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Review

The Role and Application of the AMPK-Sirtuins Network in Cellular Senescence

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Abstract

Aging and related diseases significantly affect the health and happiness index around the world. Cellular senescence is the basis of physiological aging and is closely related to various senile diseases. AMP-activated protein kinase (AMPK) is associated with both the regulation of cellular energy metabolism and the regulation of cellular senescence. Another set of proteins, sirtuins, has also been demonstrated to play an important role in cell senescence. However, it is not clear how AMPK and sirtuins coordinate to regulate cellular senescence. Herein, we summarized the role of AMPK and sirtuins in regulating metabolism, repairing DNA damage, and even prolonging human life. We have provided a detailed explanation of the clinical trials relating to the AMPK and sirtuins involved in aging. Systematically analyzing individual senescence genes and developing functional reference notes will aid in understanding the potential mechanisms underlying aging and identify therapeutic targets for both anti-aging interventions and age-related illnesses.

Keywords: AMPK; sirtuins; aging; mTOR; senescence

1. Introduction

Globally, there has been a consistent increase in the aging population, as depicted in Fig. 1. In China, the population aged 60 years or older comprises around 264 million people, accounting for 18.7% of the total population, according to the 7th national census data. China has the largest elderly population in the world. Aging diseases also place a considerable economic burden on China [1]. Age-related diseases, such as cardiovascular diseases, coronary heart disease, cancer, diabetes, and arthritis, significantly impact the population's health [2]. Furthermore, aging exacerbates the severity of various diseases [3]. Clinical research is increasingly focused on understanding how to counteract cellular senescence to enhance the quality of life.

Cellular senescence is defined as a stress response in which cultured cells irreversibly lose their ability to proliferate, leading to cell cycle arrest, also known as the onset of senescence [4]. While aging and cellular senescence are distinct processes, they are intricately interconnected [5]. As an organism undergoes senescence, its overall functionality diminishes, consequently heightening the risk of mortality [5]. Previous research has indicated that there are numerous mechanisms of cellular senescence, including autophagy of cells, DNA damage, abnormalities in telomerase function, and genetic abnormalities related to carcinogenesis and oncogenesis [6].

Telomere shortening is a predominant factor in cell cycle progression. Cellular autophagy is an important process in eukaryotes that regulates homeostasis *in vivo*. Autophagy is associated with various causes, including inflammatory and immune mechanisms, oxidative stress, caloric restriction (CR), mitochondrial function or structure disruption, and cellular hormonal disorders. These factors can influence autophagy and, consequently, cellular senescence [7]. In the context of anti-aging research, cellular senescence can be regulated indirectly or directly by various substances, such as the insulin/insulin-like growth factor signaling pathway (IIS), The Mammalian Target of Rapamycin (mTOR), nicotinamide adenine dinucleotide (NAD^+), AMP-activated protein kinase (AMPK) and sirtuins, among other pathways [8].

AMPK regulates energy metabolism throughout the body and plays a role in the cellular senescence process by maintaining intracellular energy balance and vital activities [9]. The sirtuin family is involved in cellular biological activities, such as metabolism, inflammatory responses, DNA

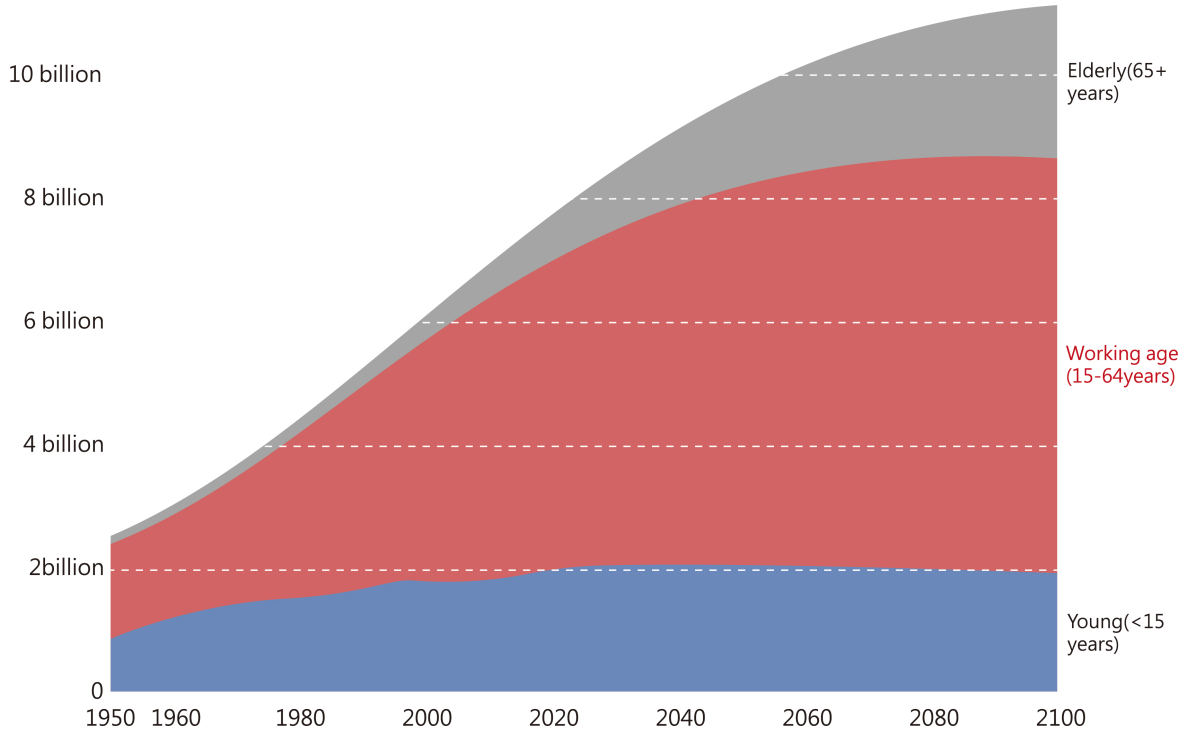

Fig. 1. Population size of young, middle-aged, and older people, 1950–2100. Historical estimates for 1950–2015 and estimates based on United Nations (UN) medium-term scenario projections to 2100. Source: United Nations Century Population Prospects (2017).

repair, and more. Furthermore, the sirtuin family is also involved in the cell cycle and apoptosis, which significantly affects cellular senescence [10]. In recent years, clinical research has demonstrated that the AMPK and the sirtuin family are highly influential in the progression of senescence [11]. This review focuses on their impact on cellular senescence progression as well as their interactions and regulation to enhance cell resistance to senescence.

2. AMPK and Aging

2.1 Introduction

AMP-activated protein kinase (AMPK) is an ATP-dependent protein kinase closely related to biological energy metabolism. AMPK is a heterotrimeric complex composed of three subunits: α, β, and γ. The α subunit is a catalytic subunit, whereas the β and γ subunits are regulatory. There are two α subunits (α1 and α2), two β subunits (β1 and β2), and three γ subunits (γ1, γ2 and γ3). The α subunits have an N-terminal domain and a C-terminal domain, which links the β and γ subunits and binds to AMP. The C-terminal region of the β-subunit acts as a scaffold and interacts with the C-terminal regions of the α and γ subunits. The distribution of these subunits varies slightly among mammals, with γ2 being abundant in the human heart and γ3 being present only in skeletal muscle [12]. Thus, the various subunits of AMPK are differentially expressed between mammalian tissues.

