Sirtuin signaling in cellular senescence and aging

Shin-Hae Lee, Ji-Hyeon Lee, Hye-Yeon Lee & Kyung-Jin Min*
Department of Biological Sciences, Inha University, Incheon 22212, Korea

Sirtuin is an essential factor that delays cellular senescence and extends the organismal lifespan through the regulation of diverse cellular processes. Suppression of cellular senescence by Sirtuin is mainly mediated through delaying the age-related telomere attrition, sustaining genome integrity and promotion of DNA damage repair. In addition, Sirtuin modulates the organismal lifespan by interacting with several lifespan regulating signaling pathways including insulin/IGF-1 signaling pathway, AMP-activated protein kinase, and forkhead box O. Although still controversial, it is suggested that the longevity effect of Sirtuin is dependent with the level of and with the tissue expression of Sirtuin. Since Sirtuin is also believed to mediate the longevity effect of calorie restriction, activators of Sirtuin have attracted the attention of researchers to develop therapeutics for age-related diseases. Resveratrol, a phytochemical rich in the skin of red grapes and wine, has been actively investigated to activate Sirtuin activity with consequent beneficial effects on aging. This article reviews the evidences and controversies regarding the roles of Sirtuin on cellular senescence and lifespan extension, and summarizes the activators of Sirtuin including Sirtuin-activating compounds and compounds that increase the cellular level of nicotinamide dinucleotide. [BMB Reports 2019; 52(1): 24-34]

INTRODUCTION

The Sirtuin family is nicotinamide dinucleotide (NAD+)-dependent deacetylases having remarkable properties in preventing diseases and reversing some aspects of ageing. Sirtuins are known to regulate diverse cellular processes including DNA repair, fat differentiation, glucose output, insulin sensitivity, fatty acid oxidation, neurogenesis, inflammation, and aging (1-3). Research interests increased after a report showed that extra copies of SIR2, a member of Sirtuin in budding yeast Saccharomyces cerevisiae, extended the lifespan by 30% by preventing the formation of extrachromosomal DNA circles (4). The main activity of Sirtuins is deacetylation (5, 6); recent studies have indicated other enzymatic activities, including O-ADP-ribosylation, demalonylation, desuccinylation, and depropionylase (7). Thus, a recent proposal has renamed Sirtuin as deacylase, and not deacetylase.

Unlike budding yeast, multicellular organisms have more than one Sirtuin in their genome. Caenorhabditis elegans has four Sirtuins (sir-2.1, sir-2.2, sir-2.3, and sir-2.4), where sir-2.1 is the most similar to the S. cerevisiae SIR2. In an experiment using sir-2.1 fused with mCherry fluorescence marker, sir-2.1 was shown to be expressed in the nerve cells of the head, hypodermis, muscle and intestinal cells in C. elegans (8). The expression of sir-2.1:mCherry was found to be partially nuclear-localized when excess food is available, and was localized in the nuclei of intestines and muscles under nutrient deprived conditions (8). Drosophila melanogaster has five Sirtuins (dSirt1, dSirt2, dSirt4, dSirt6, and dSirt7), of which SirT1 (better known as dSir2) is most similar to S. cerevisiae SIR2 (9), and high levels are found in the nuclei of neurons and fat bodies (10). Although recently reported that fly dSirt4 contains a mitochondrial targeting sequence (11), the dSirt4 knockout fly appeared healthy, and the mitochondria respiratory function was not disrupted (11). In mammals, there are seven Sirtuins (SIRT1-SIRT7) having different profiles of enzymatic activity and subcellular compartmentation (12). SIRT1, having the highest sequence homology to yeast SIR2, is predominately found in the nucleus but also shuttles between the cytoplasm and nucleus (13). SIRT2 is generally found in the cytoplasm, but binds to the chromatin during mitosis (13). SIRT3 resides in the mitochondria and is translocated to the nucleus in response to stress (such as DNA damage) (13, 14). SIRT4 and SIRT5 are localized in the mitochondria and SIRT6 and SIRT7 are mostly localized in the heterochromatin regions and nucleoli, respectively (13). The main activity of SIRT4 and SIRT6 are ADP-ribosylation, whereas SIRT5 exerts demalonylation and desuccinylation activities (15). The subcellular localization and enzymatic activities of Sirtuins are summarized in Table 1.

ANTI-AGING EFFECTS OF SIRTUIN

Sirtuin in cellular senescence

Cellular senescence is a physiological phenotype aimed at
Table 1. Properties and functions of Sirtuins related with senescence and aging

| Sirtuin | Cellular localization | Activity | Functions in cellular senescence and aging |
|---------|-----------------------|----------|------------------------------------------|
| Yeast   | SIR2                  | Nucleus  | Deacetylase                              |
|         |                       |          | DNA damage repair                        |
|         |                       |          | Replicative lifespan extension           |
|         |                       |          | Cell cycle arrest                         |
| C. elegans | sir-2.1 | Nucleus and cytoplasm | Deacetylase | Lifespan extension |
|         | sir-2.2 | Mitochondria | Unknown | Lifespan extension |
|         | sir-2.3 | Mitochondria | Unknown | Lifespan extension |
|         | sir-2.4 | Nucleus | Unknown | Stress resistance |
| Drosophila | Sirt1 (dSir2) | Nucleus and cytoplasm | Deacetylase | Lifespan extension |
|         | Sirt4 | Mitochondria | Unknown | Lifespan extension |
| Mammal  | SIRT1 | Nucleus and cytoplasm | Deacetylase | Lifespan extension |
|         | SIRT2 | Cytoplasm | Deacetylase | DNA repair |
|         | SIRT3 | Mitochondria | Deacetylase | Cell cycle arrest |
|         | SIRT4 | Mitochondria | ADP-ribosyl-transferase | Mitochondrial function |
|         | SIRT5 | Mitochondria | Deacetylase | Oxidative stress |
|         | SIRT6 | Mitochondria (chromatin) | Deacetylase | Fatty acid oxidation |
|         | SIRT7 | Nucleolus | Deacetylase | Apoptosis |

