Essay

MSc Biomedical Sciences

Cellular Senescence:

Spotlight on the Mitochondria

---

by

Sara Nankova Mavrova (S2769514)

May 2016

Courtesy of Joel Dubin, MadMicrobe Studios

Group: Cellular Senescence and Age-related Pathologies (ERIBA)

Supervision: Dr. Marco Demaria

Examiners: Dr. Floris Foijer, Dr. Marco Demaria

rijksuniversiteit groningen

Table of Contents

Abstract

In order to respond to internal and environmental stress, cells developed different stress response mechanisms. Such a mechanism is cellular senescence, which leads to permanent cell-cycle arrest of proliferative cells. Not so long ago, it has become evident that senescent cells have serious alterations in their gene expression and function. Furthermore, paradoxical to their anti-tumorigenic function, these cells can promote tumor formation and progression along with other disorders. This feature of the senescent cells is due to their altered secretome, called senescence associated secretory phenotype (SASP). Recent studies identified that SASP is regulated by the activity of the mammalian target of rapamycin complex 1 (mTORC1) and can be abrogated by the administration of rapamycin or complete clearance of the mitochondria. In this essay, the molecular mechanisms behind the SASP regulation will be discussed together with the mitochondrial involvement in this process.

Introduction

Cellular senescence is a process that leads to permanent cell cycle arrest in response to different stress conditions (Campisi and d'Adda di Fagagna 2007; Kuilman et al. 2010). The senescence process is restricted to proliferative tissues, where it acts as a preventive mechanism for cancer development (Campisi & d'Adda di Fagagna, 2007). Cellular senescence was described for the first time by Hayflick in an experiment with human fibroblasts serially passaged in cell cultures (Hayflick & Moorhead, 1961). The senescence observed in this experiment was due to telomere attrition, but now we know that there are other triggers of cellular senescence (Carlos Lopez-Otın, Maria A. Blasco, Linda Partridge, Manuel Serrano, 2013). DNA damage induces activation of DNA-damage response (DDR) - the main cause for the cell cycle arrest. Persistent DDR can lead to the activation of senescence and contributes to the maintenance of this state.

According to the free radical ageing theory (Harman 1956), reactive oxygen species (ROS) play a key role in disrupted cellular homeostasis due to accumulated oxidative damage. ROS are formed as a normal by-product of the oxygen metabolism and can react with all major macromolecules, including DNA. Hydrogen peroxide, which is the most abundant type of ROS in the cell, is also an inducer of DNA damage. As a non-renewable macromolecule, DNA can accumulate oxidative damage over time, which can activate the DDR. The fact that mitochondria are the main source of ROS in the cell turned the spotlight on these organelles, as important participants in the process of senescence. Recent findings (Correia-Melo et al., 2016; Wiley et al., 2016) show that apart from the ROS production, mitochondria can contribute to the senescent state through previously unknown molecular pathways.

In this essay I am going to discuss the features of senescent cells and the molecular pathways that regulate their function. The main focus will be on recent findings about the role of mitochondria in establishment, maintenance and phenotype of the senescent state.

Hallmarks of senescent cells

Senescent cells share similar characteristics, which include: cell-cycle arrest (hereafter also referred as growth arrest), markers specific for senescence, resistance to apoptosis, and altered gene expression and secretory phenotype.
Unlike quiescence, the growth arrest of senescent cells is a permanent and irreversible process. Senescent cells are typically arrested in G_1 phase of the cell cycle (Brookes et al., 2015) and remain metabolically active in their non-proliferative state. However, there is evidence that some oncogenes can induce senescence in cells in G_2 phase (Di Micco et al., 2006).

Senescent cells can be identified in cultures and *in vivo* by several markers. Most widely accepted, as a senescent marker, is the activity of senescence-associated β-galactosidase (SA-βgal). SA-βgal probably originates from the lysosome (Lee et al., 2006) and can be detected by histochemical staining. Furthermore, the cyclin-dependent kinase inhibitor (CDKI) $p16^{INK4a}$ is expressed by a large group of senescent cells and often correlates with the

presence of active SA-βgal (Krishnamurthy et al., 2004). The third most prominent marker of senescent cells is persistent DNA-damage response (DDR), which is associated with maintenance of the senescent state through the activation of p53 (Fumagalli et al., 2012; Rodier et al., 2009).

Cells that undergo permanent cell cycle arrest due to irreparable damage have two options: to succumb to apoptosis or become senescent. The mechanism behind this cell fate decision is not clear yet. However, it is probably dependent on the cell type and molecular pathways that are regulated by p53 (Seluanov et al., 2001). Remarkably, most senescent cells are resistant to apoptosis, which is at least in part, dependent on the cell type (Hampel, Malisan, Niederegger, Testi, & Jansen-Durr, 2004).

A prominent feature of the senescent cells is their gene expression, altered in the direction of down-regulation of cell-cycle activators and up-regulation of cell-cycle inhibitors (Campisi & d'Adda di Fagagna, 2007). Senescent cells often express p16, which is a regulator of the retinoblastoma protein (pRB) and p21 - a target of p53. Both p16 and p21 are CDKIs and act as activators of pRB, but their mechanisms of action are different (Campisi, 2013). When active, pRB leads to suppression of cell-cycle promoting factors, such as replication-dependent histone genes, cyclin A, cyclin B and PCNA (Narita et al., 2003).
Surprisingly, a large portion of the up-regulated genes in senescent cells are coding for secretory proteins that can influence the cellular microenvironment and in many cases induce immune response. The secretome of senescent cells includes key components of the Wnt, IGF1, TGFβ, and interleukin (IL) signaling cascades and it is used for intra- and intercellular communication (Kuilman & Peeper, 2009). By their secretory function, senescent cells can alter the microenvironment, which in turn regulates cellular behavior.