AMPK is essential for providing energy for cellular activities as it is sensitive to changes in AMP/ATP and ADP/ATP ratios [13]. AMPK is widely distributed in all eukaryotes and can be activated in response to hypoxia, ischemia, or nutritional deficiency. This leads to a decrease in ATP concentration and a rapid increase in 5′-AMP levels. The interaction of 5′-AMP with the γ subunit of AMPK allows it to respond to changes in the ATP/AMP ratio. Activated AMPK reduces ATP consumption and increases ATP production, leading to a more stable energy metabolism [14].

2.2 AMPK-Associated Pathways During Cellular Senescence

AMPK has been shown to increase metabolism [15], promote cellular autophagy, reduce oxidative stress, and inhibit inflammation and endoplasmic reticulum stress [16], all of which can have a significant impact on the progression of cellular senescence through various pathways (Table 1, Ref. [16–22]).

2.2.1 CREB/CRTC-1 Signaling Pathway

cAMP-response element binding protein (CREB) is a well-studied regulatory protein that plays a crucial role in gene transcription and has been associated with memory. Low *CREB* activity can lead to long-term memory impairment, whereas high activity may improve age-related deficits in neuronal excitability and cognitive performance [23].

Studies have demonstrated that the cyclic adenylate response element binding protein (CREB)-regulated tran-

Table 1. Pathways of AMPK regulation of aging.

AMPK-regulated substances	Role	AMPK on its direction of action	Experimental subjects	References
CRTC-1/CREB signaling	AMPK can inhibit the CRTC-1/CREB pathway to achieve life-extending effects	Inhibition	*Caenorhabditis elegans*	[16,17]
mTOR	Suppression of autophagy	Inhibition	Mice	[18]
FOXO axis	Increased antioxidant effect	Promotion	*Caenorhabditis elegans*	[16]
p53	Enhancing self-efficacy	Promotion	Mice	[16]
NF-κB	Associated with immune response, inflammatory response, etc.	Inhibition	Mice	[16,19]
Nrf2/SKN-1	Improving cellular defense function under oxidative stress	Promotion	*Caenorhabditis elegans*	[16,20]
ULK1	Increase in self-longing	Promotion	Mice	[21,22]

AMPK, AMP-activated protein kinase; CRTC-1, CREB-regulated transcriptional coactivator-1; CREB, cAMP-response element binding protein; mTOR, The Mammalian Target of Rapamycin; FOXO, Forkhead box class O; NF-κB, The nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, Nuclear factor-erythroid 2-related factor-2; ULK1:unc-51-like kinase 1; SKN-1, C. elegans skinhead family member 1.

scriptional coactivator-1 (CRTC-1) is the primary long-lived target of AMPK and calcineurin in Caenorhabditis elegans. The phosphorylation status of CRTC-1 is regulated in an antagonistic manner by both AMPK and calcineurin, which modulate their activity and exert effects on cellular senescence. AMPK and calcineurin share a common signaling pathway that interacts with CRTC-1 and is involved in mediating longevity. Calcineurin is a protein phosphatase that activates CRTC-1 by dephosphorylating it, thereby directly antagonizing AMPK. Conversely, CRTC-1 can be directly phosphorylated by AMPK. Our research shows that CRTC-1 is a direct target of AMPK and associates with the CREB homolog-1 (CRH-1) transcription factor *in vivo*. Activation of AMPK or inactivation of calcineurin reduces the activity of CRTC-1 and CRH-1. Several studies have shown that activation of AMPK or inactivation of calcineurin slows down senescence in *C. elegans* and that both regulate cellular senescence through post-translational modifications of CRTC-1 [17].

Studies have demonstrated that AMPK activation promotes the transcriptional reprogramming of metabolic genes through the CRTC-1/CREB pathway, which is associated with cellular senescence. Interestingly, the AMPK/AAK-2 signaling pathway is also closely linked to cellular senescence. CRTC-1 is a direct phosphorylation target of AAK-2/AMPK in *C. elegans*, and studies have shown that suppressing the CRTC-CREB pathway through AAK-2/AMPK signaling prolongs the lifespan of *C. elegans*. These findings suggest that factors inhibiting CRTC-induced CREB activation are critical in regulating the senescence progression and lifespan [16].

2.2.2 mTOR Signaling Pathway

The Mammalian Target of Rapamycin (mTOR) is a serine-threonine kinase that exists as the catalytic subunit of at least two protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). When activated, mTORC1 regulates metabolism and cellular physiological processes by promoting protein translation and cell growth. mTORC1 also regulates autophagy, the ability to degrade cells and maintain cellular homeostasis when energy levels are low. mTORC2 is involved in cytoskeletal reorganization and cell survival [24].

AMPK can directly inhibit mTORC1 through phosphorylation of Raptor, and by suppressing the inhibitory effect of mTORC1 on cellular autophagy, a lifespan extension effect can be achieved [18]. AMPK can also indirectly inhibit mTORC2 by activating tuberous sclerosis 2 (TSC2) [25].

In addition, mTOR inhibition reduces mRNA translations, protein toxicity, and oxidative stress, thereby delaying senescence. Activating cellular autophagy can lead to the removal of damaged proteins, thereby extending lifespan [26]. Moreover, inhibition of mTOR activity facilitates the self-renewal of mouse hematopoietic stem cells. It has also been reported that activation of the mTOR pathway could inhibit sirtuins family (SIRT1), leading to impairment of cellular autophagy and counteracting effects on resistance to cellular senescence. Studies have shown that inhibition of mTOR activity enhanced SIRT2 activity, thereby extending the lifespan of yeast. Resveratrol also suppresses mTOR activation and improves cell aging [27].

2.2.3 FOXO Signaling Pathway

Forkhead box class O (FOXO) is an insulin-sensitive protein that plays a critical role in cellular physiological processes such as cell proliferation, apoptosis, reactive oxygen species (ROS) response, cellular senescence, cancer, and regulation of the cell cycle and metabolism [28]. Studies have shown that under dietary restriction conditions, AMPK/aak-2 and *FOXO/daf-16* can increase the lifespan of worms [29]. AMPK γ2 can activate FOXO, which can enhance resistance to oxidative stress and prolong the organism's lifespan. Additionally, the *FOXO/4E-BP* signaling pathway can stimulate autophagy and maintain muscle deformation stability in Drosophila that have undergone

muscle senescence. AMPK can also enhance autophagy via FOXO and the ULK1/mTOR pathway to achieve lifespan extension [16].

SIRT1 can increase resistance to oxidative stress in mammalian cells by regulating FOXOs. SIRT2 enhances FOXO3 by deacetylating FOXO3, leading to cell cycle arrest and resistance to oxidative stress [27]. FOXOs can inhibit cellular triglyceride accumulation via the sirtuin pathway [30].

