopic level, oxidation of lipids can alter fluidity and cause severe physiology and membrane damage, something very evident in some diseases as well [66]. At the cellular level, ROS and OS induce multiple related cellular fates such as apoptosis, necrosis, autophagy and senescence, which can adversely affect tissue structure and function. Here, we focus on ROS-induced cellular senescence, its connection to age-associated pathologies and plausible role of senescence in ageing itself.

5. ROS, oxidative stress and cellular senescence

The phenomenon of cellular senescence, originally described by Hayflick and Moorehead in human lung fibroblasts [67], is now known to be a major cellular phenotype involved in cancer and age-related pathologies [68,69]. In addition, recently it was reported that cellular senescence also plays an important role in normal development including embryogenesis [70-72]. Senescence observed by Hayflick is known as replicative senescence or Hayflick Limit, which is caused by progressive telomere shortening in the somatic cells that undergo mitotic cell divisions and lack telomerase [68,73]. Besides fibroblasts, cellular senescence occurs in multiple cell types, such as epithelial cells, endothelial cells, lymphocytes and chondrocytes, and possibly even post-mitotic cell such as neurons and glial cells [74]. It is very well known that the senescent phenotype is also induced by non-telomeric signals which include various types of stresses, including oxidative stress [68,75]. Stress-induced senescence is variously termed such as stress-induced premature senescence (SIPS) or simply as premature senescence (PS). For the purpose of this review, SIPS/PS is included under cellular senescence, which also includes other forms of senescence induced by non-telomeric signals such as oncogene-induced senescence (OIS), and telomere attrition-induced senescence (replicative senescence). It is important to note that SIPS/PS and OIS or other forms of senescence also include to some extent telomere dysfunction/damage [76,77], which is likely to be amplified by persistent stress signals.

Cells undergoing cellular senescence exhibit stable growth arrest, enlarged, vacuolated and flattened morphology but are metabolically active [78]. Senescent cells are commonly identified using senescence-associated β-galactosidase (SA-β-gal) marker, which can be detected by a simple histochemical staining of cells grown in culture or in in vivo tissue sections [79]. The other less commonly used marker of senescence is presence of the senescence-associated heterochromatin foci (SAHF) in cells undergoing senescence [80]. SAHF are DNA domains stained densely by 4',6'-diamidino-2-phenylindole (DAPI) and are enriched for histone modifications including lysine9-trimethylated histone H3 manifest increased methylation of histone H3 on Lys9 (H3K9me) [80]. Among molecular markers, a key tumor suppressor, p16 (aka p16INK4a or CDKN2A) is known to be upregulated in most senescent cells [81,82].

Apart from stable growth arrest phenotype mediated by overexpression CDK inhibitors such as p16, senescent cells are known to overexpress a variety of secreted molecules, which are collectively described as SASP factors [83]. The SASP factors include many pro-oncogenic growth factors, proteases and pro-inflammatory factors such as cytokines, and chemokines, which acts via autocrine

and paracrine activities [83]. Although many of the SASP factors are conserved, some may vary across different cell types and importantly under different senescence-inducing signals [69,83-85].

Oxidative stress caused by oxygen, hydrogen peroxide and tert-butylhydroperoxide is known to induce premature senescence in human and mouse cells [86,87]. These reagents and other stressors including activated oncogenes such as H-Ras^{V12} generates ROS [88], which lead to DNA damage response (DDR) and induction of cellular senescence [89,90]. The ROS-induced senescence proceeds via mitochondrial and non-mitochondrial pathways, which likely converge at the known molecular players of the cellular senescence such as p53, pRB, p16 and p21 [91,92] (Figure 1). While the induction of p53 and p21 is primarily related to DDR [89,90], the upregulation of p16 and subsequent increase in hypo-phosphorylated state of pRB may be related to the epigenetic dysregulation of polycomb group proteins, in particular BMI1, which is known to epigenetically silence *p16INK4a* locus via polycomb repressive complex (PRC)-mediated histone modifications [93]. Interestingly, BMI1 is known to inhibit ROS and participate in DNA repair function [94]. It was also shown to localize to mitochondria and regulate mitochondrial function [94]. Thus, downregulation of BMI1 during senescence [95], may further contribute to the ROS-mediated induction of cellular senescence. Recent studies suggest that ROS-induced senescence includes positive feedback loops reinforcing and further amplifying senescence signals, for example- SASP factors promote ROS generation and senescence via autocrine and paracrine mechanisms [96,97], and ROS generates more mitochondrial (mt) mutations and ROS, which may further increase intracellular ROS, DDR and ultimately the senescent phenotype [76,91,92,98] (Figure 1).