permanent cell cycle arrest, and is morphologically identified as flattening, increased size of nucleus and nucleoli, and the appearance of vacuoles in the cytoplasm (16). In addition, several biomarkers developed for cellular senescence are targeted towards the senescence-associated β-galactosidase (SA-β-gal), telomere attrition, senescence-associated heterochromatic foci, cell cycle arrest in the G1 phase, and accumulation of DNA damage with the high level of ATM, p53, p16, and p21 (17). Although cellular senescence is considered to be a beneficial process to suppress the accumulation of aberrant cells caused by stress in young organisms, it is detrimental in older organisms to induce age-related phenotypes. In addition, senescent cells are known to be increased by aging (18).

Although still under debate and not fully defined, growing evidences have shown that Sirtuin is an essential factor in delaying cellular senescence and extending organismal lifespan. Especially, the role of Sirtuin on the protection from cellular senescence has mainly been investigated with mammalian SIRT1 and SIRT6. The levels of Sirtuins, including SIRT1 and SIRT6 but not SIRT2, are reported to decrease in senescent cells of mouse embryonic fibroblasts, lung epithelial cells, human endothelial cells and macrophages exposed to oxidants (19-22). In addition, the reduction of SIRT1 and SIRT6 using pharmacological inhibitors, siRNA or miRNA, promotes premature senescence-like phenotypes in endothelial cells (23-25). Conversely, the overexpression of SIRT1 and SIRT6 suppresses the cellular senescence in angiotensin II-treated human coronary artery endothelial cells, primary porcine aortic endothelial cells, and stress-exposed lung cells (22, 26-28). Taken together, these results support that Sirtuins have a role in cellular senescence.

The Sirtuin-related suppression of cellular senescence is mainly mediated through the prevention of telomere attrition and the promotion of DNA damage repair. Sirtuins play vital roles in sustaining genome integrity, by contributing in maintaining the normal chromatin condensation state, and responding to DNA damage and repair. Especially, the nuclear form of Sirtuins, such as SIRT1, SIRT6 and SIRT7, act as transcriptional regulators to suppress gene expression by stabilizing the chromatin structure (2). SIRT1 deacetylates histones H3, H4 and H1 and more than 50 non-histone proteins, including DNMT1, transcription factors and DNA repair proteins (29). Similar to mammalian Sirtuins, Drosophila
Sirtuin and its activators: promising targets for longevity
Shin-Hae Lee, et al.

DSir2 is also involved in the epigenetic inheritance of silent chromatin states (30), and the mutation of dSir2 was reported to suppress the heterochromatin-mediated silencing phenomenon known as position effect variegation (31). SIRT1 and SIRT6 are known to regulate the expression of telomere reverse transcriptase required for telomere elongation (32), and to deacetylate histone 3 lysine 9 (H3K9) and H3K56 resulting in maintaining the telomeric integrity (33). In addition, SIRT1 and SIRT6 were shown to be recruited to the damaged sites and promote DNA repair through deacetylating the repair proteins such as poly (ADP-ribose) polymerase (PARP)-1, Ku70, NBS, and Werner (WRN) helicase (34-37). SIRT4 also plays a role in DNA damage by regulating the mitochondrial glutamine metabolism (38). Furthermore, Sirtuins modulate cellular senescence through the deacetylation of a variety of signaling molecules such as FOXO, NFκB, and p53. SIRT1 deacetylates FOXO3 and FOXO4, potentiating the FOXO-induced cell cycle arrest (39, 40), and deacetylates all the major acetylation site of p53 (41), thereby suppressing the oncogene- or stress-induced cellular senescence (27, 42). Furthermore, SIRT6 regulates the RelA subunit of NFκB by modifying the cellular senescence-related gene expression (43).

In addition to the suppression of senescence of mitotic cells, Sirtuin also modulates the senescence of stem cells, and is required for the maintenance of stem cell self-renewal (44). The expression level of SIRT1 is reported to be higher in embryonic stem cells, but decreases in differentiated cells through the miRNA-mediated post-transcriptional regulations (45). Reduction of SIRT1 resulted in increased DNA damage, and induced aging phenotypes in hematopoietic stem cells and endothelial progenitor cells (46, 47), whereas an overexpression of SIRT1 delayed the senescence of bone marrow-derived mesenchymal stem cells (48). In addition to SIRT1, SIRT3 (a mitochondrial type of Sirtuin) is also highly expressed in hematopoietic stem cells (49), suggesting that SIRT3 might also function in stem cells.