2.2.4 The *p53* Signaling Pathway

p53 is a transcription factor that regulates the transcriptional process of target genes [24]. As a well-known anti-oncogene, its main functions are to prevent rapid cell proliferation and induce apoptosis [31]. *p53* is a mediator of aging mainly through regulating cellular autophagy [6]. Activated AMPK can increase *p53* expression and induce autophagy by acting in conjunction with mTOR [31]. Moreover, AMPK promotes *p53* activity through phosphorylation at *Ser-15* [32]. Forced expression of AMP α2 increases *p53* gene transcription and improves *p53* protein phosphorylation at *Ser-46*, thereby triggering apoptotic cell death [16].

During CR, AMPK activation leads to the activation of *p53*, causing cell cycle arrest and promoting cell survival. Continuous activation may lead to irreversible cell cycle arrest and promote the occurrence of senescence [33]. In addition, when *p53* is activated, it inhibits mTOR and therefore suppresses senescence [34].

SIRT1 deacetylates *p53*, reducing DNA damage and stress-mediated cellular senescence. The stimulation of *p21* by *p53* inhibits the activity of cyclin-dependent kinases (CDKs) and regulates cell cycle arrest during cellular senescence. Studies have shown that SIRT1 can inhibit cellular senescence by decreasing the expression of *p53* and *p21*, thereby promoting the differentiation of adipose cells [27]. The *p53* gene can also prevent DNA damage, promote DNA repair, and prevent mutation.

2.2.5 NF-κB Signaling Pathway

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a nuclear transcription factor that regulates gene expression and plays a critical role in apoptosis and inflammatory signaling in various diseases [35]. Studies have shown that the NF-κB pathway is effective in countering skin aging [19]. AMPK can enhance cell resistance by stimulating the *FOXO/DAF-16*, Nrf2/SKN-1, and SIRT1 signaling pathways. Furthermore, AMPK can suppress the NF-κB signaling pathway to inhibit the inflammatory response. Over a decade ago, it was observed that the DNA binding activity of the NF-κB complex increased significantly with age in various mouse and rat tissues. In summary, the AMPK-SIRT1-NF-κB signaling pathway plays a key role in suppressing the immune response [16].

2.2.6 Nrf2/SKN-1 Signaling Pathway

Nuclear factor-erythroid 2-related factor-2 (Nrf2) and C. elegans skinhead family member 1 (SKN-1) play a crucial role in the oxidative stress response of cells [16]. Nrf2 is a transcription factor that protects against oxidative damage and is involved in the prevention of age-related diseases [20]. It is also associated with the nervous system and has been shown to enhance neuronal transcription and alter neurodegenerative lesions [36]. Additionally, Nrf2 may enhance cardiac function stability in the context of abnormal hemodynamics [37] and can protect against inflammation-related diseases [38].

Activation of the Nrf2/SKN-1 signaling pathways has been shown to prolong lifespan in various organisms, and AMPK-mediated activation of the pathways promotes cellular antioxidant and anti-inflammatory responses, potentially contributing to lifespan extension [16]. However, elucidating the exact mechanism requires further research.

2.2.7 ULK1 Signaling Pathway

ULK1 is a serine/threonine protein kinase that is a direct mammalian homolog of yeast *Atg1*. ULK1 has five homologs: ULK1, ULK2, ULK3, ULK4, and serine/threonine kinase 36 (STK36), with ULK1 and ULK2 thought to be involved in signaling associated with autophagy. Cellular autophagy can be severely affected when ULK1 is absent, indicating the important role of ULK1 in autophagy [21]. Phosphorylation of ULK1 by AMPK can increase autophagy and prolong the lifespan [22]. Furthermore, the AMPK/ULK1 pathway may play a role in tumor suppression and regulation of cellular metabolism in physiological processes. It has also been demonstrated that AMPK can indirectly inhibit ULK1 by suppressing mTOR, which inhibits autophagy and leads to anti-aging effects [39].

3. Sirtuins and Aging

3.1 Introduction

Nicotinamide adenine dinucleotide (NAD^+) is a coenzyme for dehydrogenase and is also known to be an electron-delivering body. NAD can be reduced to NADH with H^+ and can be synthesized into ATP in the respiratory chain. A decline in NAD^+ levels has been linked to a number of age-related diseases [40].

In cell biology, NAD^+/NADH dinucleotides are essential for driving a wide range of reduction-oxidation reactions. Studies have shown that elevated levels of NAD can counteract the onset of aging [41]. The precursor of $NADP^+$ and nicotinamide adenine dinucleotide phosphate (NADPH) is NAD^+. $NADP^+$ and NADPH play a key role in the response to resistance to ROS and in various cellular biosynthetic pathways [42].

Oxidative stress can result in an accumulation of ROS, which can cause damage to cellular proteins, lipids, and

DNA, ultimately leading to cellular damage. Cellular damage can contribute to the development of various pathologies such as aging, cancer, neurodegenerative diseases, cardiovascular diseases, and diabetes. The levels of NAD^+ directly determine the level of activity of the sirtuin family, which comprises seven members. Studies have shown that the sirtuin family catalyzes a variety of reactions related to NAD, such as deacetylation, diacylation, and ADP-ribosylation. Notably, it also has the ability to deacetylate histone and non-histone targets [43].

In mammalian SIRT1–*7*, each member has a catalytic domain that binds NAD^+. Each protein is predominantly located in a different subcellular compartment, which is closely related to intracellular biological functions [43]. Specifically, the seven sirtuin family proteins (SIRT1–7) are located in different subcellular compartments. SIRT1 and 2 are mostly located in the cytoplasm, SIRT3, 4, and 5 in the mitochondria, and SIRT1, 2, 6, and 7 in the nucleus.

SIRT1 is a well-known long-lived protein and plays a key role in mediating cell death, viability, and cardiovascular disease. SIRT2 is essential for the regulation of the cell division cycle. Improved expression of SIRT3 has been shown to be associated with human lifespan and also protects cardiomyocytes from senescence and oxidative stress while inhibiting myocardial hypertrophy. Recently, SIRT6 has also been shown to reduce myocardial hypertrophy, while SIRT7 is thought to regulate apoptosis and stress responses in the heart [44].

Thus, the sirtuin family is not only linked to antioxidant and oxidative stress responses but also to longevity, mitochondrial function, DNA damage repair, and metabolism. In the following sections, we explore the function of SIRT1–7 in cells and the relationship between the sirtuin family and aging in more detail.

3.2 Sirtuins and Aging

Sirtuins are often referred to as longevity proteins, and their activity is dependent on NAD^+. Each member of the sirtuin family has unique characteristics in combating aging (Table 2, Ref. [27,45–52]). The specific roles and characteristics of SIRT1–7 will be discussed in detail below.

3.2.1 SIRT1

SIRT1, the most extensively studied of the sirtuin family, is located on chromosome *10q21.3* and consists of eight introns and eleven exons. The protein structure of SIRT1 contains 747 amino acid residues and a catalytic core region surrounded by variable NH_2 and COOH-terminal structural domains of approximately 250 amino acids, which provide active regulatory sites for cellular activities such as metabolic regulation, gene transcription, and post-translational modification. SIRT1 alters its transcriptional and enzymatic activity and protein levels through acetylation and deacetylation of these substrates.

