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Review

Mitochondria: Are they causal players in cellular senescence?☆

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ABSTRACT

Cellular senescence entails an irreversible cell-cycle arrest characterised by drastic cytomorphological and metabolic changes. In recent years, the implications of cellular senescence in physiological and pathological settings, such as ageing and cancer, have gained firm ground. It is, therefore, important to understand the mechanisms underpinning the establishment and maintenance of senescence. Age-dependent alterations in cellular metabolic processes are greatly driven by changes in mitochondrial function and homeostasis. Classically, mitochondrial dysfunction has been implicated in cellular senescence mainly by promoting oxidative damage-induced cell-cycle arrest; however, emerging data suggests that other mitochondrial-dependent factors play an important role in the induction of senescent phenotypes. Here we review the role of mitochondrial homeostatic mechanisms, mitochondrial metabolites and ROS generation in the signalling pathways leading to the induction and maintenance of cellular senescence and discuss how this may contribute to the ageing process. This article is part of a Special Issue entitled: Mitochondrial Dysfunction in Aging.

1. Introduction

Cellular senescence was first described as the irreversible cell-cycle arrest resulting from prolonged replication of cells in culture [43]. This form of senescence, termed replicative senescence, was later shown to be a result of telomere attrition occurring during DNA replication with each cell division [40]. Various reports have since demonstrated that cells can also enter senescence in response to other stimuli such as oncogene activation and a variety of other stresses [69], these forms of senescence are termed "oncogene-induced senescence" and "stress-induced premature senescence" respectively. During senescence, cells develop a distinctive phenotype marked by changes in cell morphology, which include larger cellular volume and flattening of the cytoplasm [9], increased lysosomal content [20] and activity of the lysosomal enzyme β-galactosidase at pH 6.0, also known as senescence-associated β-galactosidase (SA-β-gal) [24]. The senescent phenotype is also characterised by altered chromatin and nuclear structure, altered gene expression, protein processing and metabolism [13,84,106,125]. Interestingly, senescent cells have been shown to produce increased levels of Reactive Oxygen Species (ROS) when compared to their younger counterparts and to secrete a plethora of growth factors, extracellular matrix (ECM) degrading proteins and pro-inflammatory cytokines, collectively known as the Senescence-associated Secretory phenotype (SASP) [21]. Both the senescence-associated pro-oxidant and pro-inflammatory phenotypes have been shown to not only stabilise senescence in an autocrine fashion [2,59,90], but also to induce paracrine senescence which may contribute to the detrimental effects of senescence during ageing [1,85]. The pro-oxidant phenotype of senescent cells has been associated with mitochondrial dysfunction during senescence [3,49,50,79,91,139], suggesting that mitochondria may play a role in the process.

Mitochondria have long been considered the "power-house of the cell" due to their primary function in energy production through oxidative phosphorylation and generation of adenosine triphosphate (ATP). Due to its unique evolutionary origin, mitochondria structure and biochemistry are very similar to bacteria [34]. From a structural perspective, the mitochondrion (the basic unit of mitochondria) contains two membranes (the outer and the inner membrane) that separate two distinct compartments: the inter-membrane space and the matrix. The inner membrane is highly folded into cristae and harbours the electron transport chain (ETC) where oxidative phosphorylation takes place [75]. Further to regulating energy production, mitochondria couple many other fundamental cellular processes, including regulation of cellular metabolism, cell-cycle control and cell-death [86]. Here, we review the role of mitochondria in cellular senescence, particularly the impact of mechanisms regulating mitochondrial homeostasis, mitochondrial metabolites and ROS generation in the signalling pathways responsible for the induction and maintenance of cellular senescence.

2. The role of mitochondria in senescence

Generation of ATP by mitochondria occurs *via* oxidative phosphorylation which requires reduction of oxygen to promote oxidation of