Sirtuin in organismal lifespan
In addition to the roles in cellular senescence, it is well established that Sirtuin regulates the organismal lifespan in several animal models. Increased expression levels of Sirtuin, especially yeast SIR2 and its homologues, extends the lifespan of budding yeast S. cerevisiae, worms C. elegans, fruit flies D. melanogaster, and mice (4, 10, 50, 51). The first investigation for the longevity effect of SIR2 was established using the yeast model system almost 20 years ago, in which the complex of SIR2/3/4 extended the replicative lifespan of S. cerevisiae by silencing the HM loci and preventing a/a co-expression; SIR2 alone also extended the lifespan by repressing the recombination and generation of toxic rDNA circles (4). The longevity effect of SIR2 has been confirmed in higher organisms, while there are different mechanisms of exerting longevity effects in yeast, including changes in mitochondrial function and biogenesis, suppression of inflammation, and regulation of genomic stability (52). A 7-fold overexpression of sir-2.1 extended the mean lifespan of worms by 14.8-50.5% (50), whereas a low-copy overexpression of sir-2.1 extended the lifespan by 26.2% (53). In addition, sir-2.1 mutation resulted in decreased lifespan of C. elegans (4, 54, 55). In Drosophila, overexpression of dsir2 using a P-element mediated insertion of the UAS sequence upstream of dsir2 extended the lifespan (10), whereas dsir2 null mutants showed a shortened lifespan (31). Of note, the overexpression of dsir2 in the pan-neuronal cells or fat body extended the lifespan up to 52% and 32.2%, respectively, but the dsir2 induction in motoneuron or muscles had no effect on the lifespan (10, 56). These results indicate that the prolongevity effect of Sirtuin is tissue-specific. Similarly, mice overexpressing SIRT1 specifically in the hypothalamus has increased median lifespan by 16% in females and 9% in males (57).

Sirtuins other than SIRT1 are also reported to exert a prolongevity effect. The transgenic male mice overexpressing SIRT6 showed a significantly longer lifespan than wild-type mice by 16% (51), whereas the SIRT6- and SIRT7-deficient mice lived shorter than controls (43, 58). In addition, a polymorphism in SIRT3 has been reported in European centenarians (59). In Drosophila, the overexpression of dSirt4 (which has a mitochondria-targeting sequence) in the whole body or fat body, was reported to extend the lifespan and increase the resistance to starvation (11). The expression of dSirt4 was induced by starvation in the fat body, and a deficiency of dSirt4 resulted in decreased fertility, locomotion activity, and lifespan (11).

The molecular targets of this longevity effect of Sirtuins have been actively investigated. Sirtuins are found to especially interact with all the major conserved longevity pathways, such as AMP-activated protein kinase (AMPK), insulin/IGF-1 signaling (IIS), target of rapamycin (TOR), and forkhead box O (FOXO). Of these, FOXO transcription factor is the most fascinating target of Sirtuin. In C. elegans, the extension of lifespan by elevation of sir-2.1 was shown to be dependent ondaf-16, the homologue of FOXO in worms (50, 53, 60). Loss of daf-16 using mutants or RNAi treatment abolished the lifespan extension of sir-2.1 overexpression (50, 53, 60). Daf-16 was reported to physically interact with Sir-2.1 under heat stress (60), and sir-2.1 reduction completely prevented the subsequent activation of Daf-16 target genes, although reduction or overexpression of sir-2.1 had no effect on the nuclear translocation of Daf-16 (61). Conversely, dSir2 is also shown to be important for dFOXO-dependent lifespan extension in Drosophila. Lifespan extension by the overexpression of constitutively active dFOXO in the adult fat body was abrogated when dSir2 was knocked down using dSir2 RNAi (56).

Considering that FOXO is a major component in the IIS cascade to promote lifespan extension and stress resistance,
several evidences have reported the association of the IIS pathway with the prolongevity effect of Sir2uin. In C. elegans, Sir-2.1 does not interact physically with Daf-16 when the expression of insulin-like receptor (daf-2) was decreased via daf-2 RNAi (60), and the deletion of sir-2.1 had no effect on the lifespan of a long-living daf-2 mutant (55). In addition, deletion of daf-2 did not result in further extending the lifespan of worms overexpressing sir-2.1 (50), and the reduction of dSir2 by mutation or RNAi expression showed a decrease in starvation survival and systemic insulin signaling in Drosophila (62). These results indicate that the lifespan extension by IIS reduction is associated with SIR2. In mammals, the relationship of IIS and Sir2uin has also been well investigated. SIRT7 is reported to play a crucial role in metabolic homeostasis and IIS (63, 64). In addition, SIRT6 transgenic mice express lower serum levels of IGF1, higher levels of IGF-binding protein 1, and altered phosphorylation levels of major components of IGF1 signaling (51).

AMPK signaling belongs to the protein kinase family and restores cellular energy levels. Increased AMPK activity is known to extend the lifespan of some model organisms. The mutation of AMPK (aak-2) in C. elegans abrogated the lifespan extension by sir-2.1 expression (65), indicating that AMPK also contributes to the Sir2uin-induced lifespan extension. SIRT1 activates AMPK through the direct deacetylation of LKB1, a regulator of AMPK, and AMPK is known to activate SIRT1 through the elevation of NAD+ levels (51). In addition, AMPK contributes to the prolongevity effect of IIS, suggesting that these longevity pathways intricately cross-talk with each other.

Apart from these, several other molecules are also reported to mediate lifespan extension by Sir2uin overexpression, including 14-3-3, kat-1, hcf-1, and cts-1 in C. elegans. The 14-3-3 protein is a small acidic protein that alters the subcellular localization of its target. The mutation of par-5 and ltt-2, encoding the two proteins of a conserved 14-3-3 family in worms, abolished the lifespan extension by sir-2.1 overexpression (60, 67). In addition, in vivo GST-pull down assay and immunoprecipitation assay revealed that Sir-2.1 directly interacts with PAR-5 and FTT-2 (67), and both of which are necessary for the SIR2.1-mediated transcription activation of the DAF-16 target genes sod-3 and hsp-16.2 (61). These results support the concept that the lifespan-extension effect of SIR2 is mediated by the 14-3-3 protein. In addition, a study of mutant screening reported that loss-of-function mutations of ketoacyl thiolase (kat-1) resulted in premature aging and fully suppressed the lifespan extension exerted by overexpression of sir-2.1 (68). Also, host cell factor 1 (hcf-1), a nuclear co-repressor of FOXO, was shown to act downstream of sir-2.1 to modulate the lifespan in C. elegans (69). Furthermore, mitochondrial regulators such as cts-1 and lzo-1, and the mitochondrial unfolded protein response (UPRmt) gene hsp-6, were reported to increase by sir-2.1 overexpression, and the knock-down of UPRmt regulator ubl-5 using RNAi almost completely suppressed the lifespan extension by sir-2.1 overexpression, thereby indicating that the effects of sir-2.1 are dependent on UPRmt (70).