6. ROS, mitochondria, and cellular senescence

Since mitochondria are major producer of ROS, it is not surprising that several studies have linked mitochondrial dysfunction to senescence and ageing [58,99]. Recent studies suggest that ROS can trigger DDR via damage to telomeric as well as non-telomeric DNA, and that DDR can also generate ROS via a positive feedback mechanism [76]. The positive feedback mechanism was shown to be mediated by a p53-dependent signaling pathway that include p21, GADD45A, p38, GRB2, TGFBR2, and TGFβ in human diploid fibroblasts [91]. As indicated earlier that intracellular ROS also include non-mitochondrial ROS. Hence, non-mitochondrial ROS may also participate or collaborate with mt ROS in induction of cellular senescence.

A recent study suggests that mitochondria, and mt ROS are essential for induction of senescent phenotype as defined by common markers such as SA-β-gal, and generating a full spectrum of senescent features [100]. In this study, it was shown that depletion of mitochondria using CCCP (Carbonyl cyanide m-chlorophenyl hydrazone), an uncoupler that induces mitochondrial depolarization, and degradation via proteasome-mediated pathways and autophagy, leads to cell cycle arrest but no SA-β-gal induction and absence of most of the SASP factors [100]. Cells also did not upregulate p21 and p16 as commonly observed with senescence induction [100]. The authors further suggested an essential role of mTORC1, which may integrate DDR signals, and PGC-1β in mitochondrial biogenesis and induction of senescence. While this study points to important role of mitochondria in senescence, artificially induced depletion of mitochondria may impact other cellular functions, which could override some of the phenotypes associated with senescence. Recently, it has been argued that mitochondrial effectors other than the ROS, such as mitochondrial dynamics, altered redox state, defective ETC and altered metabolism also plays equally important role in the induction

of cellular senescence [101]. It has also been found that mitochondria while essential for senescence induction, mitochondrial dysfunction may induce senescence with a distinct secretory (SASP) phenotype, that lacks IL-1 arm but retains growth arrest, which appears to be due to the decreased NAD+ (Nicotinamide adenine dinucleotide) /NADH ratio and AMPK (AMP-activated protein kinase)-mediated p53 induction [92].

It is known that levels of NAD+, which regulates NAD+ -dependent histone/protein deacetylases known as sirtuins (SIRTs), decline during the ageing process, and that the NAD+ deficiency results in mitochondrial dysfunctions via PGC-1α (PPAR-γ coactivator 1 alpha) -dependent and -independent pathways [102,103]. It was also shown that NAD+ repletion restores mitochondrial function in a SIRT1 dependent manner [102,103]. The conserved energy sensor AMPK regulates SIRT1 activity by increasing NAD+/NADH ratio and decreasing the concentration of the NAM (nicotinamide) [104,105]. Interestingly, SIRT1 also activates AMPK via deacetylation of LKB1 [105,106]. Thus, the AMPK-NAD+-SIRT1 and SIRT1-LKB1-AMPK signaling pathways play an important role in mitochondrial function and energy metabolism. In addition, recently, it was reported that NAD+ repletion not only restores mitochondrial function but also improves stem cell function resulting in a healthy life span in mice [107]. Since, it was also shown that NAD+ repletion prevented or delayed senescence of muscle, neural and other adult stem cells [107], it is likely that NAD+/NADH ratio plays an important role in mitochondrial dysfunction induced senescence and its deleterious downstream effects on lifespan including age-related tissue degeneration. In summary, mt ROS, and mitochondrial and telomere dysfunctions induce cellular senescence, which is a prime cause of age-related pathologies (Figure 1).

7. Oxidative stress, senescence and age-associated pathologies

As described above, increased levels of ROS due to oxidative stress and vice versa results in different cellular fates that are detrimental to cellular and tissue physiology, and organismic well-being. In particular, cellular senescence directly or indirectly modulates age-associated physiological and pathological traits [108-111]. In addition, therapy-induced senescence may also cause accelerated ageing via a compromised immune system [112]. Related to cellular senescence, oxidative stress and mt ROS can also trigger telomere shortening and dysfunction [113], and telomere shortening is considered one of the hallmarks of ageing, and has been linked to the several age-associated traits (normal and pathological) [58,114]. Such associations strongly suggest a causative role of oxidative stress, ROS and cellular senescence in the ageing process. In this review, we briefly cover the effects of oxidative stress and cellular senescence on cancer, cardiovascular diseases and neurodegenerative disorders such as Alzheimer's Disease (AD) and Parkinson's Disease (PD).

