SIRT1 and AMPK in regulating mammalian senescence: a critical review and a working model

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Abstract

Ageing in mammals remains an unsolved mystery. Anti-ageing is a recurring topic in the history of scientific research. Lifespan extension evoked by Sir2 protein in lower organisms has attracted a large amount of interests in the last decade. This review summarizes recent evidence supporting the role of a Sir2 mammalian homologue, SIRT1 (Silent information regulator T1), in regulating ageing and cellular senescence. The various signaling networks responsible for the anti-ageing and anti-senescence activity of SIRT1 have been discussed. In particular, a counter-balancing model involving the cross-talks between SIRT1 and AMP-activated protein kinase (AMPK), another stress and energy sensor, is suggested for controlling the senescence program in mammalian cells.

1. Cellular senescence

Senescence, originating from the Latin word *senex*, refers to a physiological program towards permanent cell cycle arrest. The first evidence of cellular senescence dates back to fifty years ago when Hayflick and Moorhead observed that after a limited number of divisions, embryo-derived fibroblasts entered an irreversible state of growth arrest [1]. Similar phenomena have been subsequently observed in human hepatocytes, keratinocytes, lymphocytes, smooth muscle and endothelial cells [2-6], leading to the concept of 'Hayflick limit', i.e. the finite replicative life span or the restricted cumulative population doubling of somatic cells *in vitro.* Morphologically, senescent cells are characterized by cellular enlargement and flattening with a concomitant increase in the size of the nucleus and nucleoli, augmented number of lysosomes and Golgi, and the appearance of vacuoles in the cytoplasm [7].

For a number of years, the occurrence of senescence *in vivo* has been questioned, due mainly to the lack of specific markers. In 1995, Dimri and colleagues reported that several types of human senescent cells expressed a β-galactosidase that was detectable by a histochemical assay at pH 6 [8]. An age-dependent increase in this senescence-associated β-galactosidase (SA-ß-Gal) staining was observed in human skin. On the other hand, this marker was absent in pre-senescent and quiescent fibroblasts, and terminally differentiated keratinocytes. Since then, other senescence biomarkers have been identified, including telomere attrition [9,10], active checkpoint kinase ATM [11], heterochromatin proteins [12], and the cyclin-dependent kinase (CDK) inhibitors p21CIP1 (p21), p16INK4a (p16) and p27KIP1 (p27) [13-16], most of

which are actively participated in pathways that affect cellular senescence. Using selective markers, an age-dependent increasing of senescent cells has been validated various mammal tissues *in vivo* [11,17-19]. Cellular senescence is now considered to the hallmark of mammalian ageing.

The physiological roles of senescence remain controversial. In general, can be viewed as an alternative response program to cellular stresses and damages otherwise may cause programmed cell death [20]. When confronted with metabolic hyperglycemia) and/or environmental stressors (e.g. oxidative stress), a self-protective mechanism of senescence may be initiated to halt the energy-consuming process of proliferation (Figure 1). However, a feedback-regulation should be in place to prevent cellular senescence from becoming irreversible (as is the case with terminal ageing of the organism), which, most of the time is independent of the initial stress conditions. The duration of cell survival in the non-dividing state after cessation of proliferation also a characteristic of longevity [21]. The fate of senescent cells in the living is largely unknown. The persistent presence of senescent cells can exert adverse on tissue functions, thus representing a pivotal underlying cause of ageing-related dysfunctions and diseases [22]. For example, in healthy postnatal tissues, the endothelial cells (referring to those line the inner surface of blood vessels) are mostly quiescent and rarely proliferate. In response to vascular injury, tissue ischemia, or other stress conditions, a remarkable phenotypic plasticity allows them to proliferate rapidly. However, their regenerative capacities declines progressively and coincides with the development of senescence [23]. Senescent endothelial cells in vascular

lesions release less available nitric oxide for vasodilator regulation, and become pro-inflammatory, pro-thrombotic and pro-atherosclerotic, which accelerate the development of cardiovascular diseases [24,25].