Deliberations on the role of Sir2uin on lifespan extension

Although numerous evidences indicate that overexpression of Sir2uin delays the cellular senescence and extends the lifespan of organisms, several reports have challenged this theory (71, 72). The outcrossing abrogated the lifespan extension phenotype of geln3 worm strains, used for overexpressing Sir2uin 10-30 fold, indicating that the longevity effect of this Sir2uin overexpressing strain is due to a lack of genetic background standardization (73). In addition, geln3 strain also has an unlinked dhy mutation which attributes to the longevity effect of the strain (73), indicating that the lifespan extension of geln3 is also due to incorrectly matched controls. The report also showed that overexpression of dSir2 using outcrossed dSir2EP2300 and the two newly constructed lines containing inducible UAS-dSir2 under ubiquitously expressing Tubulin-CaI4 driver did not extend the lifespan compared to the flies expressing the Gal4 driver only (73). Independently, a reduction in the expression of dSir2 using Sir2 RNAs in the fat body did not affect the lifespan of flies (56). Later, this argument was also refuted. Viswanathan and Guarente showed that geln3 worms still have long lifespan after outcrossing compared to outcrossed control lines (74).

These contradictory results concerning the effect of Sir2uin overexpression on lifespan might be explained by the extent of overexpression of Sir2uin. Whitaker et al. showed that highly expressed (45-fold increase) dSir2 in the whole fly shortened the lifespan, but modest levels (2-11-fold increase) of dSir2 resulted in extended lifespan (75). Thus, they asserted that dSir2 expression in previous reports might not be relevant each other, since the extent of overexpression varied depending on the controls that were used for comparison. In the previous reports showing the lifespan extension by dSir2, the dSir2 was overexpressed 3-4-fold (10, 56, 76). The dose-dependent effect of Sir2uin was also presented in a mice model. Alcendor et al. showed that 2.5-7.5-fold mild increase of SIRT1 in mouse heart prevented age-related cardiac hypertrophy by eliciting an increase in the level of antioxidant enzymes, but a 12.5-fold increase of SIRT1 increased the oxidative stress and promoted cardiac hypertrophy (77).

Calorie restriction

Calorie restriction (CR), also known as dietary restriction, is a proven intervention to extend lifespan in almost all animal models including non-human primate, which experimentally means a reduction in calorie intake by 10-50% compared to the ad libitum intake without malnutrition (78). Although still controversial, it is believed that the beneficial effects of CR on lifespan extension and prevention of age-related diseases is mediated by the induction of Sir2uin (79, 80). In many animal models, the expression and activity of Sir2uin was reported to be increased by CR and nutritional deprivation, and the
activation of Sirtuin by CR was mediated by the upregulation of AMPK and increase of NAD+ levels (81). In C. elegans, the expression levels of sir-2.1 tagged with mCherry fluorescence increased due to starvation in the intestine and muscle cells, but not in nerve cells (8). In D. melanogaster, dSIR2 expression increased in the flies fed low-calorie food (82). In mammals, the levels of Sirtuins (except SIRT4) were also reported to increase after CR (52), which is known to be tissue specific (83). Increased levels of SIRT1 by CR were observed in white adipose tissue, skeletal muscle, kidney, brain and intestine, (81, 83, 84). However, in liver, the response of SIRT1 expression to CR is debatable; one paper showed induction of SIRT1 in the liver of CR-experienced mice (81), but another report showed a reduction in levels (83). Furthermore, the latter report also showed that knock-out of SIRT1 in liver is dispensable in this tissue (83). In addition, SIRT6 is indirectly activated by CR by upregulating the SIRT1, FOXO3a, and nuclear respiratory factor 1 (NRF) (85). SIRT3 are induced by CR in diverse tissues, including muscle, white adipose tissue, and liver (86); SIRT3 is suggested to be essential for cochlea neurons against oxidative damage (87). These studies indicate that the expression of Sirtuin is regulated by CR with tissue-specificity, suggesting that a more elaborate investigation is required to understand Sirtuin regulation by CR.

Numerous researches have also reported the requirement of Sirtuins in the lifespan-extension effect of CR in various organisms. In budding yeast, CR does not extend the lifespan of SIR2 mutant strain (88), and the lifespan extension by CR was reported to require the nicotinamidase PNC1, an enzyme that recycles NAD+, which is critical for Sirtuin-dependent functions (89). This indicates that SIR2 is indispensable for mediating the positive effects of CR in yeast. In C. elegans, the requirement of Sirtuin is dependent with respect to the strain and genetic background. The strain of eat-2 such as ad465, ad113, ad116 that have defective pumping and are considered as the CR model of worms, lived longer than the wild-type N2 strain (55). The sir-2.1 mutation suppressed this longevity effect of eat-2 in ad465 and ad113 strain (55) but not in the ad116 strain, in which CR is more extreme (90). In Drosophila, the lifespan extending effects of CR were partially mediated by dSIR2 (10, 76), and mammalian Sirtuins are well known to mediate the beneficial effect of CR (79). SIRT1 knockout mice did not normally display the metabolic response triggered by CR, and the increased physical activity by CR mice was not seen in SIRT1 knockout mice (91). Knockout of SIRT3 increased the oxidative stress and damage by CR (92). In addition to these CR-related outputs, SIRT1 knockout mice failed to show the lifespan extension in response to CR (91), indicating that SIRT1 mediates the lifespan-extension by CR.