7.1. Oxidative stress, senescence and cardiovascular disease (CVD)

Short telomere length is associated with cardiovascular risk factors and common cardiovascular diseases, such as atherosclerosis, heart failure, and hypertension [115]. Telomere shortening and dysfunction is a known feature of senescent cells undergoing replicative senescence, however, telomere dysfunction may also result due to chronic oxidative stress. DNA damage signals and inflammatory diseases. It is thought the SASP factors secreted by senescent cells via autocrine and paracrine activities can accelerate degenerative and proliferative activities in various tissues and tissue environment, which may contribute to cardiovascular diseases [115]. Senescence of human umbilical

venous endothelial cells (HUVECs) has been proposed to be involved in endothelial dysfunction, which may contribute to the atherosclerosis during ageing process [116]. Senescent HUVECs in atherosclerotic lesions are expected to express increased levels of pro-inflammatory molecules, which may further contribute to the pathogenesis of CVDs. Several studies have shown significant increase in the levels of many pro-inflammatory cytokines such as TNF-α and IL-6 levels amongst older individuals as well. Damage to the arterial walls has been linked to the production of ROS by the endothelium which promotes oxidation modifications of low-density lipoproteins (LDL). The LDL eventually migrates from the blood stream into the sub endothelial space of the arterial wall, exhibiting a crucial step in the initiation of atherosclerosis [117]. It has been observed that increased TNF-α activates NADPH, leading to oxidative stress through increased production of ROS. NF-kB, a redox-sensitive transcription factor, has been shown to engage in activating various inflammatory responses inside the arterial wall which promotes translocation of immune cells and cytokines to the region. TNF-α actually regulates NFκB expression. In a comparative study of young versus old healthy individuals, both NFκB and NADPH levels were elevated [118]. It is important to note that NFκB is a major transcription factor that has been proposed to be the master regulator of the SASP phenotype in senescent cells [119-122]. Klotho, a senescence suppressor protein extends the lifespan of mice and its disruption results in atherosclerosis and endothelial dysfunction [123-125]. It was recently shown to suppress TNF-alpha-induced expression of adhesion molecules and NFκB activation [126].

Another protein that plays a role in ROS production and involved in CVDs is p66Shc that is encoded by the ShcA gene [127]. The p66shc adaptor protein, which controls oxidative stress response and life span in mammals [128], is known to regulate cell fate including apoptosis and senescence in fibroblasts [129]. It is also known to be overexpressed in patients with coronary artery disease [130]. In comparison, numerous in vitro studies have supported that fact that knockdown of p66Shc reduces intracellular free radicals even under high oxidative stress. In some mouse models, the lack of p66Shc gene displayed a prolonged lifespan and increased resistance to apoptosis. Vessel impairment has been shown to be a result of increased presence of ROS and reduced NO bioavailability [131]. The $p66Shc^{-/-}$ mice exhibit significantly decreased myocardial injury and provided greater protection against age related endothelial dysfunction due to low ROS levels [132]. Thus, many important regulators of cellular senescence are dysregulated in CVDs and these regulators are potential targets for therapeutic interventions.

7.2. Oxidative stress, senescence and Alzheimer's disease

Ageing is a major risk factor for AD, which is the most prevalent form of dementia and affects almost five million Americans with the majority of them being over 65. The disease is characterized by selective neuronal death mainly in the hippocampus and nearby brain regions. The other factors include formation of senile plaque from accumulating amyloid B peptide (AB) and neurofibrillary tangles due to tau protein dysfunction. Since the brain has a high oxygen consumption and glucose demand to meet, it creates an environment that is very susceptible to oxidative imbalance. In addition, a significant decrease is observed in mitochondrial cytochrome oxidase (complex IV) in hippocampal neurons [133]. The lack of this key enzyme in ETC leads to greater ROS levels and reduction in energy stores. Not surprisingly, an increasing amount of evidence suggests that ROS generation may play a critical role in the disease [134]. The accumulation of AB peptides fragments is generally due to a mutation of its precursor APP or other AD associated genes. This leads to the formation of a "sticky"