In addition to acting as a barrier of unlimited cell growth and division, senescence functions as a tumor suppressive mechanism to restrict excessive cell growth. Senescent cells are present in pre-malignant tissues [26]. Escaping from senescence, or immortality, is important for malignant transformation [26-29]. However, the additional levels of complexity suggest that senescence functions as a biological 'double edged sword' during tumorigenesis. On the one hand, it prevents activated oncogenes from initiating a clone of neoplastic cells and limit the replicative capacity of an incipient tumor cell [30,31]. On the other hand, although senescent cells themselves cannot become neoplastic, they promote the growth of nearby preneoplastic cells and in this way may contribute to the age-related increase in tumor incidence [32,33].

2. Sirtuins - the longevity regulator

Sirtuins are a family of NAD^+-dependent protein deacetylases that exert multiple cellular functions by interacting with, and deacetylating a wide range of signaling molecules, transcription factors, histones and enzymes [34-36]. *Sir2* (silent information regulator 2), the first gene discovered in this family, was originally shown to regulate transcriptional silencing at cell-mating loci, telomeres, and ribosomal DNA (rDNA) in yeast, through deacetylation of the epsilon-amino groups of lysines in the

amino-terminal domains of histones [37-42]. The anti-ageing effects of *Sir2* was firstly demonstrated by Kaeberlein et al, who showed that the integration of extra copies of *Sir2* extended lifespan up to 30% in *S. cerevisiae* [43]. Similar effects of *Sir2* on lifespan extension were subsequently observed in *C. elegans* and *Drosophila melanogaster* [44-47].

Sirtuins are highly conserved during the evolution from bacteria to humans. In mammals, the family is represented by seven members assigned as SIRT1-7, which share the catalytic domain of ~275 amino acids with Sir2 [48-51]. SIRT1-7 show diverse cellular localizations. SIRT6 and SIRT7 are localized in the nucleus, while SIRT3, SIRT4 and SIRT5 reside in the mitochondria. [51]. SIRT1 and SIRT2 shuttle between the cytoplasm and the nucleus. Mammalian sirtuins have been implicated in a wide range of biological processes, including metabolism, cell survival, development, chromatin dynamics and DNA repair. SIRT1 is the mammalian ortholog most highly related to Sir2. Protein expression and transcription levels of SIRT1 decline with ageing in animals and human tissues, including lung, fat, heart and blood vessels [52-54]. Sirtuins have attracted considerable interest because of their role as the longevity regulator and their therapeutic potentials for the prevention/treatment of ageing-associated medical complications, in particular cardiovascular diseases, diabetes and neurodegenerative disorders.

In 1935, McCay and co-workers reported that long-term caloric restriction without malnutrition almost doubled lifespan in rats [55]. This lifespan-extending effect of caloric restriction has been confirmed in a wide range of organisms ranging from

yeast to rodents, and primates [56-60]. These observations lead to the concept that caloric restriction regulates lifespan by reducing the metabolic rate and thus diminishing the accumulation of macromolecule damage over time. In higher organisms, caloric restriction is the only non-genetic intervention capable of promoting longevity and reducing the incidence of age-related disorders, such as diabetes, cardiovascular disease, and cancer. Both Sir2 and SIRT1 have been implicated in the anti-ageing activity of caloric restriction. Decreasing the expression of Sir2 blocks the lifespan-extending effect of caloric restriction in *S. cerevisiae* and *Drosophila* [46,61]. Likewise, in rodents, caloric restriction stimulates SIRT1 expression in a variety of tissues, including brain, visceral fat, kidney and liver [56]. Mice lacking SIRT1 fail to show an increased activity in response to caloric restriction [62]. By contrast, elevation of SIRT1 expression results in a beneficial phenotype resembling that of caloric restriction [63-65]. Despite these suggestive findings, there is yet no foolproof evidence that mammalian sirtuins are either indispensible or sufficient to lifespan extension in response to caloric restriction. In fact, the available evidence suggests that sirtuins mediate the anti-ageing effects of caloric restriction largely by regulating cellular energy metabolism in ways that directly benefit normal physiology [66-70].