Regulation of SASP

The regulatory mechanisms of the secretory phenotype of senescent cells are complex and depend on the trigger of senescence. Senescent cells induced by DNA damaging agents, critically short telomeres, or oncogene activation have persistent DDR, which stimulates the secretion of factors associated with inflammation, modulation of the extra cellular matrix, and proliferation (Rodier et al., 2009; Salama et al., 2014). Because this secretory profile is specific to senescent cells, it has been named senescence-associated secretory phenotype (SASP).
The inflammatory components of SASP are shown to be up-regulated by two transcription factors: NF-κB and C/EBPβ (Acosta et al., 2013; Kuilman et al., 2008). A direct transcriptional target of C/EBPβ is IL-6, which is the most prominent pro-inflammatory cytokine of the SASP. The expression of IL-6 is controlled by persistent DDR by pathways independent of p53. Epithelial and endothelial cells express IL6-R and gp130 signaling complex and can be direct targets of IL-6 secreted by neighboring cells. Both NF-κB and C/EBPβ are shown to be regulated by IL-1, thus inducing the secretion of IL6 and IL8. Acosta et al. (Acosta et al., 2013) have shown that IL-1 maturation is promoted by inflammasome through caspase 1. The presence of IL-1 can affect neighboring cells expressing the cell-

surface receptors IL-1 receptor/Toll-like receptor superfamily, which trigger the NF-κB pathway (Mantovani, Locati, Vecchi, Sozzani, & Allavena, 2001).

Functions of senescent cells and SASP

The SASP is primarily a protective response, whose beneficial effects started recently to emerge. Apart from a communication signal, SASP can promote proliferation and tissue repair and has an important role in wound healing (Demaria et al., 2014). Furthermore, proteases secreted from senescent cells limit the formation of fibrosis and thus help to preserve the function of damaged tissues (Jun & Lau, 2010).

Apart from its beneficial effects, the chronic presence of pro-inflammatory SASP can alter the tissue microenvironment and structure and contribute to the development and progression of degenerative diseases and cancer.
Factors secreted by senescent cells can promote tumor development and malignancy. Such effects have been observed in a number of tissues, including breast, skin, prostate, pancreas, and oropharyngial mucosa (Coppé Jean-Philippe , Pierre-Yves Desprez, Ana Krtolica, 2010). Furthermore, the SASP components IL-6, IL-8 and GROα (growthrelated oncogene) can stimulate epithelial-to-mesenchymal transition, which is an important step in the development of metastatic carcinoma (R. M. Laberge, Awad, Campisi, & Desprez, 2012). Indeed, senescent cells can create a pro-tumorigenic microenvironment by their expression of SASP components. They secrete proteases that can degrade the extra-cellular matrix (ECM), which makes it loose and facilitate cancer invasion (Coppé et al., 2008). Furthermore, senescent cells secrete multiple angiogenic factors and promote neo-angiogenesis, thus indirectly facilitating tumor vascularisation (Pacome Lecot, Fatouma Alimirah, Pierre-Yves Desprez, Judith Campisi, 2016).

SASP has been implicated in multiple degenerative processes, such as vascular calcification and development of atherosclerotic lesions, major risk factors for serious cardiovascular disease (Burton, Matsubara, & Ikeda, 2010; Gorenne, Kavurma, Scott, & Bennett, 2006). Furthermore, the expression of a SASP by astrocytes, has been proposed to contribute to neuroinflammation, shown in cultures and aged brain tissue (Salminen et al. 2011). Inflammation is associated with many neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, ALS, and MS (Glass, Saijo, Winner, Marchetto, & Gage, 2010). However, there is not enough evidence that senescent cells have a causal role in degenerative diseases (Campisi et al., 2011).

Senescent cells contribute to the process of ageing and their number increases with age. Furthermore, the fact that senescence can both suppress and promote tumor development speaks of the evolutionary reasons for its existence. These paradoxal effects of senescence can be successfully explained by the antagonistic pleiotropy ageing theory, which hypothesize that genes or mechanisms that were selected to benefit health in young age can have unselected deleterious effects in ageing (Campisi & d'Adda di Fagagna, 2007). Therefore, we can conclude that senescence is a protective process, that can be triggered by multiple stress conditions and its beneficial or deleterious effects are context-dependent.

Triggers

Mitogens and proliferation-associated signals can trigger senescence, which is consistent with its role in tumor suppression. Excessive or chronic mitogenic stimulation can be caused by oncogenes, which are mutant versions of normal genes and have the ability to transform cells in combination with additional mutations. Furthermore, cells senesce in response to overexpressed growth-factor receptors and chronic stimulation of cytokines, such as interferon-β (Campisi, 2013).

Activation of senescence can be caused by genetic events, such as telomere damage and uncapping, which leads to telomeres being recognized as double strand breaks (DSB) and induction of DNA damage response (DDR). Persistent DDR leads to the irreversible arrest of the cell cycle and senescence. Telomere damage is only one type of genomic damage, which can trigger the DDR, but severe DNA damage in other parts of the genome can also activate the senescence response through persistent DDR.