The role of Sirtuins in lifespan extension by CR has long been challenged (71). Several reports asserted that Sirtuins are not required for lifespan extension by CR in yeast, C. elegans, and fruit flies. In yeast, the SIR2 mutation suppresses the replicative lifespan extension in the CR model strain gpa2Δ and hsK2Δ, but not in fbo1Δ (93). In addition, SIR2 overexpression increases the replicative lifespan of yeast in low glucose (93). Furthermore, SIR2 mutation did not suppress the chronological lifespan extension by CR (94, 95), and deletion of all Sirtuin family in yeast did not prevent the effect of CR (96). In C. elegans, the role of Sirtuins in the lifespan extension of CR is shown as dependent with the protocols of CR. Although the lifespan extension in the pumping defective eat-2 worms requires Sir-2.1 (55), the lifespan extension by CR through food deprivation or bacteria dilution does not require Sir-2.1 (97-99). In D. melanogaster, the homozygotic mutant dsir2Δ and dsir2Δ respond normally to CR, and the CR increased the lifespan of these mutant flies (73). In addition, the dSirt4 knockout flies also responded normally to CR with the expected increase in lifespan (11). These controversial results are suggested to be due to differences in strain background, CR protocols, or Sirtuin gene redundancy (100). In addition, it is suggested that there may be both Sirtuin-dependent and -independent pathways that play a role in extending the lifespan by CR (101).

**ACTIVATORS OF SIRTUIN**

Since Sirtuin is commonly believed to mediate the beneficial effects of CR, the activators of Sirtuin are considered to mimic these beneficial effects and are hence attractive therapeutics for age-related diseases. Subsequently, high-throughput screening has identified over 14,000 Sirtuin-activating compounds (STACs).

**STACs**

In 2003, a screen for activators of the mammalian SIRT1 identified 15 small molecules including quercetin, butein, fisetin, and piceatannol (54). This study further revealed the most potent activator of SIRT1 to be resveratrol (3,5,4’-trihydroxystilbene), a polyphenol found in red wine, which extended the replicative lifespan of budding yeast by 70% at 10 μM (54). In addition, the lifespan-extension effect of resveratrol was abrogated by the SIR2 mutation (54), indicating that resveratrol extends the lifespan of yeast through the activation of Sirtuin. Interestingly, higher concentrations of resveratrol did not further increase the lifespan, and resveratrol failed to extend the chronological lifespan (54). Another study in the following year showed that fisetin, butein, and resveratrol also activated levels of Sir-2.1 of C. elegans up to 2.5-fold, and dSir2 of D. melanogaster up to 2.4-fold (102). In addition, a single amino acid of SIRT1 (E230) was proved to be critical for binding to STACs and inducing activation (103). Supplementation of resveratrol at 100 μM extended the lifespan of C. elegans and D. melanogaster by 14% and 29%, respectively, but was ineffective on the Sir-2 mutant (102), suggesting that resveratrol extends the lifespan in a Sir-2-dependent manner. However, the lifespan of transgenic worms overexpressing sir-2.1 were extended by 39% following resveratrol treatment, thereby suggesting that sir-2.1...
exerts its effect of extending the lifespan independent of resveratrol (53). In addition, the study also revealed that resveratrol-induced lifespan extension is mediated by the ER stress gene, abu-11, whose expression is regulated by sir-2.1 (53). The lifespan extending effect of resveratrol and other related STACs was also established in the short lived fish Nothobranchius furzeri (104) and in the honeybee Apis mellifera (105).

Other than resveratrol, natural compounds including cilostazol (106), paenol (107), statins (108), hydrogen sulfide (109), Icarin (110), persimmon (111), melatonin (112), and curcumin (113) are also reported as potent STACs. Some of these STACs have exhibited a prolongevity effect on model animals. For example, pretreatment with curcumin or alkylresorcinols enhanced the SIRT1 activity (113, 114), and extended the lifespan of Drosophila (114, 115).

Natural STACs are hydrophobic in nature with low solubility and low bioavailability. To overcome these weaknesses, synthetic STACs were developed by using drug design approaches. To date, more than 14,000 STACs have been synthesized up to the 5th generation, and dozens of these have been tested in animal disease models. Several STACs are also currently undergoing clinical trials (116). These synthetic STACs are reported to be beneficial for several age-related diseases, and have demonstrated protection from cancer, neurodegeneration, cardiovascular disease and diabetes, with some compounds exerting an extended lifespan. Of these, SRT1720, SRT2104, SRT1460, SRT2183, STAC-5, STAC-9, and STAC-10 have attracted attention due to their increased activity, solubility, and bioavailability as compared to resveratrol (117). Especially, SRT1720, a synthetic STAC structurally unrelated to resveratrol, has been reported to improve insulin sensitivity and mitochondrial capacity in obese rodents (118), and has extended the lifespan of mice fed a high-calorie diet, to a similar extent as resveratrol (119). In addition, SRT2104 mimics aspects of CR and extends the lifespan of male mice fed a standard diet (120). In addition to these STACs, several oxazoloi(4,5-β)pyridine and imidazo[1,2-β]thiazole derivatives have also been identified as activators of SIRT1 (121, 122). Especially, 1,4-dihydropyridine derivatives activate several Sirtuins (SIRT1-SIRT3) in a dose-dependent manner (123), and synthetic iso-nicotinamide (iNAM) also acts as a Sirtuin-activator (124).