3. Sirtuins and senescence in mammalian cells

In yeast, senescence is characterized by the accumulation of extrachomosomal rDNA circles (ERCs), which occurs in mother cells as they go through successive cell

divisions [71]. The Sir2 protein acts as a transcriptional silencer to suppress ERC formation, and as a consequence, increases longevity. The first evidence that sirtuins regulate mammalian cellular senescence was provided by Langley and colleagues, using the primary mouse embryonic fibroblasts as a model system [72]. SIRT1 antagonizes premature cellular senescence, induced by pro-myelocytic leukemia protein, by interacting with and deacetylating p53. Subsequently, the anti-senescence effects of SIRT1 have been demonstrated in other cell types, including human cancer cells (breast cancer MCF-7, lung cancer H1299 and prostate cancer cells) [73,74], human diploid fibroblasts [75] and a human umbilical vein endothelial cell line (HUVEC) [76-78]. In most of these studies, when the effects of SIRT1 are prevented with specific pharmacological inhibitors or siRNA, a premature senescence-like phenotype can be observed. Conversely, overexpression or activation of SIRT1 prevents both stress- and replication-induced cellular senescence. In HUVECs, cellular senescence occurs in parallel with an increased expression of plasminogen activator inhibitor-1(PAI-1) and a decrease in that of endothelial nitric oxide synthase (eNOS). SIRT1 exerts protective effects against endothelial dysfunction by preventing stress-induced premature senescence and deranged expression of PAI-1 and eNOS [76]. Cilostazol, a selective cAMP phosphodiesterase 3 inhibitor, exerts protective effects against endothelial senescence and dysfunction through upregulation of SIRT1, whereas sirolimus and everolimus induce endothelial senescence involving down-regulation of SIRT1 [77,78]. The latter studies suggest that p53 might be of the utmost importance in mediating the senescence signaling and the protective effect of

SIRT1. By contrast, it is not conclusive whether or not eNOS activation is directly involved in the anti-senescence activity of SIRT1, although the bioavailability of nitric oxide is impaired when SIRT1 is down-regulated [76]. Nevertheless, the increase in eNOS expression caused by caloric restriction is associated with mitochondrial biogenesis and enhanced expression of SIRT1 [79], whereas SIRT1 deacetylates and activates eNOS [80], indicating that a positive feedback mechanism exists between these two signaling molecules.

Endothelial regeneration is essential to maintain the functionality of the vasculature, in particular after mechanical endothelial injury and ischemia or during wound healing [81]. SIRT1 is highly expressed in the vasculature and regulates the proliferative activity of endothelial cells during tissue regeneration [82,83]. It has been implicated in the regeneration and proliferation of endothelial progenitor cells (EPCs) [83,84]. Exposure to high glucose accelerates EPC senescence and decreases EPC number, which is accompanied by a reduced SIRT1 expression and activity. Knockdown of SIRT1 with siRNA results in diminished EPC angiogenesis and increased senescence. SIRT1 controls the angiogenic activity of endothelial cells via a deacetylation-dependent inactivation of forkhead box O transcription factors 1 (FoxO1), a crucial negative transcriptional regulator of blood vessel development [82,85,86]. The acetylation of FoxO1 in EPC is increased significantly following exposure to high glucose. Resveratrol reduces, whereas inhibitors of SIRT1 (nicotinamide and suramine) potentiate the acetylation [83]. SIRT1 also deacetylates FoxO3 and/or FoxO4, attenuating FoxO-induced apoptosis and potentiating

FoxO-induced cell-cycle arrest [87-89]. While the implications of these FoxO modifications are still uncertain, it appears overall that deacetylation of FoxO proteins by SIRT1 promotes cell survival under stress conditions.

During the process of replicative senescence in primary porcine aortic endothelial cells, both mRNA and protein expressions of SIRT1 are progressively decreased [53]. Overexpression of SIRT1 stimulates proliferation and prevents senescence by targeting the tumor suppressor kinase LKB1. In these cells, LKB1 promotes cellular senescence and retards endothelial proliferation through activation of AMPK, a master regulator of energy metabolism. SIRT1 is activated by increases in NAD/NADH ratio [90,91], whereas AMPK senses AMP/ATP levels through its upstream kinase LKB1 [92,93]. A cross-regulation of these two pivotal energy- and stress-sensor pathways has been implied in the context of endothelial ageing [23]. The endothelium-specific elevation of SIRT1 activity protects mice from developing drug-induced premature vascular senescence [53]. There are presumably various interactions between the SIRT1 and AMPK pathways [94-97]. However, the precise connections between these two nutrient sensing enzymes in cellular senescence are largely uncharacterized and warrant further investigations.