Mitochondrial ROS

An important function of the mitochondria in the cell is the energy production in the form of ATP via oxidative phosphorylation. The synthesis of ATP requires the reduction of oxygen, which promotes oxidation of nutrients and release of ATP. Primary ROS are released from the reduction of oxygen as intermediates, which can further be used for signaling. Such ROS are hydrogen peroxide and superoxide anion radical. However, primary ROS can further react with metal ions or other reactive oxygen species and form secondary ROS, such as hydroxyl radical. These secondary ROS are highly unstable and can react with all kinds of macromolecules, thus causing cellular damage (Correia-Melo & Passos, 2015).

Oxidative stress can induce telomere damage and activate DDR, which leads to senescence (Rai et al., 2009; Von Zglinicki, 2002). Furthermore, it has been shown that telomeric DNA is more sensitive to oxidative stress, since guanine rich regions are more susceptible to oxidative modifications. The role of ROS in telomere damage and senescence has been confirmed by the increased lifespan in cell cultures when ROS have been decreased by the addition of antioxidants, reactive oxygen scavengers, and low ambient oxygen concentration (Correia-Melo & Passos, 2015).

Increased production of mitochondrial ROS in replicative senescence has been shown to contribute to the senescent phenotype by inducing oxidative damage to DNA and therefore amplifying the DNA damage signals together with the signals coming from critically short telomeres. (Passos & Von Zglinicki, 2006; Passos et al., 2007).
Furthermore, Passos and colleagues (Passos et al., 2010) have shown that ROS levels increase in senescent cells as a result of signaling through CDKN1A-MAPK14 and TGFβ pathway, which causes further DNA damage induction and DDR, creating a persistent feedback loop. These data conclude that mitochondrial dysfunction and ROS contribute to the senescence process.

Mitochondria beyond the free radical theory

Mitochondria are important source of ROS, but they are neither the only, nor the main source of reactive oxygen species. In hepatocytes, the endoplasmic reticulum and peroxisomes are shown to produce even bigger amounts of ROS than the mitochondria (Brown & Borutaite, 2012). Furthermore, non-mitochondrial sources of ROS have been implicated in senescence (Blander, De Oliveira, Conboy, Haigis, & Guarente, 2003), thus raising the question if mitochondria have a causal role in this process. In the following paragraphs I am going to discuss recently discovered molecular mechanisms that directly link mitochondria to senescence.

Mitochondria can establish and sustain senescence: molecular pathways and phenotypes

Ca^{2+}

The function of the mitochondrial electron transport chains is maintained through the proton gradient across the membrane. Disrupting this mitochondrial membrane potential can result in mitochondrial dysfunction and cellular senescence.

Mitochondria import calcium to maintain their electron transport chains (Gunter, Yule, Gunter, Eliseev, & Salter, 2004), but its accumulation can cause mitochondrial depolarization, decrease in ATP production and NAD^+ /NADH ratio, and reduced sirtuin activity, all of which can activate senescence (Ziegler, Wiley, & Velarde, 2015). Indeed, calcium accumulation in mitochondria leads to a decrease in the mitochondrial membrane potential followed by mitochondrial dysfunction, thus triggering senescence (Wiel et al., 2014). Furthermore, the decrease of calcium uptake in the mitochondria by the MCU knockdown results in escape from oncogene-induced senescence according to Wiel and colleagues (Wiel et al., 2014).

mTORC1

Several studies indicated the role of mTORC1 in the positive regulation of the SASP. Two laboratories have reported that the mTORC1 inhibitor - rapamycin has the ability to blunt SASP by inhibiting two of its inflammatory cytokines – IL-6 and IL-8 (Herranz et al., 2015; R.-M. Laberge et al., 2015). Laberge and colleagues (R.-M. Laberge et al., 2015) confirm that rapamycin, suppresses SASP by selectively targeting the SASP initiating cytokine IL-1A through suppression of mTORC1 activity. Rapamycin inhibits the translation of IL-1A, thus hampering the establishment of a signaling cascade that activates NF-κB and the expression of other SASP factors. Furthermore, Herranz et al. (Herranz et al., 2015) identify another mTOR-dependent transcript – the mRNA for the Ser/Thr-kinase MK2 (MAPKAPK2). MK2 is the target of phosphorylation of the MAP kinase p38, which have been implicated in the establishment of senescence (Serrano, 2015). By its action on downstream targets, MK2 inhibits the destabilization of multiple mRNAs encoding different cytokines. Overall, the two studies confirm that the translation of mRNAs encoding for important SASP components is directly regulated by the mTORC1 complex and can be successfully inhibited by the addition of rapamycin. Furthermore, another feature of the senescent cells - the expression of SA-βGal is abrogated. However, the cells preserve their cell cycle arrest, thus remaining senescent.

Two recent studies from the Passos and Sell laboratories show that there is a causal link between mitochondrial dysfunction and activation of senescence, which is mediated through the mTOR signaling pathway.