The requirement of SIR2 in the lifespan extension by these STACs is controversial, and several increasing evidences show that the lifespan extending effects of STACs are Sirtuin-independent. Predominantly, it is contentiously debated whether or not resveratrol and synthetic STACs directly activate SIRT1 (117). The original report reveals the activation of SIRT1 by resveratrol using a fluorescence-conjugated peptide substrate, Fluor-de-Lys (54). However, resveratrol and STACs showed no activation of Sirtuin using in vitro full-length endogenous substrates (such as p53 and PGC1α) or short, fluorescence-unconjugated peptide substrates (125-127). In addition, it was suggested that the effect of resveratrol on SIRT1 was due to the off-target effects on other enzymes such as phosphodiesterase (128). Recently, a newly developed assay for detection of Sirtuin activity, named as CycLex, revealed that SRT1720 does not activate SIRT1 (114). In addition, several reports show that resveratrol treatment does not extend the lifespan of C. elegans independent with sir-2.1 (129). Additionally, resveratrol and STACs show no beneficial effects when administrated to the obese mouse model (125). More precisely controlled protocols to detect the Sirtuin activity, and more discreetly managed experiments to investigate the role of STACs on longevity are required.

NAD+ booster

An alternative approach to activating Sirtuins is regulating NAD+ levels by activating enzymes involved in biosynthesis of NAD. NAD is an essential cofactor for electron transfer and for regulating metabolic homeostasis, and its levels decrease with aging in the liver and muscle, as could partially be explained due to the decreased activity of Sirtuin upon aging (70). The supplementation of NAD extended the lifespan of C. elegans, Drosophila, and the premature mouse model (130-132), which is mediated by Sirtuin activation (130).

During deacetylation reaction by Sirtuin, NAD+ is converted to nicotinamide (NAM), and NAM is recycled into NAD or other nicotinic acid (NA) derivatives through the NAD salvage pathway. Thus, the administration of NAM increases the level of NAD with subsequent SIRT1 activation (133, 134). NAM is also methylated by nicotinamide N-methyltransferase (NNMT) to 1-methylnicotinamide (MNA). The treatment of NAM, NA, or MNA, and the overexpression of amrt-1, the C. elegans NNMT, results in extending the lifespan of worms (135). Supplementation of NA extends the lifespan similar to the extent with sir-2.1 overexpression (135). In the mutation of amrt-1, NA and NAM are unable to extend the lifespan, but MNA is still capable of extending the lifespan of worms (135). In addition, the overexpression of nicotinamide phosphoribosyltransferase (NAMPT), the enzyme converts NAM into nicotinamide mononucleotide (NMN), is also reported to increase the SIRT1 activity (136, 137). Overexpression of the NAMPT orthologue dNAM is shown to increase the Drosophila lifespan in a dSir2-dependent manner (138). Furthermore, the inhibitor of NAD+ consuming enzyme PARPs also increases the C. elegans lifespan in a Sirtuin-dependent manner (70, 139), and the inhibition of CD38 results in NAD+ accumulation and subsequent SIRT1 activation in mice, rendering a protective effect against high-fat-diet-induced obesity (140).

An increase of NAD+ levels is also observed in energy deficient conditions, such as fasting, CR or low glucose feeding (83, 141, 142). Compounds that raise NAD levels, such as nicotinamide riboside and NMN, are potential
Sirtuin and its activators: promising targets for longevity
Shin-Hae Lee, et al.

candidates as CR mimetics that are shown to extend lifespan. Under conditions of starvation, AMPK is activated and alters the intracellular metabolism, resulting in an increase in NAD levels with a concomitant increase in SIRT1 activity (143). In yeast, the longevity effect of CR is reported to require PNC-1, a homologue of NAMPT (144). However, contrarily, several reports have revealed that the level of NAD does not increase in yeast exposed to CR (89, 145), and NAD supplementation further extended the lifespan of eat-2 mutant worms, indicating that lifespan extension by NAD worked along a different pathway from that of CR (130, 145).

CONCLUSION REMARKS
For over 20 years, the Sirtuin family has been actively investigated for its function in delaying cellular senescence and extending longevity. In addition, based on the role of Sirtuin on the beneficial effect of CR, therapeutic trials using activators of Sirtuin have actively proceeded to protect age-related diseases. Growing evidences have principally supported that Sirtuin is an attractive anti-aging molecule involved in improving health through the target molecules participating in diverse biological processes; however, the role of Sirtuin on longevity, and the longevity effect of CR, are still controversial. In addition, numerous questions remain unresolved, such as the role of other Sirtuins in addition to SIRT1 and SIRT6 on aging, the redundancy of the Sirtuin family members to regulate lifespan, whether other enzymatic activities (apart from deacetylation activity) participate in the process of aging, and whether STACs could be promoted as drugs to treat aging or age-related diseases in humans. These questions will be answered in the near future, and Sirtuin may provide the effective approach to extend lifespan and improve our quality of life.

ACKNOWLEDGEMENTS
This work was supported by Inha University Research Grant.

CONFLICTS OF INTEREST
The authors have no conflicting interests.