SIRT1 is involved in regulating several proteins, including FOXO, *p53*, nuclear factor-κB (NF-κB), nuclear factor e2-related factor 2 (Nrf2), AMPK, β-catenin, mitochondrial peroxisomes, proliferator-activated receptor γ coactivator, peroxisome proliferator-activated receptor coactivator 1α (PGC-1α), peroxisome proliferator-activated receptor-γ (PPARγ), *etc*. For example, SIRT1 deacetylates and activates FOXOs, which can be positively or negatively regulated by SIRT1 [44]. SIRT1 also affects cellular stress resistance by directly regulating *p53* and NF-κB signaling pathways [53]. SIRT1 deacetylates and inactivates *p53*, which reduces the binding of *p53* to DNA cis-elements and reduces the apoptosis-inducing activity of *p53*, thus enhancing the ability of cells to resist aging through oxidative stress [45]. However, increasing *p53* activity decreases SIRT1 expression [10].

SIRT1 also inhibits NF-κB activity in macrophages and endothelial cells through deacetylation, which is important for preventing atherosclerosis and resisting inflammatory responses in blood vessels, thus achieving the goal of preventing vascular aging [27]. Moreover, SIRT1 can also play a role in delaying the aging of the brain [45]. Additionally, overexpression of SIRT1 can inhibit myeloid cell senescence, promote cell proliferation, and suppress the apoptogens. Experiments have shown that inhibition of SIRT1 reduces bone density and accelerates bone aging, accelerating cellular senescence. Furthermore, SIRT1 can prevent age-related diseases such as diabetes, hypertension, and cancer [45].

Mitochondrial cell function is frequently disrupted with age, which promotes the production of ROS and increases oxidative stress, which severely affects cellular senescence. One of the major transcriptional coactivators regulating mitochondrial function is peroxisome proliferator-activated receptor coactivator1α (PGC-1α), which maintains mitochondrial homeostasis. Activation of SIRT1 prevents endothelial senescence through deacetylation of PGC-1α. Thus, the SIRT1-PGC-1α pathway can prevent age-related diseases and delay aging by maintaining mitochondrial homeostasis [27]. Furthermore, SIRT1 can maintain the integrity of hematopoietic stem cells and improve their ability to self-renew [54].

Overall, SIRT1 is involved in a range of pathophysiological processes such as cell differentiation, proliferation and metabolism, *DNA* repair, regulation of inflammatory responses, oxidative stress, and immune responses [46].

3.2.2 SIRT2

The SIRT2 gene is on chromosome *19q13. 2* and comprises 389 amino acids, is 21 kb long, contains 17 exons, and has a catalytic center of 323 amino acids. SIRT2 was identified in a yeast model and is present in the nucleus [47]. It has been shown to regulate the cell cycle and a range of biological processes, including telomere and ribosomal DNA (rDNA) silencing, information transduction of various substances, and metabolism through the deacetylation of his-

Table 2. The sirtuin family and their roles.

The sirtuins	Positioning	Role	Experimental subjects	References
SIRT1	Nucleus	Anti-aging effects on smooth muscle and blood vessels, slowing down brain aging and participating in physiological activities such as cell survival and apoptosis	Mice	[27,46]
SIRT2	Nucleus, cytoplasm	Anti-vascular senescence, regulates the cell cycle and improves autophagy	Mice	[47,48]
SIRT3	Mitochondria	Antioxidant effect, metabolic control	Mice	[50]
SIRT4	Mitochondria	Regulation of metabolism	Mice	[49]
SIRT5	Mitochondria	Regulating metabolism and enhancing autophagy	Mice	[49,51]
SIRT6	Nucleus	Genome and telomere stabilization, DNA repair, anti-aging effects on smooth muscle cells, endothelial cells	Mice	[45,52]
SIRT7	Nucleus	Anti-aging, DNA damage repair through enhanced stress resistance	Mice	[49]

SIRT1–7, Members of the sirtuin family.

tones and various transcription factors and cofactors [27].

A previous study identified an association between SIRT2 and cellular senescence, whereby inhibition of SIRT2 leads to an increased cellular oxidative stress response. This augmentation further promotes the development of cellular senescence [48]. Additionally, SIRT2 deficiency has been shown to lead to myocardial hypertrophy in mice [55], while it can control macrophage inflammatory responses by regulating *NF-κB* activity to counteract aging. SIRT2 also plays an important role in the central nervous system [49]. Interestingly, SIRT2 expression levels increase with age, and higher expression levels are associated with a higher likelihood of neurodegenerative lesions [10]. SIRT2 may increase the risk of vascular aging. Further clinical studies are needed [45].

3.2.3 SIRT3

SIRT3 is a deacetylase found in mitochondria. In an experimental study, it was observed that mice deficient in SIRT3 exhibited significantly elevated levels of mitochondrial protein acetylation when compared to mice lacking SIRT4 and SIRT5. Therefore, SIRT3 is a crucial mitochondrial deacetylase [13]. Recent research has shown that SIRT3 regulates mitochondrial function and biosynthetic pathways through lysine deacetylation and maintains basal ATP levels by regulating the electron transport chain (ETC). Several clinical studies have also shown that SIRT3 deficiency can promote the development of metabolic syndrome, which may cause cardiovascular disease. Moreover, SIRT3 levels are reduced by 50% in the kidneys of aging mice compared with young mice. Therefore, low SIRT3 expression may affect the human lifespan. Overall, SIRT3 is protective against vascular senescence in rodents and humans [49]. SIRT3 can also protect cardiomyocytes from oxidative stress-mediated cellular damage and prevent the development of cardiac hypertrophy. SIRT3 also participates in defense against several cardiac illnesses [56]. Elevated SIRT3 levels can prevent apoptosis and oxidative stress and improve the maintenance of mitochondrial homeostasis in many patients with cardiovascular diseases. Elevated SIRT3 levels also have anti-aging, anti-fibrotic, and anti-inflammatory effects [50]. The research showed that high SIRT3 expression is associated with longer a life span in mammals. SIRT3 can also maintain cellular mitochondrial function of the kidney [57]. SIRT3 is a key regulator of renal cell mitochondrial dynamics. Furthermore, the acetylase target of SIRT3 is activated in specific conditions, such as CR [58]. SIRT3 has several deacetylase targets, including acetyl coenzyme a synthase2 (AceCS2), LKB1, ornithine transcarbamylase (OTC), and FOXO3A [34]. SIRT3 is also involved in the tricarboxylic acid cycle, redox respiratory chain, and the TCA cycle [59]. Notably, the TCA cycle is crucial in the regulation of aging and maintenance of homeostasis [3]. Overall, the acetylase activity of SIRT3 plays an important role in mitochondria by regulating multiple metabolic pathways and mitochondrial functional metabolism and resisting oxidative stress, thus stabilizing cells against aging [60]. SIRT3 also promotes DNA damage repair and maintains genome stability [10].