In 2016, Nacarelli and colleagues (Nacarelli, Azar, & Sell, 2016) showed that mitochondrial stress signaling induces senescence through oxidative stress that leads to the activation of p70S6k via mTORC1. In their study, the group uses nucleoside analogs (abbreviated NRTIs by the authors) that inhibit the function of the HIV reverse transcriptase. However, these compounds also affect the γ-subunit of the mitochondrial DNA polymerase thus leading to mitochondrial toxicity and senescence. Furthermore, in patients that undergo antiretroviral therapy for HIV-1 infection, increased risk for development of age-associated comorbidites is observed due to premature cellular senescence. The downstream effects of mTORC1 activation in cells treated with NRTIs are decreased by the use of ROS scavengers or by the inhibition of the mTORC1 with rapamycin. Therefore, the authors suggest that mild mitochondrial ROS can serve as an input for mTORC1, whose action leads to premature senescence.

A new study from the Passos laboratory (Correia-Melo et al., 2016) has provided strong evidence that the pro-inflammatory SASP can be abrogated when senescent human fibroblast have been subjected to complete mitochondria clearance. Upon clearance of mitochondria, senescent cells show major changes in gene expression, reduced cell size, reduced expression of SA-β-gal, decreased formation of senescence associated heterochromatin foci (SAHF), lower ROS generation and down-regulation of p16 and p21. Furthermore, important factors of the pro-inflammatory SASP, namely IL-6, IL-8, GRO and MCP-1 (Correia-Melo et al., 2016) were not present together with decreased mTOR activity. Activation of DDR leads to increase in mitochondrial mass in ATM-Akt-mTORC1-PGC1β manner. ATM phosphorylates Akt, which through a phosphorylation cascade activates mTORC1. The latter is an activator of PGC-1β, which stimulates mitochondrial biogenesis. The connection between the molecular participants in this pathway has been confirmed by the reduction of mitochondrial mass when chemical inhibition of ATM or mTORC1 was administrated. Furthermore, it has been shown that mTORC1 increases mitochondrial mass, but does not disrupt mitochondrial function. Thus, Correia-Melo and colleagues suggest that the increased ROS production causes oxidative stress and persistent activation of DDR, a vicious circle that leads to activation and maintenance of the cell cycle arrest.

AMPK

Driven by the phenotypic similarities between replicative senescence and oncogene-induced senescence (OIS) Moiseeva and colleagues (Moiseeva, Bourdeau, Roux, Deschênes-Simard, & Ferbeyre, 2009) show that oncogenes can induce an increase in mitochondrial mass, mtDNA, and ROS production prior induction of OIS and mitochondrial dysfunction. By using an oncogenic allele of Ha-Ras, they studied the role of mitochondria in OIS and suggested that mitochondrial dysfunction is involved in effector mechanisms acting downstream of p53 and Rb in senescence. Furthermore, pharmacological inhibition of the electron-transport chains (ETC) can lead to premature senescence. For example, inhibition of the ETC complexes I, II or III by rotenone, 2-thenoyltrifluoroacetone or antimycin A respectively activates senescence (Ziegler et al., 2015). Disrupting the action of the ETCs leads to depletion of ATP and increased AMP (ADP)/ATP ratio. Increased AMP to ATP ratio creates

a bioenergetic imbalance in the cell and activates AMP-activated protein kinase (AMPK). Activation of AMPK induces senescence, shown in multiple model systems (Ziegler et al., 2015). Additionally, chronic activation of AMPK increases p53 expression and phosphorylation and thus promotes p53-dependent senescence.

SASP

Recently, Wiley et al. (Wiley et al., 2016) reported for induction of cellular senescence driven by mitochondrial dysfunction. What is particularly interesting about their finding is that these cells have altered SASP that is lacking IL-6, IL-8 and IL-1β, which are important components of the SASP in senescent cells induced by DNA damage. This state was called mitochondrial dysfunction-associated senescence (MiDAS) because of its different phenotype. Furthermore, the cell-cycle arrest was caused by an increase in the NAD^+/NADH ratio, which activated AMPK. As already mentioned AMPK phosphorylates p53 and promotes senescence. Here the group identified a mechanism independent of NF-κB where p53 limits the SASP through downregulation of IL-1β and loss of IL-1-dependent inflammatory arm. In addition, senescent cells with defective mitochondria express other factors such as IL10, TNFα and CCL27. The paracrine effects of MiDAS have been shown to promote keratinocyte instead of preadipocyte differentiation. These phenotypes were observed in cells that lack the mitochondrial SIR-proteins SIR3 and SIR5, and also in cells treated with ETC inhibitors. Moreover, increase of senescent cells with MiDAS was observed in mice with mutant mtDNA polymerase with defective proofreading activity. Since, increase of mitochondria defects appears with ageing, the authors propose that MiDAS might have a role in age-dependent formation of senescent cells. However, if MiDAS appears in normal ageing has to be confirmed in future studies.

Apoptosis resistance

One of the hallmarks of senescence is apoptosis resistance. It has been previously reported that senescent cells are resistant to stress-induced apoptosis, caused by stimuli such as hydrogen peroxide, UV, staurosporine and thapsigargin (Ryu, Oh, & Park, 2007). Furthermore, it has been shown that senescent human diploid fibroblasts (HDF) have a twofold increase in Bcl-2, compared to their young counterparts (Wang, 1995). Bcl-2 is an apoptosis inhibitor, which significantly decreases when young proliferating HDFs are exposed to different pro-apoptotic stress conditions. However, in senescent cells a failure to downregulate the expression of Bcl-2 is a potential cause for resistance to stress-induced apoptosis. Additionally, apoptosis is significantly enhanced in senescent HDFs with a Bcl-2 conditional knockout (Ryu et al., 2007).

