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Oxaloacetate decarboxylase FAHD1 – a new regulator of mitochondrial function and senescence

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ABSTRACT

FAHD1, a member of the FAH superfamily of enzymes, was identified in a proteomic screen for mitochondrial proteins with differential expression in young *versus* senescent human endothelial cells. FAHD1 acts as oxaloacetate decarboxylase, and recent observations suggest that FAHD1 plays an important role in regulating mitochondrial function. Thus, mutation of the nematode homolog, *fahd-1*, impairs mitochondrial function in *Caenorhabditis elegans*. When FAHD1 gene expression was silenced in human cells, activity of the mitochondrial electron transport (ETC) system was reduced and the cells entered premature senescence-like growth arrest. These findings suggest a model where FAHD1 regulates mitochondrial function and in consequence senescence. These findings are discussed here in the context of a new concept where senescence is divided into deep senescence and less severe forms of senescence. We propose that genetic inactivation of FAHD1 in human cells induces a specific form of cellular senescence, which we term *senescence light* and discuss it in the context of mitochondrial dysfunction associated senescence (MiDAS) described by others. Together these findings suggest the existence of a continuum of cellular senescence phenotypes, which may be at least in part reversible.

Ageing is a standard feature of biological organisms, which is pronounced by a progressive functional impairment of multiple cells and tissues (Tosato et al., 2007). Degenerative diseases, as well as cancer occur during ageing, establishing a challenge to our society. Increasing evidence suggests a central role for metabolic regulation in the control of ageing and lifespan (Trifunovic et al., 2004; Dillin et al., 2002; Schloesser et al., 2015; Herker et al., 2004; Hütter et al., 2007; Kozieł et al., 2014; Fabrizio and Longo, 2007; Wiley et al., 2016; Sohal and Orr, 2012). Several aspects of mitochondrial metabolism, such as energy production, accumulation of reactive oxygen species (ROS), and fatty acid metabolism were identified as critical processes of lifespan regulation. However, molecular mechanisms and the impact on ageing of individual mitochondrial proteins are not fully understood (Dillin et al., 2002; Schloesser et al., 2015; Kozieł et al., 2014; Sohal and Orr, 2012).

1. Cellular senescence as a tool to study ageing

Ageing in mammals (and probably other vertebrates) is linked to an accumulation of senescent cells with age, which is associated with hyperplastic, as well as degenerative age-related pathologies (Campisi, 2013). The phenomenon of cellular senescence was firstly described by Hayflick in 1965, and proposed to represent a common process that links ageing, cancer, and degeneration. He described that human diploid fibroblasts (HDFs) have a limited replicative capacity in culture (Hayflick, 1965) (now referred to as the “Hayflick limit”), and thereby established the concept and category of replicative cellular senescence (Hayflick, 1965; Baker and Sedivy, 2013). A general hallmark of cellular senescence is the long-term exit from the cell cycle, initiated for example through the expression of cell cycle inhibitors (Campisi, 2005). Aside of an altered morphology, which is enlarged and flattened, cells show a specific expression pattern of genes coding for cell-cycle inhibitors and/or activators (Zhang et al., 2003; Mason et al., 2004; Trougakos et al., 2006; Jackson and Pereira-Smith, 2006). The

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investigation of metabolic and other changes in senescent cells of human origin has contributed significantly to better understanding of ageing in humans.

1.1. Triggers of cell senescence

A first molecular explanation for the limited replicative potential of human diploid cells in cell culture was telomere shortening (telomere-initiated cellular senescence) (Harley et al., 1990). However, besides the natural phenotype described by Hayflick (Hayflick, 1965), it is now well understood that in addition to telomere shortening, many extrinsic stressors may induce senescence-like phenotypes, referred to as stress-induced premature senescence (SIPS) and oncogene-induced senescence (OIS), respectively. Replicative senescence and oncogene-induced senescence are known to act as efficient tumour suppressor mechanisms, limiting the growth of tumour cells under certain conditions (Liu et al., 2018; Schosserer et al., 2017). DNA damage is known to contribute to senescence-associated growth arrest in SIPS, replicative senescence (in the form of telomeric DNA damage), and OIS (DNA damage because of replication stress) (Robles and Adami, 1998; Sedelnikova et al., 2004; Wang et al., 2009). In addition, there are other types of senescence that occur in the absence of DNA damage (Wiley et al., 2016; Zwerschke et al., 2003; Muñoz-Espín et al., 2013; Stöckl et al., 2006), which will be discussed in detail below. In case of substantial DNA damage, DNA replication in senescent cells cannot be initiated anymore, detectable by the lack of bromodeoxyuridine (BrdU) and/or ^{3}H-thymidine incorporation. Two major pathways can independently give rise to growth arrest in cellular senescence: the p16^{INK4a}/pRB and p53/p21 pathways (Campisi and d'Adda di Fagagna, 2007; Campisi, 2001). The p53 pathway (Sherr and McCormick, 2002) is usually triggered by a DNA damage response (DDR) (Fig. 1). DDR can be caused by many insults, including telomere dysfunction, ultraviolet or ionizing radiation, DNA damaging chemicals used for cancer therapy (therapy-induced senescence, TIS) (Gonzalez et al., 2016), oxidative stress, or the expression of certain oncogenes. Unrepaired DNA damage is associated with the expression of γ-H2AX (a phosphorylated form of histone variant H2AX) and the DDR proteins NBS1, MDC1, and 53BP1 involved in DNA repair processes. Alternatively, p53 can be stabilized by the tumour suppressor protein p14^{ARF} ("alternative reading frame", a second gene product encoded by the INK4 locus also coding for p16^{INK4a}), which inactivates the E3 ubiquitin-protein ligase HDM2 thereby preventing proteasome-dependent degradation of p53 (Espinosa et al., 2003) (Fig. 1).