3.2.4 SIRT4, 5

SIRT4 is primarily located in the matrix of mitochondria consisting of structural domains and catalytic centers [61]. Firstly, it is involved in the regulation of cellular metabolism [49], providing a carbon source for the citric acid cycle by activating the glutamine cycle [59], and also inhibits β-oxidation of fatty acids by inhibiting peroxisome activated receptor α (PPARα) [13]. Therefore, SIRT4 can intervene in metabolism by regulating glutamine catabolism, fatty acid oxidation, and amino acid catabolism. However, it is worth noting that SIRT4 plays an opposite

role to SIRT3 and SIRT5 when involved in cellular regulation [13]. SIRT4 also plays a key role in the regulation of insulin secretion and in the maintenance of stable glucose metabolism [57]. Secondly, SIRT4 expression reduces cell mortality after DNA damage [62] and enhances inhibition of cancer cell metastasis and invasion [63]. In summary, SIRT4 can regulate protein function, cell metabolism, and DNA damage [60].

SIRT5 is a NAD-dependent protein lysine demethylase, desuccinylase, and deglutarylase, primarily located in the mitochondrial matrix, cytoplasm, and nucleus. SIRT5 catalyzes demethylation, desuccinylation, and deglutarylation of mitochondrial enzymes through various metabolic pathways related to glycolysis, fatty acid oxidation, and the urea cycle. However, mitochondrial SIRT5 has a few targets, including carbamoyl phosphate synthetase 1 (CPS1) [64] and succinate dehydrogenase (SDH) [65]. SIRT5 is highly expressed in the brain, heart, liver, and kidney [64]. SIRT5 can regulate amino acid catabolism and regulate ammonia production and ammonia-induced autophagy by regulating glutamine metabolism in non-hepatocytes. In addition, SIRT5 controls ammonia levels through interaction with the mitochondrial GLS and desuccinylation. In addition to reducing ammonia production, SIRT5 can also reduce autophagy and mitochondrial autophagy [51]. Recent studies have suggested that SIRT5 can be considered a tumor promoter or suppressor and may be associated with tumor formation. Moreover, it may inhibit the development of neurodegenerative diseases [66]. Peroxisome proliferator-activated receptor co-activated receptor-1α (PGC-1α) and AMP-activated protein kinase (AMPK) are major regulators of SIRT5. AMPK overexpression inhibits SIRT5 expression [67]. Moreover, SIRT5 is associated with glycolysis [57]. In addition, SIRT5 plays an important role in the TCA cycle and the electron transport chain (ETC) [66]. Overall, SIRT5 is involved in glycolysis, the TCA cycle, fatty acid oxidation, electron transport chain, ketone body formation, nitrogenous waste management and ROS detoxification, and oxidative stress. Therefore, SIRT5 plays an important role in regulating metabolism and maintaining cellular homeostasis.

3.2.5 SIRT6, 7

SIRT6 is a class III histone deacetylase found in the nucleus. SIRT6 exhibits deacetylase activity that can activate free histones when packaged into nucleosomes and undergo deacetylation. SIRT6 activity is nucleosome-dependent and can only participate in activation when bound to nucleosomes. SIRT6 regulates mitosis and maintains its fidelity [68]. SIRT6 has been shown to possess the ability to inhibit premature senescence and exert anti-denervation effects on endothelial cells and blood vessels. Furthermore, it has demonstrated the capacity to prevent the progression of atherosclerosis and the senescence of smooth muscle cells associated with aging [52]. SIRT6 also controls gene expression changes associated with aging by modulating nuclear factor *kB* [69]. Clinical studies have shown that SIRT6 deficiency can substantially increase the probability of severe metabolic disorders, genomic instability, inflammatory diseases, and oncological diseases in mice. In contrast, SIRT6 overexpression extends the lifespan of mice [70]. Notably, SIRT6 is a chromatin-associated protein that stabilizes the genome and regulates telomeric chromatin in human cells. This ensures efficient telomere replication and prevents structural abnormalities of telomeres [71]. SIRT6 also regulates the homeostasis of renal function [57]. Overall, SIRT6 mainly promotes the repair of DNA damage and maintains genomic stability [10]. Recent experiments have shown that SIRT6 overexpression increases the lifespan of male mice by approximately 16% [69].

SIRT7 is a class III histone deacetylase primarily found in the nucleus. It functions in chromatin and is involved in cellular transformation, prevention of fatty liver disease, and is also associated with tumor formation *in vivo* [68]. For example, SIRT6,7 may enhance the invasion and metastasis of osteosarcoma cells [72]. SIRT7 is also crucial for ribosomal DNA transcription, has acetylase activity and can regulate the transcriptional activity of RNA polymerase I [57]. In addition, SIRT7 also exhibits deacetylase, desuccinylase, and deglutaminase activity [73]. Most importantly, it is involved in a number of stress responses in the endoplasmic reticulum and reduces DNA damage, and improves cell survival under genomic stress conditions [49].

4. Co-Regulation of Cellular Senescence by AMPK and Sirtuins

Studies have shown that AMPK interacts with the sirtuin family. Moreover, AMPK can directly or indirectly influence the sirtuin family to prolong lifespan. The findings indicate that the activation of AMPK and the sirtuin family can increase the lifespan of various organisms. AMPK can regulate lifespan and CR in worms and *Drosophila*. Similarly, SIRT1 is closely associated with the aging process in many organisms, including flies, *C. histolytica*, mice, worms, and mammals [27].

Metabolism (anabolism and catabolism) is a constant cell self-renewal that takes place in an organism, and it is associated with resistance to cellular senescence. Metabolism is a vital function in living organisms. Metabolism ensures continuous growth of cells and human life by maintaining physiological homeostasis. Therefore, metabolism is associated with cellular senescence. Anabolism involves the use of energy from adenosine triphosphate (ATP) or nicotinamide adenine dinucleotide phosphate (NADPH) to produce macromolecules in a biosynthetic process, whereas catabolism involves breaking down large components into smaller compounds that can be used directly in metabolism. Phosphatidylinositol 3-kinase/protein kinase