Mitochondria are important regulators and inducers of apoptosis and a decrease in the mitochondrial membrane potential ($\Delta\Psi_m$) has been previously shown to be an early apoptotic event directly modulated by Bcl-2 and Bax proteins. Indeed, this is the case in young HDFs, exposed to pro-apoptotic signals. However, in senescent HDFs, decrease in $\Delta\Psi_m$ as well as downregulation of Bcl-2 have not been observed (Ryu et al., 2007). In addition, apoptosis resistance is dependent on the cell type and the nature and intensity of the stress. One example is the activation of apoptosis in epithelial cells after exposure to the compound ceramide, while senescent human fibroblasts are insensitive to it (Campisi & d'Adda di Fagagna, 2007).

Concluding remarks

In this essay, I reviewed the most prominent features of senescent cells and the molecular pathways that regulate their function. In addition, I discussed recent findings in the field, implicating mitochondria and the mTORC1 complex in the establishment and maintenance of the senescent state and its secretory phenotype. As we already saw, mitochondria are active participants in the regulation of senescence and SASP.

Mitochondrial dysfunction is shown to increase with ageing and is a cause for multiple age-dependent disorders in post-mitotic tissues (Green, Galluzzi, & Kroemer, 2011). It is generally accepted that mitochondria have a central role in ageing by being both contributors and targets of the ageing process. Recent findings point at the important role of mitochondria in the establishment and maintenance of senescence (Correia-Melo et al., 2016; Wiley et al., 2016). In addition, the process of senescence is restricted to proliferative tissues and the number of senescent cells increases with ageing (Shawi & Autexier, 2008), which leads to tissue deterioration. Therefore, we can speculate that mitochondrial dysfunction is involved in age-dependent deterioration of both differentiated and proliferative tissues.

Recent study (Correia-Melo et al., 2016) indicated that increase of the mitochondrial mass in senescent cells is a downstream effect of the action of the mTORC1 complex, which stimulates mitochondrial biogenesis through the activation of PGC-1β. Furthermore, removal of mitochondria or chemical inhibition of ATM or mTORC1 abrogated SASP and "reversed" the senescent phenotype of senescent human fibroblasts, without re-initiating the cell cycle. In addition, Wiley and colleagues (Wiley et al., 2016) showed that dysfunctional mitochondria can induce senescence with a distinct SASP, which lacks the IL-1-dependent inflammatory arm and expresses other factors such as IL10, TNFα and CCL27. Three other studies reported for the regulatory role of mTORC1 in SASP expression and senescence activation (Herranz et al., 2015; R.-M. Laberge et al., 2015; Nacarelli et al., 2016). These findings establish a molecular network of the interplay between mTORC1, mitochondrial damage and ROS production in the induction of senescence and regulation of the SASP (Fig.1).

According to the present literature, mitochondria are just one of the downstream targets of mTORC1. There are other mTORC1 molecular targets that regulate SASP independently of the mitochondrial function. Therefore, it is an interesting observation that senescent cells subjected to mitochondrial clearance have very similar phenotype to that of senescent cells treated with rapamycin. This puts forward the question: Are mitochondria simply a target of mTORC1 or are they involved as a causal modulator of mTORC1 activity? It is possible that there is a feedback loop where mTORC1 and mitochondria actively communicate and regulate the state and the secretory phenotype of senescent cells. Further studies are necessary to clarify this connection.