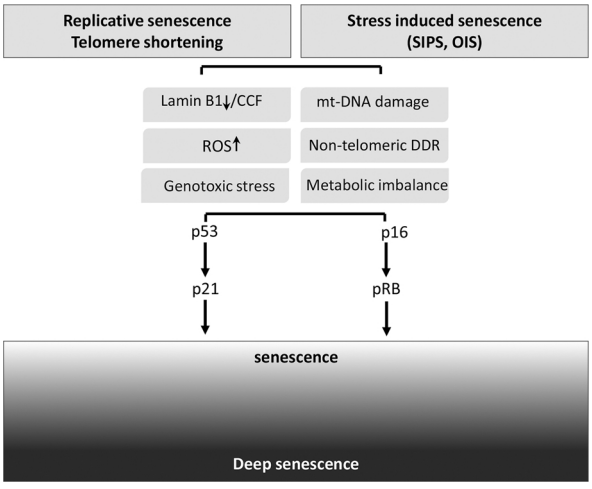

Fig. 1. Hallmarks of replicative senescence and stress-induced senescence. Replicative senescence (RS), stress-induced premature senescence (SIPS) and oncogene-induced senescence (OIS) are triggered by quite diverse stimuli, all of which induce substantial DNA damage, either at telomeres (RS) or at random sites in the genomic DNA (SIPS, OIS). In response to damage to nuclear DNA, elevated ROS production and mitochondrial DNA damage are triggered which act in a positive feedback loop (Passos et al., 2010), along with the appearance of metabolic imbalance. Furthermore, the expression of lamin B is reduced, causing damage to the nuclear envelope with subsequent release of cytosolic chromatin fragments (CCF). At the molecular level, a DNA damage response (DDR) is mounted, involving several kinases which lead to phosphorylation of p53 and activation of p53 target genes such as p21. Subsequently, expression of p16 is activated and the p53/p21 pathway collaborates with the p16/pRb pathway to trigger irreversible growth arrest. In RS and OIS, further passaging of p16-positive cultures can lead to "deep senescence".

1.2. Senescence outcomes

The identification of explicit molecular markers of cellular senescence is challenging. Despite several identified senescence markers, there is no marker which would exclusively recognize the senescent state. A commonly used and relatively specific marker to identify senescent cells is senescence-associated β-galactosidase (SA-βgal) (Dimri et al., 1995; Biran et al., 2017). Its activity reflects increased lysosomal biogenesis, that can be detected by histochemical staining dependent on the pH (Dimri et al., 1995; Lee et al., 2006). A crucial feature of many senescent cells is the senescence-associated secretory phenotype (SASP) (Malaquin et al., 2016; Coppé et al., 2008), which is a complex pro-inflammatory response in which senescent cells secrete a large number of cytokines, growth factors, and proteases (Braig et al., 2005; Chen et al., 2005). Powerful paracrine activities are associated with components of SASP to support tissue repair, and to either inhibit or promote the development of cancer (Campisi, 2013; Muñoz-Espín and Serrano, 2014). Cytoplasmic chromatin fragments (CCF), which leak out from the nucleus due to changes in the nuclear envelope, have been found in senescent cells and were shown to support sustained activation of the SASP (Ivanov et al., 2013; Dou et al., 2017), involving the activation of the cGAS/STING pathway (Li and Chen, 2018).

Several studies demonstrated that senescent cells positive for p16^{INK4a} accumulate with age in multiple tissues (Campisi, 2013; Robles and Adami, 1998). The mechanisms by which p16^{INK4a} gene expression is upregulated in senescent cells is largely unexplored, but probably involves repression of the p16 promoter by pRB in combination with the transcriptional repressor Bmi1 (Ressler et al., 2006). In healthy young cells the expression of p16^{INK4a} is undetectable or low, whereas in senescent cells its expression is detectable (Campisi, 2013; Baker et al., 2016a; Romagosa et al., 2011; Lin et al., 1998; Krishnamurthy et al., 2006; Janzen et al., 2006; Molofsky et al., 2006). Clearance of p16^{INK4a} expressing cells was associated with a delay in age related pathologies in mice (Baker et al., 2016a,b), suggesting that senescent cells contribute actively to tissue ageing. In replicative senescence and in several types of SIPS, activation of the p16-pRB pathway occurs usually secondary to occupation of the p53 pathway (Stein et al., 1999; Jacobs and de Lange, 2004) (Fig. 1). However, the same stimuli producing a DDR which can trigger the p53 pathway can also engage the p16-pRB pathway (Fig. 1). In this scenario, the CDK inhibitor p16 keeps pRB hypo-phosphorylated and active. Subsequently the transcription factor E2F is prevented from transcribing genes necessary for proliferation. On the other hand, E2F can also limit cell cycle progression by inducing p14^{ARF} gene expression, which in turn activates the p53 pathway. Thus, the p53 and the pRB pathway can also be regulated interactively (Campisi and d'Adda di Fagagna, 2007; Sherr and McCormick, 2002), orchestrating the entry into cellular senescence (Fig. 1).