plaque, which interferes with neuronal communication and potentially initiates AD progression [135]. AB that is present in the mitochondria was associated with increased inflammation and ROS production through the disruption of protein transportation and lipid polarity eventually leading to the deterioration of cognitive function from neuronal apoptosis [136]. Another perspective corresponds to a more age-related mitochondrial dysfunction as the primary root cause from an accumulation of mt DNA mutations as well as affecting tau protein functionality [137]. In addition to selective cell death, p16-dependent senescence of astrocytes has been linked to sporadic form of AD [138]. It has been also reported that microglial cell senescence is exacerbated by the presence of amyloid and is associated with telomere shortening and that could be a contributing factor in the pathogenesis of AD [139]. In summary, although neuronal cell death may be the primary cause of AD, senescence in astrocytes and microglial cells may also contribute to AD development and progression [140].

7.3. Oxidative stress, senescence and Parkinson's disease

PD is another neurodegenerative disease that is characterized by a selective loss of Dopamine neurons in the substantia nigra pars compacta (Snpc) region of the brain. The symptoms include rigidity, slower voluntary movement, and bradykinesia. Although the majority of PD cases observed are sporadic, about 15% account for familial forms of PD and are linked to a few disease associated genes [141]. However, in both forms oxidative stress appears to be the underlying mechanism of action that ultimately initiates cell death pathways. The initial sign of PD formation may be due to the dopamine molecule itself. Auto oxidation of dopamine occurs spontaneously and produces changes in brain mitochondria permeability [142]. Oxidized dopamine is eventually converted to neuromelanin where it accumulates in the Snpc but not before there is a generation of ROS from ferritin catalyzed reactions and NADPH depletion [143]. A reduction in Complex I activity of the respiratory chain has been observed in patients with sporadic PD [144]. One of the well-studied biomarkers of PD is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) a neurotoxin that inhibits complex 1 activity in the form of 1-methyl-4-phenylpyridinium (MPP+), disrupting electron flow and producing ROS [145]. Other evidences of mitochondrial dysfunction include the mutation of genes such as alpha-syn, PINK1, and Parkin. Overall, these genes have been observed to play crucial roles in oxidative protection or basic mitochondrial functioning. Similar to AD, protein accumulation also occurs in PD in the form of α-synuclein. The protein mainly functions in neuroplasticity and pre-synaptic signaling. However, accumulation of α-synuclein has been observed to regulate mitochondrial membrane permeability and reduce complex I activity thereby increasing ROS levels. The genes of Parkin and PINK1 are located in the mitochondria and seem to demonstrate distinct roles in the organelle's normal functioning. In damaged mitochondria, PINK1 normally serves as a neuroprotective agent by accumulating on the outer membrane and recruiting Parkin to initiate mitophagy [146]. Generally, mutations in PINK1 are associated with autosomal recessive PD. Parkin mutations are associated with impaired complex 1 activity and high vulnerability to oxidative stress due to the accumulation of impaired mitochondria in the neuron causing cellular damage [147]. Thus, genetic dysfunction in either of these proteins can cause severe cellular damage to dopamine neurons through decreases in membrane potential and ROS generation. Recently, it has been proposed that environmental stressors associated with PD may act in part by eliciting senescence in glial cells and the glial cell-SASP within non neuronal glial cells in the ageing brain [148].

7.4. Oxidative stress, senescence and type 2 diabetes (T2D)

Senescent cells can act as a driver and amplifier of many age-related diseases including T2D [112]. SASP factors induced by senescent cells in peripheral adipose tissue and senescent pancreatic beta cells may act as driver of the T2D pathogenesis, while metabolic and signaling changes induced by high circulating glucose, growth hormone and lipid metabolites may further induce formation of senescent cells [112,149]. It has been speculated that high glucose may induce premature senescence in pre-adipocytes, fat cells and fibroblasts via increased ROS and mitochondrial dysfunction [149,150]. Adipose tissue dysfunction resulting from senescent pre-adipocytes may result in obesity [151]. Obesity, which is known to be associated with a pro-inflammatory state, possibly caused by SASP factors from the senescent cells is also considered a contributing factor in the development of insulin resistance [112,149]. Accordingly, certain anti-diabetic drugs such as metformin may alleviate diabetes and diabetic complications via inhibition of NFκB-mediated secretion of SASP factors by senescent cells [152]. Extension of lifespan in diabetic patients and mice model [153], and decreased incidence of cancer in patients treated by metformin is possibly related to the inhibition of SASP factors by metformin [149].