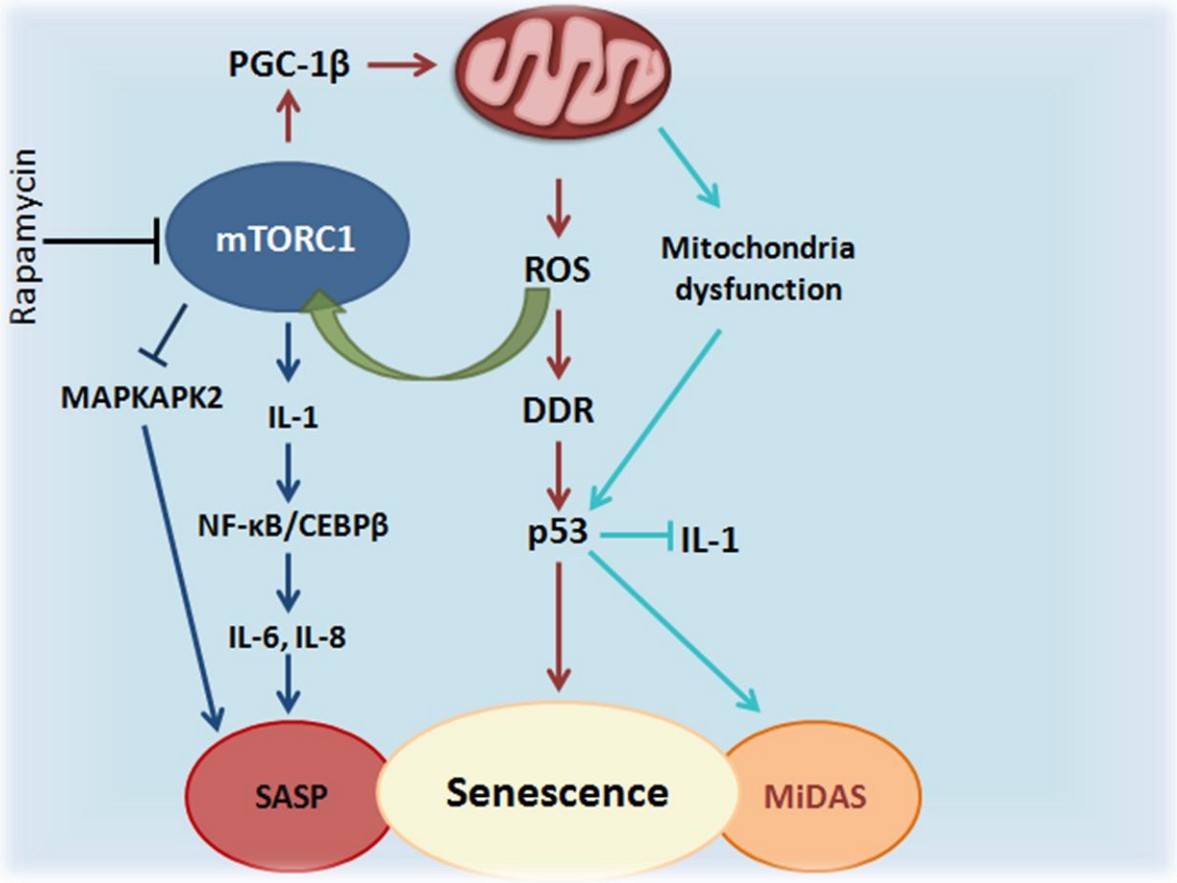

Figure 1 Graphical representation of identified interacting molecular pathways between mitochondria and mTORC1. Different colors of the arrows indicate different pathways. MTORC1 induces the expression of IL-1and thus positively regulates other SASP components through the activation of NF-κB and CEBPβ (dark blue arrows). MTORC1 can be inhibited with rapamycin, which abrogates the SASP. Additionally, mTORC1 activates PGC-1β (red arrows) and thus boosts mitochondria biogenesis and increases total mitochondrial mass. This leads to overproduction of ROS, which can activate DDR (red arrows) and also serves as an input for mTORC1 activation (green arrow). Furthermore, mitochondrial dysfunction activates senescence through the p53 pathway (blue arrows). P53 inhibits IL-1-dependent inflammatory arm, thus leading to mitochondrial dysfunction-activated senescence with a distinct secretory phenotype, called MiDAS (blue arrows).

Used terms

Replicative senescence

Replicative senescence, or also known as the Hayflick limit, is the inability of cells to divide indefinitely. This phenomenon is caused by the telomere shortening, which appears with each replication cycle. In this case, senescent cells are old cells that reached the end of their replicative capacity.

Oncogene-induced senescence

Oncogenes are the mutant forms of normal genes and have the ability to transform cells in combination with other mutations. Overexpression of oncogenes can induce senescence in

order to preserve the cell from excessive mitogenic stimulation that can lead to oncogenic transformation.

Premature senescence

Premature senescence is stress induced senescence that has some phenotypic differences with the replicative senescence. Unlike replicative senescence, premature senescence appears independently of the replicative capacity of the cells and can be induced because of the presence of irreparable damage.

References

Acosta, J. C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J. P., … Gil, J. (2013). A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nature Cell Biology*, *15*(8), 978–90. http://doi.org/10.1038/ncb2784

Blander, G., De Oliveira, R. M., Conboy, C. M., Haigis, M., & Guarente, L. (2003). Superoxide dismutase 1 knock-down induces senescence in human fibroblasts. *Journal of Biological Chemistry*, *278*(40), 38966–38969. http://doi.org/10.1074/jbc.M307146200

Brookes, S., Gagrica, S., Sanij, E., Rowe, J., Gregory, F. J., Hara, E., & Peters, G. (2015). Evidence for a cdk4-dependent checkpoint in a conditional model of cellular senescence. *Cell Cycle*, *14*(8), 1164–1173. http://doi.org/10.1080/15384101.2015.1010866

Brown, G. C., & Borutaite, V. (2012). There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells. *Mitochondrion*, *12*(1), 1–4. http://doi.org/10.1016/j.mito.2011.02.001

Burton, D. G. A., Matsubara, H., & Ikeda, K. (2010). Pathophysiology of vascular calcification: Pivotal role of cellular senescence in vascular smooth muscle cells. *Experimental Gerontology*, *45*(11), 819–824. http://doi.org/10.1016/j.exger.2010.07.005

Campisi, J. (2013). Aging, cellular senescence, and cancer. *Annual Review of Physiology*, *75*, 685–705. http://doi.org/10.1146/annurev-physiol-030212-183653

Campisi, J., Andersen, J. K., Kapahi, P., & Melov, S. (2011). Cellular senescence: A link between cancer and age-related degenerative disease? *Seminars in Cancer Biology*, *21*(6), 354–359. http://doi.org/10.1016/j.semcancer.2011.09.001

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

Carlos Lopez-Otın, Maria A. Blasco, Linda Partridge, Manuel Serrano, and G. K. (2013). The hallmarks of aging. *Cell*, *153*(6). http://doi.org/10.1016/j.cell.2013.05.039

Coppé Jean-Philippe , Pierre-Yves Desprez, Ana Krtolica, and J. C. (2010). The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu Rev Pathol.*, 99–118. http://doi.org/10.1146/annurev-pathol-121808-102144.The

Coppé, J.-P., Patil, C. K., Rodier, F., Sun, Y., Muñoz, D. P., Goldstein, J., … Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biology*, *6*(12), 2853–68. http://doi.org/10.1371/journal.pbio.0060301