Additional studies have shown decreased hetero-chromatinization in senescence, in particular replicative senescence. This is particularly predominant in senescent cells that were further cultivated for extended periods of time once growth arrest was established. Such cells are non-dividing but highly metabolically active and enter into a distinct, later stage of senescence often referred to as "deep senescence" (Baker and Sedivy, 2013). Chromatin changes typically occur in silenced areas of

the genome, which contain retro-transposable elements (RTE) the expression of which is accelerated in deep senescence. In young and healthy cells, expression of RTEs is strongly restrained at the chromatin level; upon activation they can destabilize the host genome. If such changes would occur *in vivo* as well, they may further contribute to age-associated tissue degeneration and pathology (Sedivy et al., 2013).

2. The role of mitochondria in ageing and cellular senescence

According to the mitochondrial theory of ageing (Sohal and Orr, 2012; Wei et al., 2001; Sanz and Stefanatos, 2008), the process of ageing is associated with the accumulation of molecular damage caused by mitochondrial ROS, generated as products of the mitochondrial electron transport chain (ETC). In general, increased oxidative damage and decreased oxidative capacity are characteristics of age-associated mitochondrial dysfunction (Cui et al., 2012). With age, mitochondria lose their respiratory activity, produce increasing levels of ROS (Chistiakov et al., 2014), and accumulate damaged mitochondrial DNA (mtDNA) (Arnheim and Cortopassi, 1992). The theory also predicts that a dysfunctional ETC will increase mtDNA mutations, which in turn, will further impair ETC function (Miquel, 1991; Berlett and Stadtman, 1997; Beckman and Ames, 1998). In a mouse model with proof-reading deficient mitochondrial DNA polymerase (referred to as mutator mice), it was shown that a dysfunctional ETC is mainly responsible for premature ageing in such mice (Trifunovic et al., 2005), showing that impaired ETC leads to an increased sensitivity to oxidative stress, inducing cell death (Trifunovic et al., 2005). In humans, mitochondrial dysfunction is a hallmark of skin ageing, which has been correlated with reduced proteasome function in the elderly skin (Kozieł et al., 2011). It has been shown that in samples from aged human donors, mitochondrial membrane potential was decreased, and ROS levels were increased (Kozieł et al., 2011) with respect to samples from young donors. The excessive generation of ROS is one of the major consequences of mitochondrial dysfunction, and is believed to be a major driving force behind ageing in general (Fig. 1). Accordingly, increased resistance to oxidative stress was found to correlate with longevity in different model organisms and long-lived mutants (Finkel and Holbrook, 2000).

Beyond their role in ROS production, mitochondria are implicated in the modulation of ageing and lifespan in many species, where they play an essential role in many cellular processes, such as energy metabolism, cell signalling, cell differentiation, cell death and cellular senescence. Several aspects of mitochondrial metabolism contribute to the modulation of ageing and longevity in model organisms including mammals (López-Otín et al., 2013) and contribute to age-related diseases, including cancer. Moreover, chronic obstructive pulmonary disease, diabetes, intestinal barrier dysfunction, osteoporosis, and vascular disease (atherosclerosis), are believed to be consequences of defects in mitochondrial function (Boland et al., 2013; Ahmad et al., 2015; Montgomery and Turner, 2015; Chen et al., 2008; Lane et al., 2015). In addition, downregulation of mitochondrial oxidative phosphorylation proteins, accompanied by mitochondrial dysfunction, has been identified in the rare segmental premature ageing disorder of Hutchinson-Gilford progeria syndrome (HGPS) (Rivera-Torres et al., 2013), suggesting a direct link between mitochondrial function and healthy ageing in humans.

2.1. Mitochondrial quality control in age-related neurodegeneration

Mitochondrial dysfunction is also involved in neurodevelopmental (such as Autism, Schizophrenia) (Rossignol and Frye, 2014; Manji et al., 2012) and neurodegenerative (Huntington's, Parkinson's, and Alzheimer's) diseases (Lane et al., 2015; Lustbader et al., 2004; Gorman et al., 2016). Quality control systems, such as degradation of intracellular components (autophagy) and especially the selective autophagic degradation of mitochondria (mitophagy) affect neuronal homeostasis (Martinez-Vicente, 2017) and are believed to deteriorate during ageing. Mitophagy, which can be in turn further impaired by oxidative damage and cellular energy deficits (Kerr et al., 2017), represents a critical process required to maintain cellular function, by maintaining mitochondrial quality control and homeostasis. Defective mitochondria have been associated with ageing and age-related diseases, and are eliminated selectively by functional mitophagy (Um and Yun, 2017). Together with mitophagy, active mitochondrial dynamics (i.e. shifting between fusion and fission actions), is required to maintain a healthy mitochondrial network (van der Bliek et al., 2013), which is also required for healthy ageing (Zemirli et al., 2018). Bioenergetics deficits, decreased mitochondrial membrane potential, and increased ROS production are other impacts contributing to age-related neurodegenerative disorders (Lane et al., 2015; Fang et al., 2017). However, mechanistic links between mitochondrial dysfunction and neurodegeneration remain still largely elusive.