7.5. Oxidative stress, senescence and cancer

Oxidative stress and cellular senescence both can be pro-oncogenic or anti-oncogenic depending on the cellular context and stage of cancer development, and therapeutic interventions. While multiple mechanisms drive tumorigenesis, cancer cells exhibit sharp difference in metabolic and signaling pathways in order to fuel the energetically expensive conditions required for their proliferation and survival. Cancer cells in general have a more sustained oxidative stress environment due to overproduction of intracellular and mitochondrial ROS, which can result in accumulation of mitochondrial and nuclear DNA mutations, and genomic instability [154]. It is believed that increased mitochondria- and NOX-generated ROS activate hypoxia inducible factors (HIFs) and PI3K pathways, and metabolic adaptation that results in enhanced proliferation, increased cell survival, migration and invasion, and inhibition of cell death pathways that are necessary for tumorigenesis [154]. In addition to HIFs and PI3K pathways, ROS also activates an inflammatory network of transcription factors such as NFκB, STATs, NRF2, p53, AP-1 and PPARϒ, which can further cause oxidative stress-related changes in tumor cells, neighboring cells and tumor microenvironment leading to tumor invasion, metastasis, angiogenesis, cancer stem cell survival and therapy resistance [155]. However, the increased ROS also acts as a double edge sword as it makes cancer cells more susceptible to induction of cell death pathways and cell senescence by therapeutic interventions [156]. In particular, because cancer cells are dependent on increased anti-oxidant capacity, they are more vulnerable to exogenous ROS-generating drugs or reagents targeting anti-oxidant pathways [156]. However, cancer cells may further exhibit redox adaptation resulting in drug resistance, which may require development of novel reagents that target redox adaptive response in cancer cells [156].

With respect to cancer, it has been argued that cellular senescence is a potent tumor suppressor mechanism, as the growth inhibitory phenotype is likely to put a road block into tumor cell proliferation, which will limit tumor growth and acquisition of more aggressive features by cancer cells [68]. It is known that senescence bypass is required for cancer development, and replicative immortality, which results due to senescence bypass and telomerase re-expression is considered one

of the important hallmarks of the cancer cells [157]. In addition, most DNA damaging and oncogenic signals strongly provoke senescence-like phenotype as an initial response in vitro and in vivo, suggesting potential tumor suppressor role of senescence [158]. More recent views on senescence highlight the pro-oncogenic role of senescent cells via a variety of SASP factors, which can further abet oncogenic phenotypes in a variety of cancers [83,109,159]. The SASP factors are broadly categorized into three categories- 1. Inflammatory chemokines and cytokines such as IL-1, IL-6 and IL-8; 2. Growth factors such as CSFs (colony stimulating factors) and VEGF (vascular growth factor); 3. Secreted Matrix remodeling proteases such as MMPs (matrix metalloproteases) [83,109,159]. The SASP factors have a myriad of pro-oncogenic effects on pre-neoplastic and neoplastic cells such as promoting proliferation, invasion and migration [84,160] . These factors can also induce epithelial to mesenchymal transition (EMT), metastasis and angiogenesis, and cause inflammation to create a permissive microenvironment by disrupting the tissue architecture for tumor progression and metastasis [109,161]. The therapy-induced senescence can paradoxically result in tumor progression, treatment resistance and recurrence via SASP factors [162]. Recently, it was also shown that senescence can augment cancer stem cell phenotype [163,164].

The SASP factors mediate pro-oncogenic effects of senescence via cell non-autonomous mechanisms which involve a paracrine signaling [83,96,165]. Interestingly, it has been shown that certain SASP factors can feedback to induce senescence in neighboring cells and regulate senescent phenotype via a positive feedback loop involving induction of ROS [76,91,92,98] (Figure 1). In summary, the senescent phenotype, while meant to be a tumor suppressive mechanism, can act to amplify and abet cancer development and possibly play a role in disease recurrence.