Correia- Melo, C., Marques, F. D., Anderson, R., Hewitt, G., Hewitt, R., Cole, J., … Passos, J. F. (2016). Mitochondria are required for pro- ageing features of the senescent phenotype. *The EMBO Journal*, e201592862. http://doi.org/10.15252/embj.201592862

Correia-Melo, C., & Passos, J. F. (2015). Mitochondria: Are they causal players in cellular senescence? *Biochimica et Biophysica Acta - Bioenergetics*, *1847*(11), 1373–1379.

http://doi.org/10.1016/j.bbabio.2015.05.017

Demaria, M., Ohtani, N., Youssef, S., Rodier, F., Toussaint, W., Mitchell, J., … Campisi, J. (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Developmental Cell*, *31*(6), 722–733. http://doi.org/10.1016/j.devcel.2014.11.012

Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., … d'Adda di Fagagna, F. (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature*, *444*(7119), 638–642. http://doi.org/10.1038/nature05327

Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov, J. M., … d'Adda di Fagagna, F. (2012). Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. *Nature Cell Biology*, *14*(4), 355–65. http://doi.org/10.1038/ncb2466

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., & Gage, F. H. (2010). Mechanisms Underlying Inflammation in Neurodegeneration. *Cell*, *140*(6), 918–934. http://doi.org/10.1016/j.cell.2010.02.016

Gorenne, I., Kavurma, M., Scott, S., & Bennett, M. (2006). Vascular smooth muscle cell senescence in atherosclerosis. *Cardiovascular Research*, *72*(1), 9–17. http://doi.org/10.1016/j.cardiores.2006.06.004

Green, D. R., Galluzzi, L., & Kroemer, G. (2011). Mitochondria and the Autophagy–Inflammation–Cell Death Axis in Organismal Aging, (August), 1109–1112. http://doi.org/10.1016/S1095-668X(02)00387-1

Gunter, T. E., Yule, D. I., Gunter, K. K., Eliseev, R. A., & Salter, J. D. (2004). Calcium and mitochondria. *FEBS Letters*, *567*(1), 96–102. http://doi.org/10.1016/j.febslet.2004.03.071

Hampel, B., Malisan, F., Niederegger, H., Testi, R., & Jansen-Durr, P. (2004). Differential regulation of apoptotic cell death in senescent human cells. *Experimental Gerontology*, *39*(11-12 SPEC. ISS.), 1713–1721. http://doi.org/10.1016/j.exger.2004.05.010

Harman, D. (1992). Free radical theory of aging. *Mutation Research/DNAging*, *275*(3-6), 257–266. http://doi.org/10.1016/0921-8734(92)90030-S

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

Herranz, N., Gallage, S., Mellone, M., Wuestefeld, T., Klotz, S., Hanley, C. J., … Gil, J. (2015). mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nature Cell Biology*, *17*(9), 1205–17. http://doi.org/10.1038/ncb3225

Jun, J.-I., & Lau, L. F. (2010). The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nature Cell Biology*, *12*(7), 676–85. http://doi.org/10.1038/ncb2070

Krishnamurthy, J., Torrice, C., Ramsey, M. R., Kovalev, G. I., Al-regaiey, K., Su, L., & Sharpless, N. E. (2004). Ink4a/Arf. *Journal of Clinical Investigation*, *114*(9), 1299–1307.

http://doi.org/10.1172/JCI200422475.The

Kuilman, T., Michaloglou, C., Mooi, W. J., & Peeper, D. S. (2010). The essence of senescence. *Genes and Development*, *24*(22), 2463–2479. http://doi.org/10.1101/gad.1971610

Kuilman, T., Michaloglou, C., Vredeveld, L. C. W., Douma, S., van Doorn, R., Desmet, C. J., … Peeper, D. S. (2008). Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network. *Cell*, *133*(6), 1019–1031. http://doi.org/10.1016/j.cell.2008.03.039

Kuilman, T., & Peeper, D. S. (2009). SMS-ing cellular stress, *9*(FEBRUARy). http://doi.org/10.1038/nrc2560

Laberge, R. M., Awad, P., Campisi, J., & Desprez, P. Y. (2012). Epithelial-mesenchymal transition induced by senescent fibroblasts. *Cancer Microenvironment*, *5*(1), 39–44. http://doi.org/10.1007/s12307-011-0069-4

Laberge, R.-M., Sun, Y., Orjalo, A. V, Patil, C. K., Freund, A., Zhou, L., … Campisi, J. (2015). MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nature Cell Biology*, *17*(8), 1049–1061. http://doi.org/10.1038/ncb3195

Lee, B. Y., Han, J. A., Im, J. S., Morrone, A., Johung, K., Goodwin, E. C., … Hwang, E. S. (2006). Senescence-associated β-galactosidase is lysosomal β-galactosidase. *Aging Cell*, *5*(2), 187–195. http://doi.org/10.1111/j.1474-9726.2006.00199.x

Mantovani, A., Locati, M., Vecchi, A., Sozzani, S., & Allavena, P. (2001). Decoy receptors: A strategy to regulate inflammatory cytokines and chemokines. *Trends in Immunology*, *22*(6), 328–336. http://doi.org/10.1016/S1471-4906(01)01941-X

Moiseeva, O., Bourdeau, V., Roux, A., Deschênes-Simard, X., & Ferbeyre, G. (2009). Mitochondrial dysfunction contributes to oncogene-induced senescence. *Molecular and Cellular Biology*, *29*(16), 4495–4507. http://doi.org/10.1128/MCB.01868-08