2.2. Mitochondrial damage in cellular senescence

Senescence can be driven by ROS contributing to a sustained DNA damage response, thus leading to a steady growth arrest (Hewitt et al., 2012; Passos et al., 2010). In this model, ROS, derived from oxidative phosphorylation, induce cumulative oxidative damage to proteins, lipids, and nucleic acids. In support of this hypothesis, a complete depletion of mitochondria by forced mitophagy in human cells rendered these cells unresponsive to several senescence inducing stimuli (Correia-Melo et al., 2016), suggesting that mitochondria are essential players in cellular senescence. Consistent with this notion, mitochondrial damage is postulated as potential regulator of cellular senescence (Trifunovic et al., 2004; Dillin et al., 2002; Lane et al., 2015).

3. Senescence in the absence of DNA damage

The activation of a DDR is widely considered as a cause and hallmark of cellular senescence. DDR is triggered by DNA double strand breaks, which are themselves induced by various extrinsic and intrinsic factors (Bielak-Zmijewska et al., 2018). As discussed above and in Fig. 1, it is known that DNA damage is a central trigger of many cellular senescence types (replicative senescence, telomere dysfunction induced senescence, OIS, SIPS). However, recent data indicate that in some models, senescence can be induced in the absence of DNA damage. Thus, both developmental senescence (Muñoz-Espín et al., 2013), and senescence induced by inhibition of the mitochondrial electron transport chain (Wiley et al., 2016; Stöckl et al., 2006) occur in the absence of detectable DNA damage.

3.1. Developmental senescence

A potential role of cellular senescence in development was suggested by the finding that cellular senescence is involved in the formation of two embryonic structures in the mouse, the endolymphatic sac of the inner ear and the mesonephros (Muñoz-Espín et al., 2013; Storer et al., 2013); in these instances, senescence occurs in the absence of DNA damage. The exact mechanisms underlying developmental senescence are currently unclear; however, it was revealed that p16 expression is not upregulated during developmental senescence, whereas expression of p21 is upregulated independently of p53 and required for the senescent phenotype. It was hypothesized that senescent cells are subsequently eliminated by macrophage infiltration, to support correct tissue remodelling in embryonic tissue of the mouse. Responsible molecular pathways may include TGF β/SMAD and PI3K/FOXO signalling (Muñoz-Espín and Serrano, 2014). The fate (cell cycle re-entry, cell death, clearance by immune cells, others) of senescent cells occurring transiently at defined states during murine embryogenesis is currently unclear, and more studies are warranted to fully appreciate the mechanism underlying this interesting phenomenon.

3.2. Senescence induced by inhibition of the mitochondrial ETC

Senescence induced by inhibition of the mitochondrial ETC (Wiley et al., 2016; Stöckl et al., 2006) represents another type of senescence independent of DNA damage, which was first observed in experiments where human diploid fibroblasts were chronically exposed to low concentrations of the ATP synthase inhibitor oligomycin for two weeks which impaired cell proliferation and induced senescence like growth arrest in a subpopulation (roughly 30%) of the cells; this phenotype was independent of ROS production (Stöckl et al., 2006). Recently a similar phenotype of senescence was observed due to inhibition of ETC complex I (by either SIRT3 knock-down or by application of complex I inhibitor rotenone) and termed "mitochondrial dysfunction-associated senescence" (MiDAS) (Wiley et al., 2016). A reduced spectrum of SASP factors was noted for cells in MiDAS, as compared to replicative senescence of the same cell type. In this case, senescence was most likely induced by energy failure, as documented by a decreased NAD^+/NADH ratio. These findings suggest that a reduced level of oxidative phosphorylation can induce senescence and impair cell proliferation, in the absence of DNA damage.

3.3. Activation of AMPK contributes to senescence-associated growth arrest

A substantial energy deficit in mammalian cells leads to activation of AMP-activated protein kinase (AMPK), a heterotrimeric kinase, which is activated in cells depleted of ATP through phosphorylation of threonine-172 in the α-subunit by serine/threonine kinase 11 (STK11) (Hardie, 2018). Existing data in the literature suggest that the extent of AMPK activation differs between various forms of senescence and may also to some extent influence the outcome of the senescence-inducing stimulus. Early *in vitro* studies of HDF in replicative senescence displayed partial respiratory uncoupling of the mitochondria (Hutter et al., 2004), which was accompanied by decreased levels of NTPs (ATP, GTP, UTP and CTP), as well as highly upregulated AMP levels in senescent fibroblasts (Zwerschke et al., 2003; Wang et al., 2003). These findings provided the first evidence that changes in the cellular energy state can induce senescence. Of note, cells used in these experiments displayed a high level of DDR due to DNA damage signals emanating from eroded telomeres (see, for example, references (Zwerschke et al., 2003; Wang et al., 2003)), indicating that the DDR and AMPK activation are not mutually exclusive; instead they can simultaneously trigger a senescence response, possibly by stabilizing p53 through distinct phosphorylation events. This is supported by the finding that addition of AMP to young HDF in culture was sufficient to induce premature senescence (Zwerschke et al., 2003), most likely by activation of AMPK.