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nutrients and release of ATP. However, reduction of oxygen can generate potentially harmful intermediates such as superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which can further react with other ROS or with a transition metal to form highly reactive secondary ROS, including the hydroxyl radical(•OH) [81]. It has been suggested that primary ROS are well-controlled molecules within the cell and that their reactions with target molecules can be reversible, placing this type of ROS as important intracellular signalling molecules [23]. In contrast, secondary ROS, particularly •OH, are catalytically very reactive, not stringently controlled within the cell and therefore comprise the main damaging type of ROS [12,131]. The •OH radical is a highly reactive specie able to abstract hydrogen from nearly any C–H bond [37] and is, therefore, able to react with a vast range of macromolecules such as lipids, proteins and nucleic acids. •OH radicals are important inducers of DNA oxidation resulting in DNA lesions that often include single oxidised nucleobases, tandem base modifications, intra- and inter-strand cross-links, oligonucleotide single-strand breaks and 2-deoxyribose oxidation products [12]. Furthermore, during lipid peroxidation, allylically activated CH_2 groups present in lipids, most often in polyunsaturated fatty acids (PUFAs), are especially prone to hydrogen abstraction by •OH radicals resulting in the oxidation of these lipids in the cell [27,107]. Protein oxidation can also be induced by •OH radicals, which by abstraction of a hydrogen atom from an amino acid residue can form a carbon-centred radical derivative that can further react resulting in other forms of oxidised proteins [108].

Almost 60 years ago, Dehnam Harman had already proposed "The Free radical theory of ageing", hypothesising that ageing could be driven by free-radical associated macromolecular damage [41]. This theory was then revised to propose that mitochondria were the main drivers of the process [42]. In fact, the first experimental evidence of ROS being produced in the mitochondria was only published in the 1960s [51]. The circumstantial evidence amassed over decades in support of this theory is overwhelming, with numerous studies showing associations between oxidative stress, mitochondrial dysfunction and ageing-associated processes in humans and a variety of animal models [7]. Nonetheless, recent findings have put into question the role of oxidative stress in the ageing process. Studies using mouse models where antioxidant defence mechanisms were manipulated have generated conflicting results [80,104,120,134]. Furthermore, clinical trials where the role of antioxidant supplements was evaluated in healthy participants showed no prevention of mortality and in some cases increased mortality [36].

Despite controversy regarding the role of mitochondria and ROS generation in the ageing process, the ageing field is relatively unanimous with regard to two premises: first, that senescence can be driven by oxidative stress and second, that senescence is a contributor to the ageing process; however, not united in agreement with the hypothesis that oxidative stress and mitochondrial dysfunction are drivers of ageing. Nevertheless, evidence suggest that the role of mitochondria in senescence extends beyond induction of ROS-induced damage, particularly because mitochondria have wide-ranging cellular functions and are tightly regulated by complex quality control mechanisms. Here, we will review the evidence suggesting a role for mitochondria in cellular senescence and discuss its role during the ageing process.