8. Targeting senescent cells for a healthy lifespan

Whether senescent cells in vivo are generated due to telomere dysfunction or various stress factors including ROS, there is an irrefutable evidence in literature that senescent cells accumulate in aged and pathological tissues [79,166,167]. SA-β-gal staining together with CKI-driven senescent cell reporters such as p16-luc [168], p16-3MR (trimodality reporter) [169], INKA-ATTC [108] can be used to facilitate in vivo study of senescent cells [112,170]. The p16-3MR and INKA-ATTC reporters are designed to identify senescent cells using fluorescent markers and selectively kill senescent cells in vivo using ganciclovir and a synthetic drug AP 20187 respectively [112,170]. As discussed above, cellular senescence is thought to be a causative phenotype related to several age-associated pathological traits such as CVD, T2D AD, PD, Huntington's disease (HD), cataracts, macular degeneration, glaucoma and osteoarthritis, and physiological traits such as skin wrinkling, hair graying, reduced hearing, poor vision, diminished wound healing, sarcopenia and immune system dysfunction [108-111]. These traits are either related to upregulation (activity and/or expression) of tumor suppressors such as p16 and p53, which negatively impact the stem cell pool, or SASP factors, which promote degenerative phenotype. The hypothesis that senescent cells via these effectors promote ageing and age-related diseases is testable, albeit difficult to prove experimentally. Nonetheless, recently few remarkable studies have shown that indeed elimination of senescent cells improves healthy lifespan by decreasing the incidence of age-associated diseases.

Baker et al., showed that the p16-positive senescent cells shortens healthy lifespan in the wild type and a BubR1 progeroid mouse model, and their elimination delays most of the age-related pathological traits and imparts rejuvenation to the animals [108,171]. The authors created transgenic

mouse models using INK-ATTC transgene, which expresses GFP and FK506-binding protein-caspase 8 (FKBP-Casp8) under the control of a minimal *Ink4a* promoter that should be active only in senescent cells. Authors then administered AP20187, which activates FKBP-Casp8 to induce apoptosis specifically in senescent cells [108,171]. It was shown that elimination of senescent cells by AP20187 in these transgenic animals attenuated age-related deterioration of most organs including kidney and heart without any apparent side effects [171]. The median lifespans were increased by 24–27% depending on the background and sexes of the animals [171]. Interestingly, although p16 is a well-known tumor suppressor, elimination of p16-expressing senescent cells actually decreased tumorigenesis suggesting that perhaps in senescent cells, SASP function can override p16's tumor suppressor function [171]. In a similar mouse model it was shown that targeting senescent cells enhances adipogenesis, decreases lipotoxicity, increases insulin sensitivity, and metabolic function in the ageing animals [172]. Other recent studies demonstrated that the senolytic drugs that eliminate senescent cells via inhibition of pro-survival molecules such as p21, BCL-2, BCL-XL, PI3K and PAI2, can improve cardiac function and healthy lifespan, and delay age-related pathologies in general [173,174]. These drugs were also shown to rejuvenate organ specific stem cells, in particular hematopoietic, muscle and hair follicle stem cells [175,176].

9. Conclusion

Although the FRTA is not a perfect theory of ageing and age-related diseases, it provides a basic platform to address the potential role of oxidative stress and ROS generated by various stresses that an organism encounter during its lifespan. Produced predominantly from the mitochondria, the ROS in high doses has the potential to wreak havoc on a majority of cellular macromolecules, ultimately rendering tissues and organs susceptible to impairment. On the contrary, the mitohormosis stipulates that a balanced ROS may function as signaling molecules that contribute to longevity by instigating an adaptive response. Antioxidants, exogenous reagents and endogenous biomolecules, function in order to maintain a particular homeostatic redox balance by extinguishing the reactivity of oxidant radicals. Despite the effects of antioxidant manipulation indicating a marginal association with longevity, a dramatic difference is seen in progression of age-related diseases. Therefore, manipulation of ROS levels via known antioxidants and yet to be discovered more powerful antioxidants may provide a reasonable and inexpensive remedy to many of the age-related ailments. Although, ROS effectors can result in multitude of phenotypes including cell death, autophagy and senescence, it appears that accumulation of senescent cells may be a critical factor in normal ageing and pathological diseases that shorten a lifespan. Keeping in mind, the emerging role of senescent cells in healthy lifespan, it is not surprising that senolytic drugs are showing a great promise in experimental animal models. The next step could be combining the senoltyic drugs with reagents that affect ROS, such as antioxidants, which may prove much more potent in delaying age-related pathologies and providing a healthy lifespan and curtailing the enormous cost associated with well-being of an ageing population in the long run.

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Conflict of interest

The authors declare no conflicts of interest.

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