Further insight on this topic comes from experiments where pharmacological inhibition of mitochondrial function was used to impair the proliferation of human cancer cells. As mentioned above, inhibition of ETC complex I in human diploid lung fibroblasts (by complex I inhibitor rotenone) (Wiley et al., 2016; Gui et al., 2016) impaired cell proliferation and induced senescence, accompanied by a decreased NAD^+/NADH ratio and subsequent activation of AMPK (Schloesser et al., 2015; Wiley et al., 2016; Habib et al., 2016; Chanda et al., 2016; Edmunds et al., 2014). In contrast, when ETC complex I was inhibited by metformin in a panel of cancer cell lines, AMPK was not activated (Gui et al., 2016) although the NAD^+/NADH ratio was decreased and cells entered senescence-like growth arrest, which could be rescued by the addition of pyruvate, a key metabolite linking the TCA cycle with glycolysis.

4. FAHD1 as a regulator of mitochondrial function and senescence

To identify new mitochondrial regulators of cellular senescence, we employed an unbiased proteomics approach (Groebe et al., 2007) to identify mitochondrial proteins, the expression of which is altered with cellular senescence. Comparison of the mitochondrial proteome from young and senescent human umbilical vein endothelial cells (HUVEC) in 2D gel electrophoresis (labelled with I^{125} or I^{131}) identified a previously unknown mitochondrial protein that was differentially regulated between young and senescent HUVEC cells (Groebe et al., 2007) (Fig. 2). Subsequently, this protein was identified as fumarylacetoacetate hydrolase (FAH) domain containing protein 1 (FAHD1) (Pircher et al., 2011, 2015; Jansen-Duerr et al., 2016), localized in the mitochondrial matrix (Pircher et al., 2011, 2015; Jansen-Duerr et al., 2016), and eventually identified as eukaryotic oxaloacetate decarboxylase (ODx) (Pircher et al., 2015). Quantitation of the spots revealed no significant difference in absolute FAHD1 expression levels but an age-related difference in isoelectric point (pI) of about 0.4 pI units, suggesting differences in post-translational modification of FAHD1 through cellular senescence.

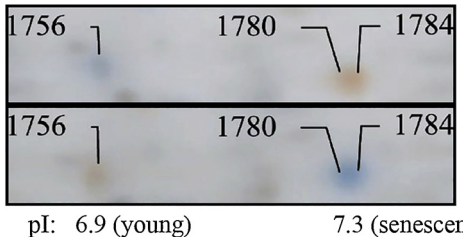

Fig. 2. Identification of FAHD1 as target for senescence-associated post-translational modification.
Representative images of high resolution 2D gels of mitochondrial preparations (10 µg each) from young and senescent HUVEC were prepared using the ProteoTope™ technique (Groebe et al., 2007).
Upper panel: Mitochondria were prepared from young and senescent human umbilical vein endothelial cells (HUVEC) and labelled with radioactive iodine isotopes I^{125} and I^{131}, respectively. 2D gel electrophoresis followed by mass spectrometric analysis of differentially expressed protein spots between young and senescent HUVEC mitochondrial extracts was performed. False colour imaging (I^{125}-labelled proteins presented in blue, I^{131}-labelled proteins in orange) for differential quantification of proteins from two samples in one experiment allowed the identification of two protein spots (#1756 and #1780/1784) as fumarylacetoacetate hydrolase domain containing 1 (FAHD1). Quantitation of the spots revealed no significant difference in absolute FAHD1 expression levels, but an age-related difference in isoelectric point (pI) of about 0.4 pI units, suggesting differences in post-translational modification of FAHD1 with cellular senescence.
Lower panel: The experiment was performed as described for the upper panel, except that mitochondrial proteins from young cells were labelled with I^{131} and mitochondrial proteins from senescent cells were labelled with I^{125} (dye swap (Groebe et al., 2007)). The pattern obtained in this experiment is complementary to the pattern shown in the upper panel, confirming that FAHD1 obtained from senescent cells migrates in IEF according to a pI of 7.3 relative to FAHD1 obtained from young cells (pI = 6.9).

4.1. FAHD1 controls oxaloacetate (OAA) levels in the mitochondrial matrix

The ODx activity, combined with its mitochondrial localization, classifies FAHD1 as a possible antagonist of pyruvate carboxylase (PC), a well-known anaplerotic enzyme in higher organisms (Owen et al., 2002; Marin-Valencia et al., 2010). Deficiency of PC was shown to cause multi-organ metabolic imbalance that predominantly manifests with lactic acidemia and neurological dysfunction at an early age (Marin-Valencia et al., 2010). These findings suggest the existence of a previously unknown level of metabolic regulation in mitochondria. In eukaryotes, two separate pools of OAA exist in the cytosol and the mitochondrial matrix, respectively. Mitochondrial OAA is a metabolite of the tricarboxylic acid (TCA) cycle, a key metabolic pathway enabling the complete oxidation of glucose, fatty acids, amino acids, and other nutrients.