In addition to SIRT1, SIRT6, a mammalian sirtuin associated with heterochromatic regions and nucleoli [98], has also been implicated in the regulation of cellular senescence. Indeed, SIRT6 depletion in mice leads to premature cellular senescence and telomere dysfunction with end-to-end chromosomal fusions, a pattern resembling the defects observed in Werner syndrome, a human premature ageing

disease [99]. SIRT6 modulates genome stability by interacting with and deacetylating histone H3 at telometic chromatin [100]. It also regulates CtIP [C-terminal binding protein (CtBP) interacting protein] to facilitate DNA end resection at the double-strand breaks (DSB) [101]. Moreover, SIRT6 forms a macromolecular complex with DNA-PK [DNA-dependent protein kinase] and promotes DSB repair [102]. The NFκB RelA subunit is also regulated by SIRT6, which enhances the NFκB-dependent gene expression changes involved in cellular senescence [103]. Mechanisms other than these have also been suggested to mediate the regulatory effects of this protein on energy metabolism in enhancing insulin signaling and glucose uptake [104]. Effects of other sirtuins, in particular SIRT2 and SIRT7, on mammalian ageing have also been proposed [105-108]. However, the functional relationship among these members of the sirtuin family remains poorly understood at this stage.

4. Anti-senescence activity of SIRT1 – focus on the cross-talks with AMPK signaling pathway

SIRT1 elicits its various effects by regulating the acetylation/deacetylation status of a wide range of protein targets involved in heterochromatin silencing, cycle progression, cell survival and metabolism [72,88,91,109-112]. By binding with and deacetylating the target proteins, SIRT1 is able to regulate their activities, intracellular localizations, stabilities and posttranslational modifications. With respect to cellular senescence, the plethora of substrates that SIRT1 targets for deacetylation includes

p53, NFκB, PGC-1α [peroxisome proliferator-activated receptor-γ coactivator 1α], eNOS, mTOR and FoxOs (Figure 1). The tumor suppressor p53 is among the first non-histone substrates identified to be functionally involved in the anti-senescence activity of SIRT1 [72]. Activation of the p53-p21 pathway acts as a major mediator of cellular senescence [113]. Deacetylation of p53 by SIRT1 results in an inhibition of DNA damage- and stress-mediated cellular senescence [114]. NF-κB is a major culprit that mediates ageing-associated pro-inflammatory responses [115]. Oxidative stress and reactive oxygen species (ROS) production modulates the promoter binding activity of NF-κB, which promotes cellular senescence by transactivating the expressions of cell cycle regulators [116]. SIRT1 physically interacts with the RelA/p65 subunit of NFκB and inhibits the transactivation potential of this protein [112]. Mitochondrial function changes during cellular senescence lead to metabolic inefficiency and increased generation of ROS [117]. SIRT1 promotes mitochondrial functions and reduces the production of ROS through regulating the master controller of mitochondrial biogenesis, PGC-1α, and the eNOS- and nitric oxide-dependent pathway [118,119]. A number of SIRT1 targets, including mTOR and FoxOs, are coordinately involved in the process of autophagy, a housekeeping process for maintaining energy balance via self-digestion [120,121]. The extension of lifespan by SIRT1 has been linked to the efficient maintenance of autophagic degradation, either directly or indirectly through a downstream signaling network [122,123].

Mammalian senescence is triggered by a complex signaling network involving the interactions of multiple proteins [19]. Unlike Sir2 in yeast, which functions

exclusively in the nucleoli and heterochromatic regions, nucleocytoplasmic shuttling of SIRT1 has been demonstrated in various mammalian cellular systems [98,124-127]. Thus, when attempting to unravel the molecular events upstream and downstream of the SIRT1 pathway in regulating cellular senescence, one has to consider searching far beyond the border of nucleus or any single cellular compartment, and cover not only long-term DNA damages and telomere shortening, but also short-term metabolic adaptations. In that context, the remainder of this article will attempt to elucidate the interplays between SIRT1 and LKB1/AMPK signaling, the two well-known stress resistance and longevity-regulating pathways.