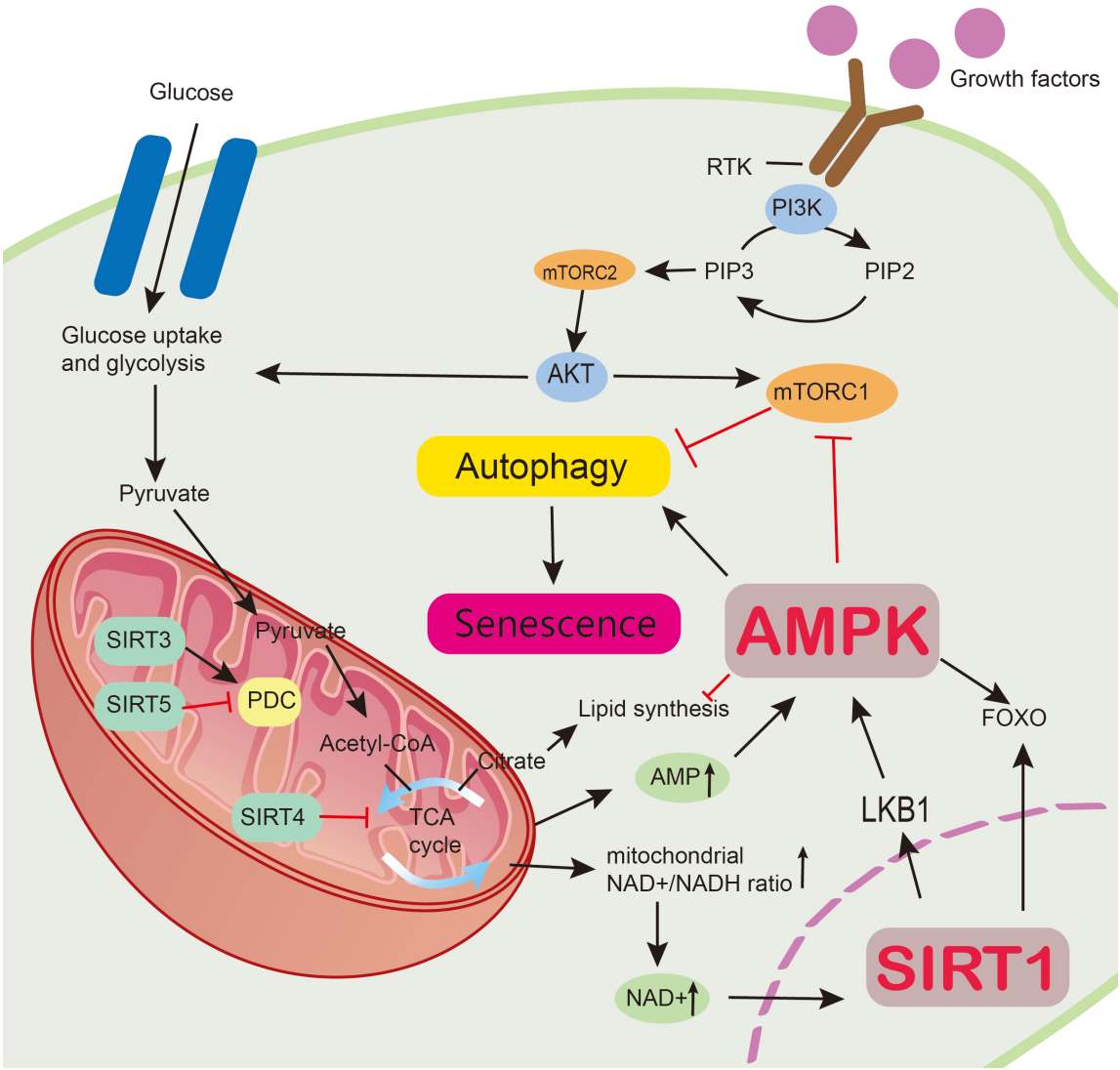

Fig. 2. Mechanism by which AMPK and sirtuin proteins regulate metabolism and resist aging. Multiple signaling pathways are involved in metabolic control, including the PI3K/AKT pathway, mTOR, AMPK, and sirtuins. Regulation of cellular senescence through the SIRT1/LKB1/AMPK pathway. AMPK, AMP-activated protein kinase; PI3K/AKT, Phosphatidylinositol 3-kinase/protein kinase B; mTOR, The Mammalian Target of Rapamycin; SIRT1, sirtuin 1; SIRT3, sirtuin 3; SIRT4, sirtuin 4; SIRT5, sirtuin 5; LKB1, liver kinase B1; FOXO, Forkhead box class O; mTORC2, mTOR complex 2; RTK, receptor tyrosine kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate.

B (PI3K/AKT), mTOR, AMPK, and sirtuins are crucial to achieving homeostasis [13]. Anabolism allows the release of growth factors that promote cell division, growth, and differentiation. Growth factors belong to a family of highly effective cell surface acceptors in the receptor tyrosine kinase (RTK) family. These receptors typically promote nutrient uptake. PI3K also regulates metabolism through the interaction between RTK and growth factors, which activate the *PI3K* receptor. When energy is sufficient, RTK can activate PI3K. Activated PI3K can catalyze phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), leading to the recruitment of AKT to the cell surface by PIP3, which facilitates glucose uptake via the main glucose transporter protein GLUT1. AKT is a serine/threonine-specific protein kinase that plays an important role in many cellular processes, including glucose metabolism, apoptosis, cell proliferation transcription, and cell migration. AKT is also an activator of glycolytic enzymes. AKT can also produce pyruvate, ATP, and NADPH [74]. However, the idea that AMPK and sirtuins are directly activated by affecting ATP and NADPH through the P13K/AKT pathway has not been confirmed. In addition, AKT can trigger lipid synthesis by enhancing the effective volume of acetyl coenzyme A. When under CR, ATP levels are reduced, and AMPK will be activated. Activated AMPK can inhibit mTORC1. Studies have shown that effective inhibition of mTORC1 during energy deficiency can prolong cell survival [75]. Another key protein

that is activated when energy is low is the sirtuin family. An experimental animal study was conducted by Katharine *et al*. [76] and revealed that SIRT3 was activated during CR by comparing SIRT3 gene knock-out mice with normal mice. Activated SIRT3 was effective in reducing oxidative stress and thus delaying cellular senescence [76]. Moreover, some animal experiments have shown that a certain degree of energy restriction will delay cellular senescence. The rats in the calorie restriction experimental group lived longer than the normal group [77]. Therefore, energy restriction can delay cellular senescence by reducing oxidative stress damage. It is considered a powerful intervention to improve disease and extend lifespan in mammals [76]. In conclusion, PI3K/AKT, mTOR, AMPK, and sirtuin signaling networks can regulate metabolism and delay cellular senescence (Fig. 2).

Sirtuin SIRT1-mediated deacetylated liver kinase B1 (LKB1), belonging to sirtuin family, is the upstream kinase of AMPK and activates both LKB1 and AMPK [14]. When under CR, SIRT1 is activated and can also sense intracellular NAD^+ levels. SIRT1 activates AMPK through deacetylation of LKB1, thereby regulating cell metabolism [13]. Lan demonstrated through animal experiments that SIRT1 can deacetylate LKB1 and be activated through the regulation of *Lys-48* and activating AMPK [78]. Another animal experiment demonstrated that estrogen effectively activates the SIRT1/LKB1/AMPK pathway. Activation of this signaling pathway reduces mitochondrial dysfunction and arterial aging [79] and stimulates increased cellular autophagic activity [80], which will be beneficial in extending cell longevity. Interestingly, we also found that activation of the SIRT1/LKB1/AMPK pathway reduced triglyceride (TG) accumulation to improve obesity [81]. Therefore, the SIRT1/LKB1/AMPK pathway is crucial in the inhibition of senescence (Fig. 2).