In the TCA cycle, citrate (CIT) is produced by citrate synthase (CS) through the condensation of OAA with acetyl-CoA, a key metabolic

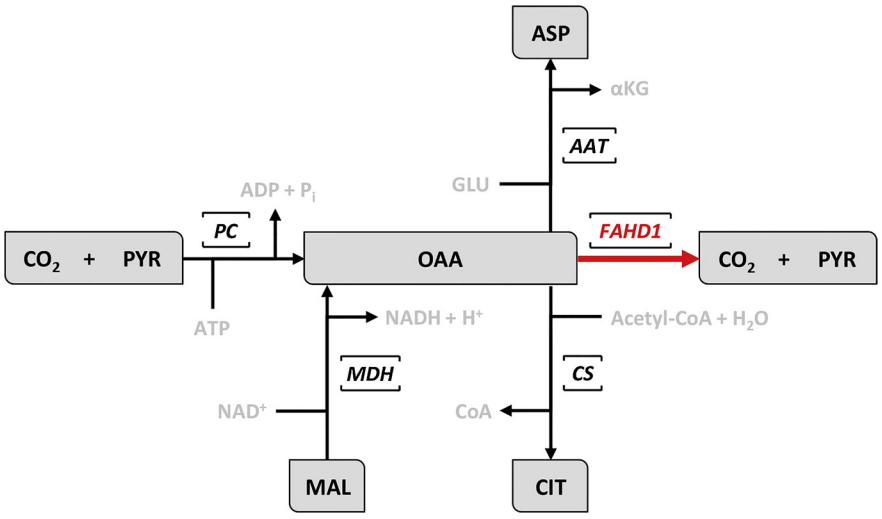

Fig. 3. OAA-producing and consuming processes in the mitochondrial matrix.
OAA is produced by the reactions catalysed by pyruvate carboxylase (PC) and malate dehydrogenase (MDH). OAA is consumed by aspartate amino-transferase (AAT) and citrate synthase (CS). The newly discovered ODx enzyme FAHD1 converts OAA back to PYR, acting antagonistic to the OAA producing reaction catalysed by PC.

intermediate resulting from the oxidation of carbohydrates and fatty acids. Whereas metabolic flux through the TCA cycle is primarily regulated by the rate-limiting enzymes isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, increasing evidence suggests that the concentration of OAA in the mitochondrial matrix is tightly regulated and contributes to flux control in the TCA cycle as well. At a given time-point, the actual concentration of OAA in the mitochondrial matrix is determined by the relative activity of OAA-producing enzymes, including malate dehydrogenase (MDH) and PC *versus* OAA-consuming enzymes, including CS and aspartate aminotransferase (AAT), which transfers an amino group from glutamate (GLU) to OAA, to yield aspartate (ASP) and α-ketoglutarate (α-KG) (Fig. 3). The concentration of both OAA precursors, such as pyruvate (PYR) and malate (MAL), and OAA derived metabolites, such as CIT and ASP, is further modulated by shuttle systems, which regulate the exchange of key metabolites between the mitochondrial matrix and the cytosol, depending on the metabolic requirements of a given cell. One example is the malate-aspartate shuttle, which exchanges MAL and ASP between the mitochondrial matrix and the cytosol. According to our hypothesis, the OAA levels resulting from the interplay between these enzymatic activities and shuttle systems can be further modulated by the recently identified ODx FAHD1 (Fig. 3).

Whereas a decrease of OAA concentration below a certain threshold will diminish TCA cycle flux due to limited substrate saturation for CS, OAA is also known as a potent competitive inhibitor of succinate dehydrogenase (SDH) (Wojtczak et al., 1969; Kotlyar and Vinogradov, 1984; Stepanova et al., 2016), the key enzyme of ETC complex II. SDH converts succinate to fumarate and thereby reduces Flavin adenine dinucleotide (FAD) to $FADH_2$, a reaction that is tightly regulated by feedback inhibition, i.e., inhibition by increased OAA levels in the mitochondrial matrix (Wojtczak et al., 1969; Stepanova et al., 2016). Energy failure induced upon inhibition of SDH is known to be associated with premature senescence in the brain and heart tissue (Stepanova et al., 2016).

4.2. Depletion of FAHD1 inhibits TCA cycle flux and impairs cell proliferation

According to our model, the TCA cycle may also be impaired by increased OAA concentration. Accordingly, sustained metabolic flux through the TCA cycle requires OAA to be in a narrow concentration range, and deviations of OAA levels in either direction potentially inhibit TCA cycle flux. Based on these considerations, we propose a new concept according to which FAHD1 evolved as a regulatory protein that modulates OAA concentration in the mitochondrial matrix (Fig. 4) and