4.1 AMPK, LKB1 and cellular senescence

AMPK is the primary regulator of cellular responses to reduced ATP levels and acts as a sensor to maintain the energy balance within a cell [128,129]. In general, activation of AMPK down-regulates synthetic pathways such as protein, fatty acid and cholesterol biosynthesis, yet switches on the catabolic pathways that generate ATP, such as fatty acid oxidation, glucose uptake and glycolysis. It achieves this not only through direct phosphorylation of a variety of key metabolic enzymes, but also by altering gene expressions in a tissue-specific manner [130,131]. Depending on the tissue types, the targeted genes include PGC-1α, the FoxO family of transcription factors, SREBP [sterol regulatory element binding protein] and ChREBP [carbohydrate response element binding protein]. AMPK is a trimeric serine/threonine protein kinase comprising a catalytic α subunit and non-catalytic β and γ subunits.

The α subunit contains an NH_2-terminal catalytic kinase domain and a COOH-terminal regulatory domain to which the β and γ subunits bind. The γ subunits contain four tandem repeats of a sequence motif "CBS domain", which represents the regulatory AMP- and ATP-binding sites of the AMPK complex. Stresses (e.g. metabolic poisoning, free radical production, heat-shock, hypoxia, or nutrient deprivation) that cause a rise in AMP/ATP ratio can activate AMPK, by facilitating phosphorylation of the α subunit at a specific residue (Thr172) located within the activation loop. This process can cause at least 50- to 100-fold activation of AMPK and is mediated by upstream kinases (AMPKK), one of which is the tumor suppressor LKB1 [92]. This complex regulatory system results in a superb sensitivity of AMPK to respond to even a very small change in AMP levels.

LKB1 is a serine/threonine protein kinase possessing proliferation-inhibitory and anti-tumor activities. It was originally discovered as a tumor suppressor gene mutated in patients with Peutz-Jeghers syndrome, a dominantly inherited human disorder characterized by an increased predisposition to cancer [132,133]. Loss-of-function of LKB1 is frequently found in non-small cell lung carcinomas [134]. Inactivation of LKB1 has also been reported in pancreatic cancers, melanomas, prostate, endometrial and cervical cancers, papillary breast cancers, testicular cancers, as well as colon and gastric cancers [135-137]. Overexpression of LKB1 suppresses cancer cell growth by inducing a G1 cell cycle arrest [138,139]. LKB1 is not only considered to be a tumor suppressor kinase that regulates cell proliferation, but also actively involved in controlling cell motility, metabolism, polarity and senescence [140-142].

LKB1 mediates AMPK activation in response to various cellular stresses- and pharmacological agents [143-150]. While activation of LKB1/AMPK signaling protects cells against energy stress by maintaining energy homeostasis and ensuring a slow consumption of energy stores [151,152], this pathway can also cause cellular senescence, cell cycle arrest and apoptosis in eukaryotic systems [153,154]. In another word, a certain level of AMPK activation is beneficial, whereas over-activation may be destructive. For example, mild energy restriction promotes AMPK activation and triggers neurogenesis, whereas severe diet restriction-induced AMPK leads to neuroapoptosis, possibly due to insufficient cell resources to reverse AMP:ATP ratio [155]. LKB1 is significantly up-regulated in senescent primary endothelial cells and overexpression of this kinase induces senescence through AMPK activation in young cells [53]. LKB1 deficiency prevents culture-induced senescence in murine embryonic fibroblasts [156]. In senescent fibroblasts, AMP:ATP ratios are two to three folds higher than those in young fibroblasts, and senescence is accompanied by a marked elevation in AMPK activity [157,158]. In mice, caloric restriction down-regulates AMPK activity in the liver [159]. Activation of LKB1/AMPK and inhibition of mTOR contribute to the premature ageing phenotype of *Zmpste24-/-* mice [153]. AMPK hyper-activation has also been reported in the skeletal muscle and liver of old rodents [160,161]. In the aorta of old mice, LKB1 and phosphorylated AMPK(Thr172) levels are much higher than those of young mice [53]. The regulation of ageing by AMPK is evolutionarily preserved. In yeast, the AMPK homologue Snf1 is a pivotal regulator of glucose-related gene expression at times of low fuel availability [162].

Snf1 is a heterotrimer composed of a catalytic α subunit (Snf1p) that phosphorylates target proteins at Ser/Thr residues, an activating γ subunit (Snf4p), and a β subunit (Sip1p, Sip2p, or Gal83). *snf1* null mutants are viable, but are unable to grow on sucrose, galactose, maltose, melibiose or nonfermentable carbon sources and do not contain any detectable peroxisomes, whereas overproduction of Snf1p causes accelerated ageing [163]. Loss of Snf4p, an activator of Snf1p, extends generational life span whereas loss of Sip2p, a presumed repressor of the kinase, causes an accelerated ageing [164].