2.1. Role of mitochondrial ROS and the electron transport chain in senescence

Data suggest that mitochondrial dysfunction is a general feature of cellular senescence and has been reported to occur independently of the nature of the senescence stimuli (*e.g.* telomere dysfunction, oncogene activation and genotoxic stress) [3,49,50,79,91,139]. It has long been shown that mitochondria are natural producers of intracellular ROS resulting from electron leakage in the electron transport chain (ETC). During this process, superoxide anions are generated and can further react to give rise to other forms of ROS [34]. During cellular senescence, an increase in mitochondrial mass, decreased mitochondrial membrane potential and defective antioxidant defence mechanisms have been linked to increased ROS levels, however the causal relationship between these processes and senescence have not yet been completely elucidated [91,96,103]. Several studies have shown that ROS can induce DNA damage and accelerate telomere shortening activating the DNA damage response (DDR) and senescence [16,95,124,126] (Fig. 1). Telomeric DNA has been shown to be especially sensitive to oxidative damage, accumulating more single-stranded breaks than the rest of the genome [92]. Consistent with a role of ROS in telomere dysfunction it has been suggested that guanine rich regions are more susceptible to oxidative modification [35] and interventions affecting both mitochondrial function and ROS generation have been shown to impact on telomere-dependent senescence *in vitro* [123]. Conversely, treatment with free radical scavengers [125], low ambient oxygen concentrations [26,98], overexpression of antioxidant enzymes [105], and mild chronic uncoupling [91] have been shown to decelerate telomere shortening and to extend the lifespan of cells in culture. Recent studies have shown that oxidative-stress induced telomere damage is irreparable and can occur irrespectively of telomere length [28,46]. The presence of a shelterin complex, a group of proteins that protects exposed telomeric ends, has been demonstrated to inhibit DNA repair mechanisms. It has also been shown that oxidative modifications of shelterin components such as the telomeric repeat binding factor-1 (TRF1) and TRF2 can affect its binding to telomeres [87] and that loss of TRF2 contributes to activation of a DDR at telomeres [121]. While ROS have been shown to promote oxidative damage with induction of a DDR and senescence, activation of major downstream effectors of the DDR during senescence can result in elevated ROS generation, suggesting that these molecules may also act as signalling factors and potential effectors/stabilisers of the senescent growth arrest. Several lines of evidence have further corroborate the idea of ROS as signalling molecules during cellular senescence: i) activation of a DDR by genotoxic stress or telomere uncapping has been reported to promote ROS generation [90]; ii) in oncogene-induced senescent cells, over-expression of activated RAS [63] or $BRAF^{V600E}$ [55] is accompanied by elevated ROS generation, iii) activation of the main DDR effectors p53 [71], p21 [72] and p16 [113] all resulted in elevated ROS production and iv) treatment with antioxidants, such as N-acetyl cysteine (NAC), is able to prevent the cell-cycle arrest, in most of the above reported cases. Indeed, major senescence signalling pathways, such as the p53 and Rb tumour suppression pathways and ROS interact to induce and stabilise the cell-cycle arrest. In oncogene-induced senescence, mitochondrial dysfunction and ROS production trigger senescence in a p53 and Rb dependent way [79], while knockdown of p53 and p21 reduces ROS generation in both telomere-dependent and -independent senescence [90]. Activation of the cell-cycle kinase inhibitor p21, downstream of DNA damage, promotes ROS generation that feeds-back into further DNA damage induction which persists even in irreversibly deep senescence. Additionally, p21 seems to be the critical mediator between the DDR, MAPK and TGF-β stress-induced signalling cascades, which have been shown to contribute to ROS generation [58,90,116]. Together these observations support a role for ROS as senescence-stabilising molecules via induction of continuous damage generation and the persistent activation of a DDR [90]. Importantly, ROS has been shown to impact on the DDR and ultimately induce senescence in a paracrine fashion [85]. The ROS-dependent paracrine senescence may be a mechanism by which senescence cells contribute to loss of tissue function during ageing.

We have discussed the signalling pathways that have linked ROS to the induction of a senescence cell cycle arrest, however other mitochondrial perturbations, namely disruption of the mitochondrial respiratory complexes and the ETC, have been associated to cellular senescence [78,79,109,135] (Fig. 1). Oxidative phosphorylation (OXPHOS) takes place in the inner mitochondrial membrane and requires four respiratory chain complexes (complex I, II, III and IV) and the ATP synthase