SIRT1 and AMPK can mutually regulate each other's activity through various pathways. AMPK enhances SIRT1 activity by increasing cellular NAD^+ levels, leading to deacetylation of the downstream target of SIRT1, PGC-1α. AMPK can lead to increased expression of nicotinamide adenine dinucleotide phosphate (NADPH), and nicotinamide phosphoribosyltransferase (NAMPT) can promote the synthesis of NAD^+. As SIRT1 is regulated by NAD^+, AMPK can regulate the activity of SIRT1 [14]. Several animal experiments had shown that when AMPK was induced to increase in mice, PGC-1α was deacetylated. In contrast, when SIRT1 was knocked out in mice, AMPK was unable to deacetylate PGC-1α. Therefore, AMPK enhances SIRT1 activity by increasing cellular NAD^+ levels, leading to deacetylation of PGC-1α, a downstream target of SIRT1 [82]. AMPK can increase cellular autophagy by regulating SIRT1 activity, indicating that AMPK can achieve resistance to cellular senescence by directly regulating SIRT1 [8]. These findings indicate that AMPK and sirtuins can inhibit cellular senescence.

5. Pre-Clinical Studies and Clinical Trials of AMPK and Sirtuins

In addition to regulating various biological signaling pathways and targets, SIRT1 can also prevent and treat age-related diseases, especially cardiovascular disease, diabetes, and neurodegenerative diseases. Although SIRT1 activation can treat aging-related illness through suppression of *p53*, this has not been recognized in clinics. In addition, SIRT1 overexpression can prolong the life span of mice, as demonstrated in an *in vivo* experiment [83]. Therefore, in-depth studies targeting AMPK and SIRT1 are needed to provide therapy for aging and aging-related diseases and the development of new drugs.

The AMPK and sirtuin family inhibit cellular senescence through multiple pathways. Some key and new clinical trials that have used AMPK and the sirtuin family are described below (Table 3). Free fatty acid receptor 4 (FFAR4) can activate the AMPK/SIRT3 signaling pathway, thus ameliorating renal tubular epithelial cell senescence after acute kidney injury [84]. Some clinical trials have found that diabetic patients have a shorter life expectancy, especially severely obese and insulin-resistant diabetic patients. However, metformin can treat pre-diabetic patients and also has an anti-aging effect on cells. Metformin inhibits inflammatory response and improves cell survival. AMPK is one of the metformin targets, indicating that metformin can inhibit cellular senescence through the AMPK pathway. Metformin can also inhibit cellular senescence through the mTOR signaling pathway [85]. Importantly, metformin can directly stimulate the activation of the longevity protein STIR1, thus inhibiting cellular senescence. Metformin can protect endothelial cells against elevated blood glucose by indirectly stimulating the downstream targets of STIR1, *p53*, and FOXO1. These findings indicate that AMPK and the sirtuin family form a network that can regulate cellular senescence, consistent with animal experiments. However, the effects of metformin on pathways associated with longevity in humans are unknown [86]. A clinical phase IV trial (NCT01765946) compared the efficacy of metformin (500 mg once daily) and placebo in patients with pre-diabetes for two months to assess the influence of metformin on longevity genes and inflammation in pre-diabetics. In that trial, longevity gene expression of SIRT1, *p66Shc*, *p53*, and mTOR was assessed in external peripheral blood mononuclear cells (PBMCs) *in vitro*. Monocyte polarization was also assessed via flow cytometry based on the expression of surface antigens (CD68, CCR2, CD163, CD206, CX3CR1) to determine the proportion of pro-inflammatory or anti-inflammatory cells. Inflammatory cytokines (TNF-α, MCP-1, IL-1, IL-6, IL-10, CCL12) were also detected in that trial. The *in vitro* research also evaluated the effects of AMPK activation or inhibition on long-lived gene and protein manifestations. The

Table 3. AMPK and sirtuins in clinical trials.

Gene/protein	Study type	Allocation	Ages eligible for study	Purpose of research	Intervention	State	Phase	Numbering
SIRT6	Clinical trial	Not applicable	60 years and older	SIRT6 expression in circulating leukocytes based on the aging quality.	Different blood sample	Completed	Not applicable	NCT0156717
SIRT1 SIRT6	Clinical trial	Not applicable	25 to 40 years	To prove that weight control is good for anti-aging.	Reduce calorie intake by 25%	Unknown	Not applicable	NCT01508091
7 sirtuins	Clinical trial	Randomized	19 to 30 years	Effect of intermittent fasting on antioxidant gene levels, sirtuins, and markers of mitochondrial biogenesis and aging.	400 IU vitamin D or 1000 mg vitamin C	Completed	Not applicable	NCT02132091
7 sirtuins	Clinical trial	Non-randomized	60 years and older	Determining whether the quality of aging affects insulin sensitivity.	Insulin perfusion at 1 $mU.kg^{-1}.min^{-1}$	Completed	Not applicable	NCT00951392
7 sirtuins	Observational	NR	20 years and older	Examine the links between arterial aging with TL, as expressed in different tissues, and LTL dynamics, as expressed in the difference between TLs of muscle and leukocytes.	NR	Unknown	NR	NCT02176941
AMPK SIRT1	Clinical trial	Randomized	40 to 75 years	Effects of metformin on longevity genes (SIRT1, *p66Shc*, *p53*, and mTOR) and inflammatory effects in pre-diabetic patients.	Metformin	Completed	Phase 4	NCT01765946
AMPK SIRT1	Clinical trial	Randomized	25 to 50 years	Effects on muscle and adipose tissue through dietary restriction and physical exercise.	Diet and exercise	Terminated	Not applicable	NCT01793896
SIRT3	Observational	NR	20 years and older	The relationship between the degree of skeletal muscle senescence and aging.	NR	Completed	NR	NCT01644279
SIRT1	Observational	NR	50 years and older	Study important factors in the pathophysiology of osteoporosis in patients with COPD.	NR	Completed	NR	NCT01067248
SIRT1 SIRT3 AMPK	Clinical trial	Randomized	65 years and older	The role of resveratrol in improving body function was investigated by looking at the link between changes in mitochondrial function and changes in body function.	Resveratrol 1000 or 1500 mg/day	Completed	Phase 2	NCT02123121
SIRT1	Clinical trial	Randomized	60 to 75 years	Exercise-related taurine supplementation in relation to metabolism in elderly sarcopenic obese women.	Taurine and exercise	Not yet recruiting	Not applicable	NCT05437952
AMPK	Clinical trial	Randomized	30 to 70 years	Using human leukocyte LC3 as a marker of autophagy and cellular senescence to validate the relationship between autophagy and senescence.	Metformin	Completed	Phase 3	NCT03309007
AMPK	Clinical trial	Randomized	40 to 75 years	The effect of metformin on people without disease	Metformin	Recruiting	Phase 3	NCT04264897
AMPK SIRT1	Clinical trial	Randomized	20 to 80 years	Acute effects of high-load resistance exercise on inflammation in young and elderly people	Resistance exercise	Recruiting	Not applicable	NCT05042167
AMPK	Clinical trial	Randomized	65 years and older	Effects on muscle in >65-year-old under different interventions	Training or high-quality protein	Recruiting	Not applicable	NCT03649698

AMPK, AMP-activated protein kinase; SIRT1–7, Members of the sirtuin family; mTOR, The Mammalian Target of Rapamycin; TL, Telomere length; LTL, Leukocyte telomere length; COPD, Chronic obstructive pulmonary disease; NR, Not reported.