Nacarelli, T., Azar, A., & Sell, C. (2016). Mitochondrial stress induces cellular senescence in an mTORC1-dependent manner. *Free Radical Biology and Medicine*, *95*, 133–154. http://doi.org/10.1016/j.freeradbiomed.2016.03.008

Narita, M., N??nez, S., Heard, E., Narita, M., Lin, A. W., Hearn, S. A., … Lowe, S. W. (2003). Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*, *113*(6), 703–716. http://doi.org/10.1016/S0092-8674(03)00401-X

Pacome Lecot, Fatouma Alimirah, Pierre-Yves Desprez, Judith Campisi, and C. W. (2016). Context-dependent effects of cellular senescence in cancer development - bjc2016115a.pdfPacome Lecot, Fatouma Alimirah, Pierre-Yves Desprez, Judith Campisi, and C. W. (2016). Context-dependent effects of cellular senescence in cancer development - bjc2016. Retrieved May 12, 2016, from http://www.nature.com/bjc/journal/vaop/ncurrent/pdf/bjc2016115a.pdf

Passos, J. F., Nelson, G., Wang, C., Richter, T., Simillion, C., Proctor, C. J., … von Zglinicki, T. (2010). Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Molecular Systems Biology*, *6*(347), 347. http://doi.org/10.1038/msb.2010.5

Passos, J. F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., … von Zglinicki, T. (2007). Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence. *PLoS Biology*, *5*(5), e110. http://doi.org/10.1371/journal.pbio.0050110

Passos, J. F., & Von Zglinicki, T. (2006). Oxygen free radicals in cell senescence: are they signal transducers? *Free Radical Research*, *40*(December), 1277–1283. http://doi.org/10.1080/10715760600917151

Rai, P., Onder, T. T., Young, J. J., McFaline, J. L., Pang, B., Dedon, P. C., & Weinberg, R. A. (2009). Continuous elimination of oxidized nucleotides is necessary to prevent rapid onset of cellular senescence. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(1), 169–174. http://doi.org/10.1073/pnas.0809834106

Rodier, F., Coppé, J.-P., Patil, C. K., Hoeijmakers, W. A. M., Muñoz, D. P., Raza, S. R., … Campisi, J. (2009). Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nature Cell Biology*, *11*(8), 973–9. http://doi.org/10.1038/ncb1909

Ryu, S. J., Oh, Y. S., & Park, S. C. (2007). Failure of stress-induced downregulation of Bcl-2 contributes to apoptosis resistance in senescent human diploid fibroblasts. *Cell Death and Differentiation*, *14*(5), 1020–8. http://doi.org/10.1038/sj.cdd.4402091

Salama, R., Sadaie, M., Hoare, M., & Narita, M. (2014). Cellular senescence and its effector programs, 99–114. http://doi.org/10.1101/gad.235184.113

Salminen, A., Ojala, J., Kaarniranta, K., Haapasalo, A., Hiltunen, M., & Soininen, H. (2011). Astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype. *European Journal of Neuroscience*, *34*(1), 3–11. http://doi.org/10.1111/j.1460-9568.2011.07738.x

Seluanov, A., Gorbunova, V., Falcovitz, A., Milyavsky, M., Zurer, I., Shohat, G., … Rotter, V. (2001). Change of the Death Pathway in Senescent Human Fibroblasts in Response to DNA Damage Is Caused by an Inability To Change of the Death Pathway in Senescent Human Fibroblasts in Response to DNA Damage Is Caused by an Inability To Stabilize p53, *21*(5), 1552–1564. http://doi.org/10.1128/MCB.21.5.1552

Serrano, M. (2015). The InflammTORy Powers of Senescence. *Trends in Cell Biology*, *25*(11), 635–636. http://doi.org/10.1016/j.tcb.2015.09.011

Shawi, M., & Autexier, C. (2008). Telomerase, senescence and ageing. *Mechanisms of Ageing and Development*, *129*(1-2), 3–10. http://doi.org/10.1016/j.mad.2007.11.007

Von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, *27*(7), 339–344. http://doi.org/10.1016/S0968-0004(02)02110-2

Wang, E. (1995). Senescent Human Fibroblasts Resist Programmed Cell Death , and Failure to Suppress bcl 2 Is Involved Senescent Human Fibroblasts Resist Programmed Cell Death , and Failure to Suppress bctl Is Involved1. *Cancer Research*, 2284–2292.

Wiel, C., Lallet-Daher, H., Gitenay, D., Gras, B., Le Calvé, B., Augert, A., … Bernard, D. (2014). Endoplasmic reticulum calcium release through ITPR2 channels leads to mitochondrial calcium accumulation and senescence. *Nature Communications*, *5*(May), 3792–3793. http://doi.org/10.1038/ncomms4792

Wiley, C. D., Velarde, M. C., Lecot, P., Liu, S., Sarnoski, E. A., Freund, A., … Campisi, J. (2016). Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. *Cell Metabolism*, *23*(2), 303–314. http://doi.org/10.1016/j.cmet.2015.11.011

Ziegler, D. V., Wiley, C. D., & Velarde, M. C. (2015). Mitochondrial effectors of cellular senescence: Beyond the free radical theory of aging. *Aging Cell*, *14*(1), 1–7. http://doi.org/10.1111/acel.12287