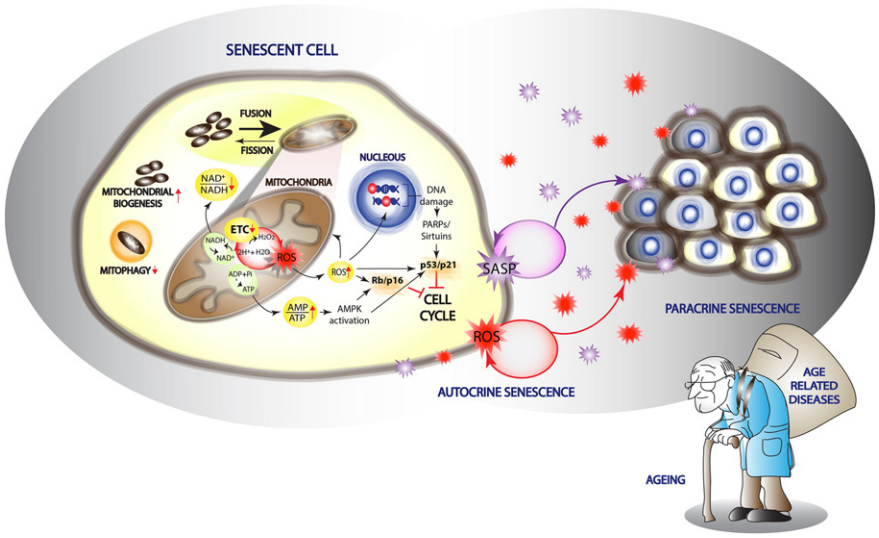

Fig. 1. Mitochondrial homeostasis impairment induces cellular senescence and may contribute to the ageing process. Deregulation of mitochondrial homeostatic mechanism: increased mitochondrial biogenesis, decreased mitophagy and decreased fission/fusion ratios have been suggested to induce cellular senescence. Perturbations on the electron transport chain (ETC) resulting in decreased ATP production and increased ROS generation can activate the Rb/p16 and the p53/p21 tumour suppressor pathways and induce a senescence cell cycle arrest. Increased ROS levels can induce telomeric and non-telomeric DNA damage activating the p53/p21 pathway via PARPs/sirtuins activation and induce a permanent cell cycle arrest. Mitochondrial metabolites imbalance, such as decreased NAD^+/NADH ratios, has been linked to senescence. Senescent cells have been shown to generate increased levels of Reactive Oxygen Species (ROS) and secrete a plethora of growth factors, extracellular matrix (ECM) degrading proteins and pro-inflammatory cytokines, collectively known as the Senescence-associated Secretory phenotype (SASP). Both ROS and the SASP have been shown to stabilise senescence in an autocrine fashion, but also to induce paracrine senescence, which may contribute to the detrimental effects of senescence during ageing.

(complex V). The electron transfer reaction occurs through the respiratory complexes and is ultimately used to produce energy under the form of ATP [102]. Perturbations in the mitochondrial respiratory complexes, either by pharmacologic inhibition or genetic manipulation, have been shown to drive cells into senescence. Inhibition of complex I by rotenone [79] or knockdown of the complex I assembly factor NDUFAF1 [78] have been shown to induce cellular senescence. A decline in complex II activity induced by desferroxamine mesylate can also drive premature senescence [135]. Compromised complex III activity by either knockdown of the mitochondrial Rieske iron-sulfur polypeptide (RISP) [79] or inhibition of this mitochondrial complex with antimycin A can also induce a senescence arrest [109]. It is still not clear how interference with the ETC, by disruption of the respiratory complexes, induces a permanent growth arrest. A possible scenario is that elevated ROS (resulting from the disruption of the respiratory complexes and increased electron leakage) in mitochondria can further decrease the ETC efficiency contributing to additional ROS generation and oxidative damage in a positive feedback loop [7]. This way, ETC perturbations may amplify mitochondrial dysfunction and induce senescence via ROS-induced damage. However, ETC inefficiency can also result in decreased ATP production, which has also been suggested to play a role in the induction of senescence [129,139]. It has been shown that the AMP/ATP ratio, a measure of cellular energy charge, increases when human fibroblasts reach replicative senescence [129,139]. Conversely, addition of exogenous AMP to the cell culture medium triggers premature senescence in young human fibroblasts [139]. Interestingly, it has been shown that reduced levels of ATP resulting from severe mitochondrial uncoupling may contribute to cellular senescence without substantially increasing oxidative stress [110]. Mechanistically, increased AMP (or ADP) to ATP ratios are known to stimulate the AMP-activated protein kinase (AMPK) [76]. AMPK activation has been reported to induce a senescence cell-cycle arrest in many cells types [48,53,74,93,97,128,129] via multiple mechanisms. Activation of AMPK is associated with increased p53 phosphorylation, increased p53, p21 and p27 protein expression and decreased retinoblastoma protein phosphorylation [93] which can promote p53- and p16-dependent senescence [52,53,128,129] (Fig. 1). Additionally, activation of AMPK has been shown to have anti-proliferative effects by down-regulating pro-proliferation genes, such as cyclin A, cyclin B1, and cyclin E [74,93,128,129]. Together, these data puts into question the hypothesis that perturbations of the ETC promote senescence mainly via ROS mediated oxidative damage and confers a possible role for cellular bioenergetics imbalance in the induction of the cell-cycle arrest and development of senescence phenotypes.

While mitochondrial ROS have been implicated in cellular senescence, non-mitochondrial sources of ROS have also been shown to play a role. NADPH oxidase-dependent ROS generation has been shown to limit the replicative lifespan of human endothelial cells in culture [67]. Human fibroblasts, expressing a temperature-sensitive simian virus 40 large T antigen, have elevated ROS production via protein kinase Cδ and p16 signalling [113]. Protein kinase Cδ has been shown to activate a non-mitochondrial source of ROS, generated by NADPH-oxidase through phosphorylation of $p47^{phox}$, an essential component of NADPH oxidase [114]. While mitochondria are traditionally regarded as the main source of intracellular ROS and, therefore, major mediators of ROS induced damage, the relative contribution of mitochondrial and non-mitochondrial sources of ROS to the induction of senescence is still to be clarified.