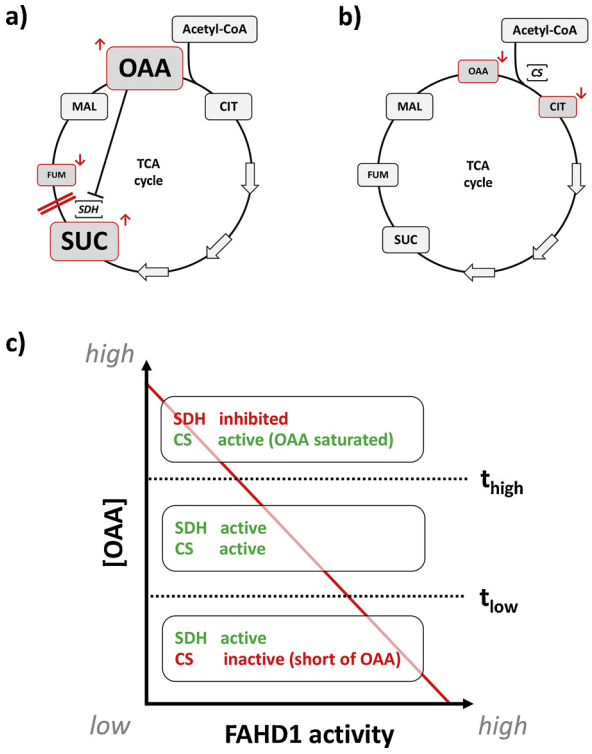

Fig. 4. Impact of the OAA rheostat on TCA cycle flux.
A high level of OAA leads to inhibition of SDH (a), whereas a low level of OAA reduces CS activity (b). These findings imply that OAA levels modulate metabolic flux through the TCA cycle in two ways (c): If OAA levels exceed the threshold level t_{high}, TCA cycle flux will be zero since SDH is inhibited CS; conversely, if OAA levels fall below t_{low}, TCA cycle flux will be zero since CS is short of its substrate OAA. Hence, sustained flux through the TCA cycle depends on OAA levels to be in an optimal concentration range, with threshold levels t_{high} and t_{low} being set by the activity of other OAA-producing and OAA-consuming processes.

therefore influences metabolic flux through the TCA cycle. In analogy to the phenotypes described for the inhibition of SDH (Wojtczak et al., 1969; Kotlyar and Vinogradov, 1984; Stepanova et al., 2016), deficiency of FAHD1 was shown to impair mitochondrial function in HUVEC (Petit et al., 2017) and in nematodes (Taferner et al., 2015). Depletion of FAHD1 in HUVEC also induced premature senescence in HUVEC by activation of the p53/p21 pathway, while no activation of DNA damage response or the p16 pathway was observed (Petit et al.,

2017).

5. The concept of *senescence light*

The data discussed in this review article suggest that cellular senescence, previously used as a common denominator for a variety of biological processes leading to terminal growth arrest of mammalian cells, turns out as a non-uniform biological pathway that is used by mammals in many divergent biological situations, ranging from ageing, response to environmental stressors, response to cancer therapy, cellular energy deficits up to normal embryonic development.

5.1. Senescence light

There is growing evidence that senescence in the absence of DNA damage represents a special condition. Besides developmental senescence, which is largely unexplored concerning mechanisms, this holds true for senescence induced by perturbations of the cellular energy metabolism. Best known examples of this phenotype to date are senescence due to inhibition of the mitochondrial ETC, and senescence due to the inactivation of genes required for mitochondrial function, such as SIRT3 and FAHD1 (Fig. 5). Although it cannot be completely ruled out, available data do not support damage to mitochondrial ETC complexes, mitochondrial structure or the mitochondrial DNA in these cases. Instead, cells seem to fall short of metabolites required for cell proliferation, such as ATP, NAD+, and precursors for nucleotide biosynthesis. We propose to term this particular type of cellular senescence

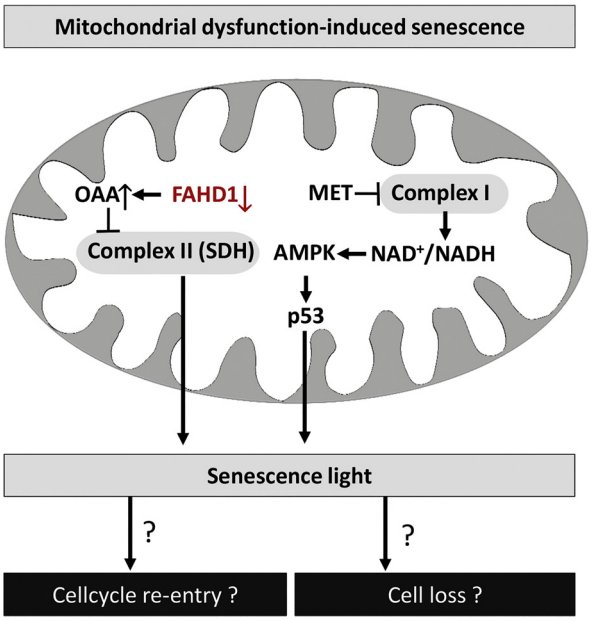

Fig. 5. Hallmarks of Mitochondrial dysfunction-induced senescence.
Mitochondrial dysfunction-associated senescence (MiDAS) is triggered by impairment of mitochondrial function and can be distinguished from other forms of senescence by the strict absence of nuclear DNA damage and by the inability of the cells to activate p16 gene expression. In cells with a defect in ETC complex I, growth arrest is triggered by AMPK-mediated phosphorylation and stabilization of p53 followed by increased p21 gene expression. According to our model, in some cellular models the inactivation of either ETC complex I (by metformin) or ETC complex II (by FAHD1 knockdown) has the potential to increase p21 gene expression in the absence of AMPK activation, suggesting additional pathways by which metabolic imbalance (e.g. lowered ratio of NAD^+/NADH or inactivation of SDH) can induce senescence-associated growth arrest. In some cellular models of SIPS and *senescence light*, cell proliferation reappears if cells are sub-cultivated after reaching initial senescence-associated growth arrest. In these cases, the fate of senescent cells (cell loss, cell cycle re-entry, others) remains to be determined.