In summary, these findings suggest that chronic activation of the LKB1-AMPK catabolic pathway may turn an originally pro-survival strategy into a pre-ageing mechanism and contribute to the progressive degeneration during cellular senescence.

4.2 Reciprocal regulations of SIRT1 and LKB1/AMPK signaling in cellular senescence

Interactions between SIRT1 and AMPK pathways occur in different types of tissues and cells [95,97,165-172]. In liver, while AMPK and SIRT1 may act in an autoregulatory loop to regulate lipid metabolism, their impacts on gluconeogenesis during fasting conditions appear to diverge [165]. In skeletal muscle, AMPK enhances SIRT1 activity by increasing cellular NAD^+ levels [94,173], and this amplification of SIRT1 and its downstream signaling pathways is impaired in AMPK-deficient states [171]. In neuronal systems, resveratrol-stimulated AMPK activity depends on LKB1 but does not require SIRT1 [174]. However, other results suggest that resveratrol

activates LKB1/AMPK signaling in both SIRT1-dependent and independent manners in HepG2 cells [95,175]. In HEK293 cells, over-expression of SIRT1 activates AMPK through LKB1 [96]. It should be noted that the regulations of growth, survival, energy metabolism and response to stresses in cancer tissues are very different from those of normal cells. Cells from SIRT1 knockout mice show either no change [174] or an increase in AMPK activity [97,176,177]. Taken in conjunction, the available evidence suggests that AMPK and SIRT1 are vital links in an orchestrated network controlling cellular homeostasis. Therefore, it is of great importance to understand the mechanisms by which they interact and the consequences of the cross-regulations under various conditions.

In endothelial cells, LKB1- and AMPK-induced senescence can be prevented by increasing the levels of SIRT1 [53]. By contrast, inhibition of SIRT1 activity or over-expression of a dominant negative deacetylase mutant, SIRT1(H363Y), induces endothelial senescence and elevates the protein levels of LKB1, resulting in a hyperactivation of AMPK [53]. These observations demonstrate the link between the anti-senescence activity of SIRT1 and the deregulation of LKB1/AMPK signaling [23]. Under normal physiological conditions, LKB1 is constitutively active [131], which makes it necessary to have a counter-mechanism available to prevent persistent or exaggerated activation of AMPK signaling. The regulation by SIRT1 of LKB1 protein stability represents such a counterbalancing mechanism (Figure 2). Deacetylation mediated by SIRT1 synergizes with the ubiquintination and degradation of LKB1 [53]. Because both acetylation and ubiquitination occur on lysine residues,

deacetylation of LKB1 by SIRT1 may control the accessibility of these residues for ubiquitination and thereby alter its stability in endothelial cells. However, how deacetylation affects the biological activities of LKB1 is incompletely understood. Structurally, LKB1 kinase domain is poorly related to other protein kinases. In particular, the NH_2- and COOH-terminal non-catalytic regions of LKB1 possess no identifiable functional domains. LKB1 shuttles between the nucleus and the cytoplasm [178]. When LKB1 is forced to remain in the cytoplasm by disruption of the nuclear localization signal, it retains full growth-suppression activity in a kinase-dependent manner [139]. It is highly possible that acetylation/deacetylation of specific residues by SIRT1 affect the intracellular localization, protein stability and/or protein-protein interactions of LKB1 in primary endothelial cells. Several other molecules involved in regulating senescence, including p53 and FoxOs, are also modulated by reversible acetylation and targeted by SIRT1 [72,179]. Changes in lysine acetylation may represent an important mechanism integrating metabolic and stress signals to govern cellular senescence and ageing.