levels of SIRT1 were significantly higher in volunteers treated with metformin after two months than those treated with a placebo. Furthermore, the placebo did not affect the expression of other genes. In addition, metformin treatment did not significantly increase mTOR protein levels [86]. However, some experiments have shown that metformin directly inhibits mTORC1 [87]. A clinical phase III trial (NCT03309007) investigated the effect of a short course of metformin treatment on cellular senescence and autophagy. This trial aimed to demonstrate the potential role of metformin in reducing inflammation, reducing muscle and tendon tissue degeneration, antitumor effects, reducing obesity and hyperglycemia, protecting cardiovascular function, and preventing neurodegeneration (age-related dementia) through the downstream induction of autophagy via AMPK and inhibiting mTOR proteins. This was a double-blinded controlled trial performed using pre-diabetic (defined as a glycosylated hemoglobin of 5.7~6.4%) admitted patients (30–70 years) with a BMI between 27 and 40 kg/m^2. The efficacy of metformin and placebo was also assessed after 12 weeks of treatment. The total daily doses of metformin and placebo were titrated to 1500 mg per day and 1944 mg per day, respectively. The impact of metformin on cellular autophagy and cellular senescence was evaluated by assessing senescence indicators, including leukocyte LC3 levels, transcription factor EB (TFEB) scores, total DNA methylation, and galactose lectin-3. LC3 data were collected at 0, 4, and 12 weeks and analyzed at 8 weeks post-sample collection. An increase in autophagic activity when LC3 puncta increases may indicate enhanced resistance to senescence. A phase III clinical trial (NCT04264897) with 148 subjects from two different research centers (Oklahoma Medical Research Foundation (OMRF) and the University of Wisconsin-Madison (UWM)) investigated groups of people more suitable for metformin treatment for 12 weeks. This was a double-blinded, placebo-controlled, two-center drug intervention trial. The participants were grouped into either an insulin sensitivity (IS) or insulin resistance group [16]. Half of each group were administered metformin, and the other half a placebo for 12 weeks. The participants participated in activities before or after the treatment to evaluate insulin sensitivity, glucose regulation, and biomarkers of aging. The participants also underwent skeletal muscle biopsies before and after the intervention to assess changes in mitochondrial function and mitochondrial remodeling before and after metformin therapy. The physical function of patients in NCT03649698 and NCT05042167 trials were studied at 12 weeks and 24 weeks after high-intensity resistance exercise, basic training, or increased dietary fiber consumption. The effect of muscle on aging and inflammatory response was assessed by measuring muscle mass, muscle strength, compliance with interventions (exercise, protein supplements, and omega-3 supplements), and functional, cognitive, and nutritional status. The changes in the effects of increased body metabolism on the organism were also assessed. Another clinical trial (NCT01793896) with middle-aged volunteers with metabolic syndrome aimed to demonstrate whether a Mediterranean diet and physical exercise increase lifespan through the activation of signaling pathways due to dietary restriction. The muscle tissue samples were obtained before and 3 months after the intervention to study AMP-activated protein kinase (AMPK) and sirtuin1 (SIRT1) expression and ROS levels. Resveratrol is a non-flavonoid polyphenolic organic compound with antitoxin effects. Resveratrol is produced by many plants. Resveratrol can be orally taken, metabolized, and eliminated in the urine or sweat. Both *in vitro* and animal studies showed that resveratrol has antioxidant, anti-inflammatory, anti-cancer, and cardiovascular protective effects. A clinical trial (NCT02123121) also showed that resveratrol could improve the function of mitochondria in the leg muscles of elderly people. The trial aimed to study the relationship between changes in mitochondrial function and changes in body function and investigate the role of resveratrol in improving body function. In that trial, control and experimental groups were set up. Patients in experimental group 1 were administered one vegetable cellulose capsule orally after a full meal (breakfast, lunch, and dinner) for 90 days, whereas those in experimental group 2 were administered one resveratrol capsule orally after a full meal (breakfast, lunch, and dinner) for 90 days (1000 mg/d). Patients in experimental group 3 were administered one resveratrol capsule orally after a full meal (breakfast, lunch, and dinner) for 90 days (1500 mg/d). The results were assessed by observing changes in cytochrome oxidase (COX), citrate synthase [62], PGC-1α, sirtuins (SIRT1 and SIRT3), AMPK, and other proteins in the muscle. Resveratrol can also enhance SIRT1-mediated deacetylation [88]. In a clinical trial registered as NCT0156717, SIRT6 leukocyte expression was measured in the blood to investigate its impact on the process of aging. Another clinical trial registered as NCT02132091 randomly assigned healthy young volunteers to undergo three weeks of intermittent fasting, receive antioxidant supplements or receive a placebo. The aim of this trial was to assess the effects of CR on resistance to aging, as well as its influence on oxidative stress. The changes in levels of active oxidants, antioxidant genes, sirtuins, and markers of mitochondrial biogenesis and aging were also assessed.

6. Summary and Outlook

The substantial increase in life expectancy and the ongoing decline in birth rates have resulted in a significant rise in the aging population. The process of aging is influenced by various factors, including environmental conditions, dietary habits, and emotional well-being. Nonetheless, cellular senescence should be completely inhibited. AMPK and the sirtuin family can inhibit cellular senescence. AMPK can maintain energy metabolism, increase cellular autophagy, reduce oxidative stress, and inhibit in-

flammation and endoplasmic reticulum stress. The sirtuin family has an anti-oxidation effect and can regulate cellular metabolism, DNA damage repair, and autophagy, which are related to cellular senescence. AMP-activated protein kinase (AMPK) and the sirtuin family can directly or indirectly inhibit cellular senescence by regulating other substances. For example, AMPK can extend lifespan by inhibiting the CRTC-1/CREB signaling pathway. AMPK can also inhibit cellular senescence by inhibiting autophagy through mTOR or increasing autophagy through ULK1. FOXO combined with nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB, Nrf2/SKN-1 signaling pathway, and AMPK can significantly inhibit cellular senescence. SIRT1 and SIRT2 can inhibit cellular senescence. Moreover, SIRT1 regulates the physiological activation of cell survival, apoptosis, resilience, and inflammation. SIRT1 activation can also extend the biological life span. SIRT1 activation also affects cellular stress resistance by directly regulating *p53* and NF-κB signaling pathways. Furthermore, the development of science and technology has improved the understanding of cellular senescence inhibition. Also, further studies should determine how to better target anti-aging treatments through the above pathways, the side effects of the treatments, and how to eliminate harmful side effects to maximize lifespan. Further clinical research is needed to confirm the mechanism by which AMPK and the sirtuin family can be used to inhibit cellular senescence in the clinical setting to improve quality of life.

Author Contributions

XP and JH designed and supervised the study. YZ,TL, YH and WW provide ideas. YH ,YZ, TL and YL reviewed the references. YH and XP wrote the manuscript. YL, YZ, WW and TL contributed to tables and figures. YZ, YL, TL,and WW reviewing the manuscript for important intellectual content. XP, WW and JH revised the manuscript. XP acquired funding. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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