"*senescence light*".

The fate of cells entering *senescence light* is not yet fully understood, probably due to methodological shortcomings. It appears possible that, in contrast to other forms of senescence which are truly irreversible, cells driven into *senescence light* might actually recover from senescence after some time and re-enter the cell cycle or might undergo cell loss/cell death (Fig. 5). Similar phenomena have been described in the literature for several forms of stress-induced premature senescence. In these cases, the senescence response can be divided into several distinct phases dictated by the experimental design: In the initial phase, a (transient) stress reponse is mounted (typically accompanied by p53 activation), which is followed by a phase of senescence "consolidation" (typically accompanied by the appearance of SA-ß-gal positive cells and in some instances increased p16 gene expression), when the stress is no longer applied. However, for some stressors (e.g. UVB irradiation (Greussing et al., 2013; Cavinato et al., 2017)), the senescence response gets "diluted", when cells are further passaged. In these settings, the fate of the formerly senescent cells is not entirely clear due to technical obstacles. In many settings of SIPS, between 30% and 95% of stressed cell populations enter the senescence phenotype. Nonetheless, when the cells are sub-cultured further, a few cells resume proliferation and after a few passages, senescent cells may have completely disappeared. It cannot be formally excluded that senescent cells just died out of the culture or even re-entered the cell cycle. Precedence for cell cycle re-entry of formerly senescent cells, referred to as "emergence" comes from studies with oncogene-induced senescence (de Carné Trécesson et al., 2011) and therapy-induced senescence (Vétillard et al., 2015) of cancer cells, where it was shown that formerly senescent cells may escape from senescence by the reactivation of the cdk4-EZH2-AP2M pathway (Le Duff et al., 2018). However, the most plausible scenario is that in SIPS senescent cells are just overgrown by descendants of a few (obviously stress-resistant) cells, where the senescence trigger was not strong enough to drive them into senescence in the first place.

DNA damage accumulation and/or DDR activation were not observed in reported cases of *senescence light*. Therefore, it is a viable hypothesis that signals triggered by DNA damage and/or an active DDR are required for canonical senescence, as opposed to *senescence light* (Fig. 6). In a model derived from these findings, we hypothesize that DDR and AMPK synergize to trigger a senescence response; in the absence of DNA damage, the extent of AMPK activation may influence the actual senescence outcome (canonical senescence or *senescence light*).

This feature of some cases of SIPS is in marked contrast to replicative senescence of human (but not mouse) cells, which appears as a very stable phenotype. Of interest, deep senescence in the strict sense refers to human diploid cells which are cultivated for months beyond the time point when they reach growth arrest and ~ 100% of the cells stain positive for SA-ß-gal activity. Such cells acquire features of deep senescence (e.g. reactivation of endogenous retroviruses due to chromatin rearrangements; see above) upon continued sub-cultivation.

5.2. Conclusions

In summary, the available data suggest that mitochondrial dysfunction can induce a spectrum of senescence-like phenotypes, dependent on the cell type used and the nature and intensity of the stressor. Senescence induced by FAHD1 inactivation in human cells may represent a new example of *senescence light*, and future work will aim at further characterization of this interesting new form of cellular senescence. Whereas the persistence of the growth arrest phenotype can be used to position a given senescence phenotype (defined by cell type and stimulus used to induce the senescence response) in a broad spectrum ranging from senescence light to deep senescence, it appears that the biological variability of senescence phenotypes is much higher than just differences in replicative potential. These findings suggest that better understanding the molecular details of a given senescence phenotype will pave the way for a better understanding of the underlying

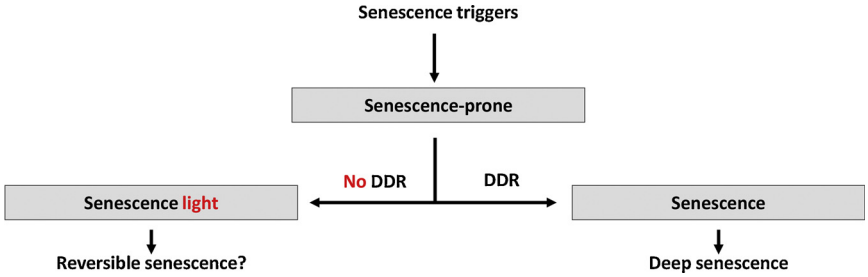

Fig. 6. The concept of *senescence light*.
Induction of senescence can be triggered by a large variety of conditions and factors in many different cell types. In our model, the outcome of senescence induction is determined by the appearance of DNA damage: In the presence of substantial DNA damage, a robust DDR is triggered which will lead to the entry of the cells into canonical senescence and irreversible growth arrest. In the absence of substantial DNA damage, cells will enter into *senescence light*, which differs from replicative senescence in various aspects, in particular by the emergence of non-senescent, proliferating cells upon extended sub-culturing. Circumstantial evidence suggests that the extent of AMPK activation may determine the percentage of cells able to resume cell proliferation.

physiological or pathological processes. Hence, more research in this exciting area is certainly warranted.

Competing interests

The authors declare that there are no competing interests associated with this manuscript.

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