REFERENCES
1. North BJ and Verdin E (2004) Sirtuins: Sirt2-related NAD-dependent protein deacetylases. Genome Biol 5, 224.
2. Toiber D, Sebastian C and Mostoslavsky R (2011) Characterization of nuclear sirtuins: molecular mechanisms and physiological relevance. Handb Exp Pharmacol 206, 189-224.
3. Guarente L (2000) Sir2 links chromatin silencing, metabolism, and aging. Genes Dev 14, 1021-1026.
4. Kaeberlein M, McVey M and Guarente L (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 13, 2570-2580.
5. Imai S, Armstrong CM, Kaebberlein M and Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403, 795-800.
6. Landry J, Sutton A, Tafrrov ST et al (2000) The silencing protein Sir2 and its homologs are NAD-dependent protein deacetylases. Proc Natl Acad Sci U S A 97, 5807-5811.
7. Grabowska W, Sikora E and Bielak-Zmijewska A (2017) Sirtuins, a promising target in slowing down the ageing process. Biogerontology 18, 447-476.
8. Bamps S, Wirtz J, Savory FR, Lake D and Hope JA (2009) The Caenorhabditis elegans sirtuin gene, sir-2.1, is widely expressed and induced upon caloric restriction. Mech Ageing Dev 130, 762-770.
9. Newman BL, Lundblad JR, Chen Y and Smolik SM (2002) A Drosophila homologue of Sir2 modifies position-effect variegation but does not affect life span. Genetics 162, 1675-1685.
10. Rognina B and Helfand SL (2004) Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci U S A 101, 15998-16003.
11. Wood JG, Schwer B, Wickremesinghe PC et al (2018) Sirt4 is a mitochondrial regulator of metabolism and lifespan in Drosophila melanogaster. Proc Natl Acad Sci U S A 115, 1564-1569.
12. Frye RA (2000) Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun 273, 793-798.
13. Michishita E, Park JY, Burneski JM, Barrett JC and Horikawa I (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol Biol Cell 16, 4623-4635.
14. Scher MB, Vaquero A and Reinberg D (2007) SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. Genes Dev 21, 920-928.
15. Du J, Zhou Y, Su X et al (2011) SirT5 is a NAD-dependent protein lysine demalonylase and desuccinyllase. Science 334, 806-809.
16. Cristofalo VJ, Lorenzini A, Allen RG, Torres C and Tresini M (2004) Replicative senescence: a critical review. Mech Ageing Dev 125, 827-848.
17. Munoz-Espin D and Serrano M (2014) Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 15, 482-496.
18. Krtolica A and Campisi J (2002) Cancer and aging: a model for the cancer promoting effects of the aging stroma. Int J Biochem Cell Biol 34, 1401-1414.
19. Sasaki T, Maier B, Barke A and Scrable H (2006) Progressive loss of SIRT1 with cell cycle withdrawal. Aging Cell 5, 413-422.
20. Anwar T, Khosla S and Ramakrishna G (2016) Increased expression of SIRT2 is a novel marker of cellular senescence and is dependent on wild type p53 status. Cell Cycle 15, 1883-1897.
21. Son MJ, Kwon Y, Son T and Cho YS (2016) Restoration
of Mitochondrial NAD(+) Levels Delays Stem Cell Senescence andFacilitates Reprogramming of Aged Somatic Cells. Stem Cells 34, 2840-2851

22. Chen J, Xie JJ, Jin MY et al (2018) Sirt6 overexpression suppresses senescence and apoptosis of nucleus pulposus cells by inducing autophagy in a model of intervertebral disc degeneration. Cell Death Dis 9, 56

23. Ota H, Akishita M, Eto M, Iijima K, Kaneki M and Ouchi Y (2007) Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 43, 571-579

24. Menghini R, Casagrande V, Cardellini M et al (2009) MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation 120, 1324-1332

25. Mostoslavsky R, Chua CF, Lombard DB et al (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124, 315-329

26. Kim MY, Kang ES, Ham SA et al (2012) The PPARdelta-mediated inhibition of angiogenesis II-induced premature senescence in human endothelial cells is SIRT1-dependent. Biochem Pharmacol 84, 1627-1634

27. Yao H, Chung S, Hwang JW et al (2012) SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice. J Clin Invest 122, 2032-2045

28. Zu Y, Liu L, Lee MY et al (2010) SIRT1 promotes proliferation and prevents senescence through targeting LKB1 in primary porcine aortic endothelial cells. Circ Res 106, 1384-1393

29. Michan S and Sinclair D (2007) Sirtuins in mammals: insights into their biological function. Biochem J 404, 1-13

30. Rosenberg MI and Parkhurst SM (2002) Drosophila Sir2 is required for heterochromatin silencing and by euchromatic hairy/E(spl) bHLH repressors in segmentation and sex determination. Cell 109, 447-458

31. Astrom SU, Cline TW and Rine J (2007) The Drosophila melanogaster sir2+ gene is nonessential and has only minor effects on position-effect variegation. Genetics 163, 931-937

32. Yamashita S, Ogawa K, Ikee T, Udono M, Fujiaki T and Katakura Y (2012) SIRT1 prevents replicative senescence of normal human umbilical cord fibroblast through potentiating the transcription of human telomerase reverse transcriptase gene. Biochem Biophys Res Commun 417, 630-634

33. Watroba M, Dudek I, Skoda M, Stangret A, Rzodkiewicz P and Szukiewicz D (2017) Sirtuin and its activators: promising targets for longevity. Aging (Albany NY) 2, 415-431

34. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431

35. Tissenbaum HA and Guarente L (2001) Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 410, 227-230

36. Kanfi Y, Naiman S, Amir G et al (2012) The sirtuin SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell 136, 62-74

37. Michishita E, Mc Cord RA, Berber E et al (2008) SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 452, 492-496

38. Jeong SM, Xiao C, Finley LW et al (2013) SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glucose metabolism. Cancer Cell 23, 450-463

39. Brunet A, Sweeney LB, Sturgill JF et al (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303, 2011-2015

40. Giannakou ME and Partridge L (2004) The interaction between FOXO and SIRT1: tipping the balance towards survival. Trends Cell Biol 14, 408-412