2.2. Role of mitochondrial homeostatic mechanisms in senescence

Eukaryotic cells are equipped with a vast range of molecular and cellular pathways that control the quality and integrity of mitochondria [112]. A tight regulation of mitochondrial content, dynamics and activity is fundamental for cellular homeostasis.

Regulation of mitochondrial content (mass) is achieved through processes of mitochondrial biogenesis and degradation and the ratio between these two cellular processes determines the amount of mitochondria within a cell [88]. Mitochondrial biogenesis is a multifactorial process which involves the integration of strictly regulated transcriptional events, lipid membrane and protein biogenesis and assembly as well as replication of mtDNA [138]. The peroxisome proliferator-activated receptor co-activator 1 (PGC-1) family which includes PGC-1α, PGC-1β and the PGC-1-related co-activator (PRC) [4,68,94] are strong activators of mitochondrial function including regulation of mitochondrial biogenesis and oxidative metabolism in a variety

of tissues [38]. The mitochondrial transcription factor A (TFAM) is another important factor regulating mitochondrial biogenesis, it is particularly involved in mtDNA transcription initiation and packaging of mtDNA into nucleoids [60]. Increased mRNA expression of PGC-1α, PGC-1β and TFAM has been observed during oncogene-induced senescence [79] and overexpression of PGC1-α has been shown to induce senescence in human fibroblasts [132], suggesting a role for mitochondrial biogenesis in promoting mitochondrial mass increase and premature induction of senescence. As opposed to *de novo* generation of mitochondria, mitochondrial degradation impairment may also contribute to an accumulation of these organelles in the cell.

Autophagy is a quality control mechanism responsible for the degradation/recycling of cellular components, with mitophagy being a special type of autophagy that promotes the degradation of dysfunctional mitochondria [57]. The E3-ubiquitin ligase protein Parkin and the PTEN-induced putative kinase 1 (PINK1) have been associated with the degradation of dysfunctional mitochondria [82,83]. When mitochondrial membrane potential decreases in mammalian cells, Parkin translocates to mitochondria and induces their removal by mitophagy and proteasome degradation. Parkin translocation from the cytosol to mitochondria is dependent on PINK1, a serine/threonine kinase, which, in functional mitochondria, is localised in the inner mitochondrial membrane [83]. The mechanism by which Parkin/PINK1 mediates mitophagy is not fully understood, but it has been shown that PINK1 translocates to the outer mitochondrial membrane, where together with Parkin, promotes the segregation of damaged mitochondria from the mitochondrial network [5]. The PINK1 kinase phosphorylates the E3-ubiquitin ligase, which subsequently mediates polyubiquitination of a subset of mitochondrial substrates that may trigger mitophagy [15,29,30,130]. The impact of mitophagy in cellular senescence remains poorly defined with some reports providing indirect or circumstantial evidence for the induction of autophagy during senescence [32]. A study has shown that induction of autophagy promotes entry into senescence [137], while another study reported that autophagy impairment induces premature senescence through a ROS- and p53-dependent mechanism, possibly via mitochondrial dysfunction in primary human fibroblasts [54]. The exact contribution of mitochondrial biogenesis and mitophagy to the establishment and maintenance of cellular senescence (Fig. 1) are still poorly understood and further studies are required to understand the kinetics and putative interactions between these two processes for the induction and maintenance of the cell-cycle arrest.