Cell cycle regulation by AMPK is mediated by inhibition of the TSC2-mTOR (mammalian target of rapamycin) pathway as well as up-regulation of the p53-p21 axis [180,181] (Figure 2). The mTOR pathway is a major controller of protein biosynthetic processes. Blockage of this pathway induces protein degradation through autophagy and the ubiquitin-proteasome system [131]. Premature ageing activates a systemic metabolic response involving induction of autophagy [153]. Actually, the physiological ageing process is associated with a declined efficiency of autophagic

degradation [121]. Although both SIRT1 and AMPK are implicated in the regulation of autophagy [176,182,183], the detailed interactions and the involvement of mTOR or other signaling molecules, such as FoxOs, NFκB and p53, remain to be elucidated. Opposing effects of the two signaling molecules have been reported in relation to p53 regulation. Persistent activation of AMPK leads to accelerated p53-dependent cellular senescence [180], whereas SIRT1 antagonizes p53-induced cellular senescence through promoting its deacetylation [72]. LKB1 acts as an upstream kinase for PTEN (phosphatase and tensin homologue), which overcomes growth/survival signaling from the PI3K/Akt pathway [184]. The balance between LKB1-AMPK and PI3K/Akt pathways may determine cell growth or death in response to the nutritional status and stress [185]. The cross-talks between SIRT1 and AMPK in controlling senescence could also converge at the level of PI3K/Akt signaling [53].

Unlike in the metabolic organ skeletal muscle, AMPK does not affect the NAD^+ biosynthetic enzyme, NAMPT [nicotinamide phosphoribosyltransferase], in endothelial cells [53]. Moreover, NAMPT expression is not different in senescent cells from that of young cells. These findings, however, cannot exclude other mechanisms that may be involved in the regulation of SIRT1 by AMPK, such as those at the posttranscriptional levels (Figure 2). For example, SIRT1 mRNA levels are regulated by the RNA binding protein HuR and by microRNA, which repress SIRT1 protein expression in response to different stress conditions [186-188]. Depending on the upstream signal, HuR causes SIRT1 mRNA to be either stabilized or degraded. During the progression of cellular senescence, the mRNA and protein levels of SIRT1

decrease progressively [53]. AMPK activation causes premature fibroblast senescence through a mechanism that involves HuR [157,189]. Moreover, AMPK inhibits the transport of HuR to the cytoplasm and thus blocks its ability to stabilize and enhance the expression of target mRNAs [189]. HuR levels are lower in senescent cells, and the over-expression of HuR restores a "younger" phenotype, whereas a reduction in HuR expression accentuates the senescent appearance [190]. Taken in conjunction, these studies are consistent with a role of HuR, possibly involving AMPK, in regulating the mRNA levels of SIRT1 during the process of replicative senescence.

5. Concluding remarks

Energy metabolism and metabolic regulators play pivotal roles in controlling longevity and cellular senescence. SIRT1 and LKB1/AMPK are the two key energy sensor systems regulating cell survival, proliferation and senescence. While acute activation of the LKB1/AMPK catabolic pathway permits a rapid adaption or resistance to external and internal stresses, sustained stimulation of the same pathway leads the cells toward a condition of irreversible senescence, which is detrimental to normal physiological functions. The anti-ageing activity of SIRT1 is achieved at least in part by fine-tuning the LKB1/AMPK pathway and preventing the transition of an originally pro-survival program into a pro-ageing mechanism, which results in systemic degeneration (Figure 3). This process is elegantly controlled by a complex network involving many signaling proteins, as well as by the ratios between low molecular weight metabolites (e.g. NAD/NADH and

AMP/ATP). Further studies on the reciprocal regulatory mechanisms and the unexplored pathways responsible for the dysregulated balance between SIRT1 and LKB1/AMPK signaling may provide important insights for temporal and quantitative control of the ageing process.

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Figures and legends:

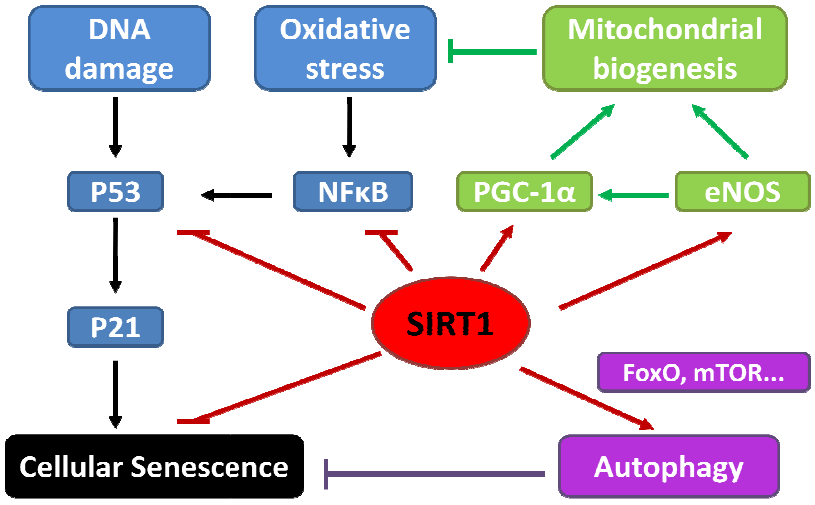

Figure 1. SIRT1 elicits the anti-senescence activity by targeting a wide range of protein substrates that are critically involved in regulating key cellular processes, such as oxidative stresses, DNA damage, mitochondrial biogenesis and autophagy.

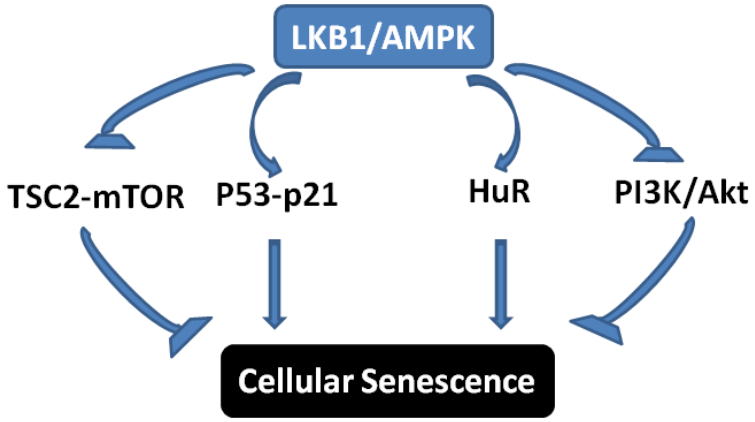

Figure 2. Several potential molecular pathways are involved in cellular senescence caused by hyperactivated LKB1/AMPK signaling.

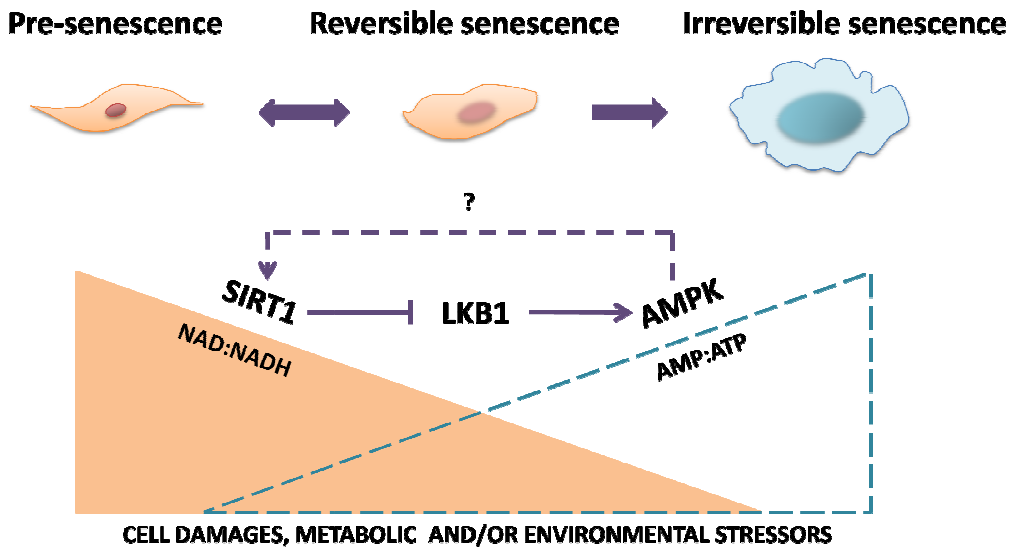

Figure 3. A model represents the reciprocal regulations of SIRT1 and AMPK pathways in mammalian cellular senescence. The progression from pre-senescence to irreversible senescence is accompanied by a decreased SIRT1 expression and activity, and an augmented AMPK function. SIRT1 counter-regulates AMPK through targeting the upstream kinase LKB1. A possible feed-back regulation of SIRT1 by AMPK is suggested for further investigations.