Further to the control of mitochondrial content via processes of mitochondrial biogenesis and mitophagy, mitochondrial network organisation has been show to play a critical role during senescence [14]. Mitochondria are highly dynamic organelles able to adapt their size, shape and organisation/structure through processes of fission and fusion [14], and alterations in mitochondrial dynamics have been shown to trigger cellular senescence [39,65,73,89,136]. Inhibition of fission promotes mitochondrial elongation and the establishment of cellular senescence. During senescence, the fission 1 (FIS1) protein is down-regulated leading to mitochondrial elongation [136]. Knockdown of the fission 1 (FIS1) protein that recruits the pro-fission protein dynamin 1-like (DNM1L or DRP1) [65] or depletion of the membrane-associated ring finger C3HC4 5 (MARCH5), a mitochondrial E3 ubiquitin ligase which blocks DRP1 [89], promotes mitochondrial elongation and induction of senescence. Mechanistically, mitochondrial elongation has been reported to be associated with a decrease in mitochondrial membrane potential and increased ROS generation, which induces DNA damage, thereby activating senescence-inducing pathways [65,136]. On the other hand, processes that stimulate mitochondrial fission have been shown to reduce senescence-associated phenotypic changes [65,136]. Overexpression of FIS1 in senescent cells is able to reverse both mitochondrial elongation and appearance of senescent phenotypes suggesting its involvement in the process [136]. Depletion of both FIS1 and the optic atrophy 1 (OPA1) protein, a critical component of mitochondrial fusion, have been shown to promote extensive mitochondrial fragmentation and markedly rescue the senescent phenotype [65]. Hence, sustained mitochondrial elongation may promote senescence-associated phenotypic changes that can be reversed by mitochondrial fission. The reason why mitochondrial elongation occurs in senescent cells in the first place is still not clear, but studies have suggested that it may be an anti-apoptotic mechanism. Upon activation of autophagy, mitochondrial elongation, promoted by protein kinase A (PKA) dependent phosphorylation of DRP1, may serve as a mechanism to avoid autophagic degradation and maintain cellular viability [33]. Corroborating this hypothesis, cytochrome c release inhibition by mitochondrial fusion enhances resistance to apoptosis [11,56]. Processes regulating both mitochondrial content (via mitochondria biogenesis and autophagy) and mitochondrial dynamics (via fission and fusion) are not mutually exclusive; indeed several studies indicate that they crosstalk to maintain mitochondrial homeostasis (Fig. 1).

2.3. The role of mitochondrial metabolites in senescence

Several reports have linked mitochondrial metabolites to cellular senescence [10,47,52,55,62,66,118]. Mitochondrial respiratory complexes produce important co-factors and metabolites not only used in cellular respiration reactions but also required for other essential cellular functions. During oxidative phosphorylation, organic molecules are oxidised in reactions that are coupled to the reduction of electron carriers such as the nicotinamide adenine dinucleotide (NAD+) [31]. The cytosolic malate dehydrogenase (MDH1) is the tricarboxylic acid (TCA) cycle enzyme that catalyses the reversible reduction of oxaloacetate to malate in the presence of reduced nicotinamide adenine dinucleotide (NADH) [31]. It has been shown that MDH1 activity is diminished as human dermal fibroblasts (HDFs) approaching their replicative limit and that knockdown of MDH1 in HDFs and IMR90 human fibroblasts result in elevated $p16^{INK4A}$ and $p21^{CIP1}$ protein levels and premature senescence [66]. Additionally, cytosolic NAD^+/NADH ratios have been reported to be decreased in replicative senescent HDFs to the same extent as following knockdown of MDH1, suggesting that cytosolic NAD^+ depletion may be a trigger of cellular senescence [66]. Furthermore, the NAD^+-dependent malic enzyme 1 and 2 (ME1 and ME2), which convert malate into pyruvate, have also been linked to senescence. Down-regulation of ME1 and ME2 trigger p53-dependent senescence, whereas enforced expression of either these malic enzymes suppresses senescence [52]. Another pyruvate metabolism related enzyme, the pyruvate dehydrogenase, has been implicated in $BRAF^{V600E}$-induced senescence by enhancing the use of pyruvate by the TCA cycle and increasing mitochondrial respiration and ROS generation [55].

Further to its co-enzymatic roles in cellular respiration, NAD^+ is an essential cofactor in many other intracellular enzymatic reactions, including those involved in the DNA repair signalling by modulating Poly-ADP ribose polymerases (PARPs) and Sirtuins activity [61]. DNA damage and a persistent DDR are potent inducers and stabilisers of cellular senescence [22,90,99]. Thus, interference with the signalling pathways involved in the DDR and DNA damage repair affects cell fate by promoting cell senescence, death or cancer [8,22,100]. PARPs and Sirtuins, a family of protein deacetylases, are important effectors of DNA damage and repair responses [18,45]. Perturbations in the PARPs and Sirtuins have been shown to impact on cellular senescence [25,61].

Imbalanced NAD^+/NADH levels have also been associated with cellular senescence (Fig. 1). Replicative senescence is preceded by a decline in the expression and activity of nicotinamide phosphoribosyltransferase (Nampt), an enzyme responsible for NAD^+ salvage from nicotinamide [118]. Chemical inhibition of Nampt activity induces premature senescence, while overexpression of Nampt delays entry into senescence and enhances resistance to oxidative stress [10,118]. The delay in senescence induction mediated by Nampt is associated with increased activity of Sirtuin 1 (SIRT1), a NAD^+-dependent enzyme that antagonizes senescence by deacetylating p53 [10,47,62,118]. Together, these data

suggest that decreased NAD^+/NADH ratios and NAD^+-dependent enzymes levels are important factors triggering cellular senescence.

Despite some evidence showing a role for mitochondrial metabolites in cellular senescence, our understanding on how these mitochondrial-associated factors contribute to a permanent cell-cycle arrest and the development of the senescent phenotype is relatively limited and further studies are required to fully understand their role in the process.

2.4. The role of senescence in the ageing process: a possible role for mitochondria?

Our understanding of the role of cellular senescence *in vivo* has considerably evolved since Hayflick's initial observation. Cumulative evidence suggests that cellular senescence is not only a tumour suppressor mechanism, but also a contributor to age-related tissue dysfunction and -diseases [119] (Fig. 1). Cells bearing senescent markers have been shown to increase with age in a variety of different tissues from mice, baboons and humans [127,44,46]. Furthermore, senescent cells have been found to be associated to multiple age-related diseases including cardiomyopathy, renal fibrosis atherosclerosis, osteoarthritis and type-II diabetes [17,70,77,111,115,133]. This has led to the hypothesis that senescent cells may not be a consequence of ageing and age-related disease but in fact play an active causal role in these processes. Recent evidence suggests that this may indeed be the case, since inducible elimination of $p16^{Ink4a}$-positive senescent cells delayed the acquisition of age-related pathologies in a progeroid mouse model with BubR1 insufficiency. Importantly, this study demonstrated that by eliminating $p16^{Ink4a}$, these mice could have not only delayed age-related degeneration but also slowed progression of already established conditions [6].

A few studies suggest causal links between mitochondria, ROS and senescence *in vivo*: in ageing mouse skin an increased frequency of senescent cells has been shown to be associated with impaired mitochondrial complex II activity. Moreover, mice carrying a heterozygous deletion of the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) show increased nuclear DNA damage and senescence markers in the skin [122]. Additionally, a mouse model with conditional deficiency of SOD2 in fibroblasts and other mesenchyme-derived cells of connective tissues was shown to have reduced lifespan and premature ageing phenotypes associated with increased expression of the senescent marker p16 [117]. Other studies have shown interactions between pathways involved in senescence and mitochondrial dysfunction. Late-generation $TERC^{-/-}$ mice, which experience generation dependent telomere shortening, have been shown to have dysfunctional mitochondria and increased ROS generation via p21 [90] and p53-dependent repression of the mitochondrial biogenesis activator *PGC-1α* [101]. The decrease in *PGC-1α* expression upon telomere dysfunction (a major inducer of senescence) in late-generation $TERC^{-/-}$ mice observed in this study seems to contradict the previously suggested role of this mitochondrial biogenesis mediator in promoting cellular senescence [79,132]. It could be argued that the loss of mitochondria observed in late-generation $TERC^{-/-}$ mice is not associated with induction of senescence but with the increased apoptosis levels also observed in these mice [19,64]. Furthermore, there are complex technical challenges which impede our ability to follow kinetically mitochondrial dynamics at the cellular level in mammalian organisms *in vivo*, and most of the data collected represents a snapshot of a highly dynamic process. As a result, further studies need to be conducted in order to understand the impact of mitochondrial homeostatic mechanisms in the induction and stabilisation of senescence *in vivo* and to resolve the contradictions between *in vitro* and *in vivo* studies.

3. Conclusions

There is increasing evidence for a causal role of senescence in age-related tissue dysfunction and pathology. There are also numerous studies (mostly *in vitro*) showing that alterations in mitochondrial homeostatic mechanisms, mitochondrial metabolites and ROS generation can lead to the activation of senescence-associated signalling pathways and promote senescence phenotypes. The role of ROS during ageing has been under intense debate with some reports supporting the hypothesis that these reactive molecules act as signalling molecules promoting longevity and others suggesting them as damage-inducing factors able to accelerate or drive the ageing process. This dichotomy can be loosely equated to the known roles of senescence *in vivo*: on one hand, senescence is beneficial for an organism (by suppressing cancer and aiding wound-healing); on the other hand, senescence is detrimental by promoting tissue dysfunction and ageing. These "trade-offs" during an organism lifespan may well explain the ageing process; however, the conciliation of all these different factors poses a major experimental challenge for the scientific community.

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