41. Luo J, Nikolaev AY, Imai S et al (2001) Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell 107, 137-148

42. Langley E, Pearson M, Faretta M et al (2002) Human SIR2 deacetylase p53 and antagonizes PML/p53-induced cellular senescence. EMBO J 21, 2383-2396

43. Kawahara TL, Michishita E, Adler AS et al (2009) SIRT6 deacetylates p53 in a stress- and dose-dependent manner. EMBO J 38, 2014-2025

44. Chen J, Xavier S, Moskowitz-Kassai E et al (2012) Sirt1 modulates premature senescence-like phenotype and defective lineage specification in Sirt1+/− mice. Stem Cell Rep 3, 44-59

45. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431

46. Rimmele P, Bigarella CL, Liang R et al (2014) Aging-like phenotype and defective lineage specification in SIRT1-deleted hematopoietic stem and progenitor cells. Stem Cell Reports 3, 44-59

47. Chen J, Xavier S, Moskowitz-Kassai E et al (2012) Sirt1 modulates premature senescence-like phenotype and defective lineage specification in SIRT1-deleted hematopoietic stem and progenitor cells. Stem Cell Reports 3, 44-59

48. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431

49. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431

50. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431

51. Kanfi Y, Naiman S, Amir G et al (2012) The sirtuin SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell 136, 62-74

52. Rimmel P, Bigarella CL, Liang R et al (2014) Aging-like phenotype and defective lineage specification in SIRT1-deleted hematopoietic stem and progenitor cells. Stem Cell Reports 3, 44-59

53. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431

54. Saunders LR, Sharma AD, Tawney J et al (2010) miRNAs regulate SIRT1 expression during murine embryonic stem cell differentiation and in adult mouse tissues. Aging 2, 415-431
Sirtuin and its activators: promising targets for longevity

Shin-Hae Lee, et al.

55. Wang Y and Tissenbaum HA (2006) Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. Mech Ageing Dev 127, 48-56

56. Banerjee KK, Ayyub C, Ali SZ, Mandot V, Prasad NG and Kolthor-Seetharam U (2012) dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner. Cell Rep 2, 1485-1491

57. Satoh A, Brace CS, Rensing N et al (2013) Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell Metab 18, 416-430

58. Vakhrusheva O, Smolka C, Gajawada P et al (2008) Sirt7 required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 15, 675-690

59. Rose G, Dato S, Altomare K et al (2003) Variability of NAD and Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. Cell 154, 430-441

60. Berdichevsky A, Viswanathan M, Horvitz HR and Guarente L (2011) Regulation of Caenorhabditis elegans lifespan by sir-2.1 transgenes. Nature 477, E1-2

61. Heidler T, Hartwig K, Daniel H and Wenzel U (2010) Sirt3 gene, human silent information regulator Sir2 homologue, and survivorship in the elderly. Exp Gerontol 38, 1065-1070

62. Banerjee KK, Ayyub C, Michan S et al (2009) Hepatic-specific SIRT1 and insulin-induced insulin receptor substrate-2 tyrosine phosphorylation. J Biol Chem 282, 34356-34364

63. Liang F, Kume S and Koya D (2009) SIRT1 and insulin metabolism pathways. Aging Cell 5, 119-126

64. Zhang J (2007) The direct involvement of SirT1 in 14-3-3 proteins to activate DAF-16 and extend life span. Cell 125, 1165-1177

65. Heidler T, Hartwig K, Daniel H and Wenzel U (2010) Caenorhabditis elegans lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. Biogerontology 11, 183-195

66. Price NL, Gomes AP, Ling AJ et al (2012) SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 15, 675-690

67. Wang Y, Oh SW, Deplancke B, Luo J, Walhout AJ and Tissenbaum HA (2006) C. elegans 14-3-3 proteins regulate life span and interact with SIR-2.1 and DAF-16/FOXO. Mech Ageing Dev 127, 741-747

68. Bendichovsky A, Nedelcu S, Boulias K, Bishop NA, Guarente L and Horvitz HR (2010) 3-Ketoacyl thiolase delays aging of Caenorhabditis elegans and is required for lifespan extension mediated by sir-2.1. Proc Natl Acad Sci U S A 107, 18927-18932

69. Rizki G, Iwata TN, Li J et al (2011) The evolutionarily conserved longevity determinants HCT-1 and SIR-2.1/SIRT1 collaborate to regulate DAF-16/FOXO. PLoS Genet 7, e1002235

70. Mouchiroud L, Houtkoooper RH, Moullan N et al (2013) The NAD(+)-Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO. Aging Cell 12, e9-e17

71. Naiman S and Cohen HY (2012) The contentious history of sirtuin debates. Rambam Maimonides Med J 3, e0022

72. Burnett C, Valenti S, Cabreiro F et al (2011) Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. Nature 477, 482-485

73. Viswanathan M and Guarente L (2011) Regulation of Caenorhabditis elegans lifespan by sir-2.1. Biogerontology 12, 987-1002

74. Palacios OM, Carmona JJ, Michan S et al (2009) Diet-dependent manner. Cell Rep 2, 1485-1491

75. Whitaker R, Faulkner S, Miyokawa R et al (2013) Increased expression of Drosophila Sir2 extends life span in a dose-dependent manner. Aging (Albany NY) 5, 682-691

76. Bauer JH, Morris SN, Chang C, Tait S and Helfand SL (2009) dSir2 and Dmp53 interact to mediate aspects of CR-dependent lifespan extension in D. melanogaster. Aging (Albany NY) 1, 38-48

77. Alcendor RR, Gao S, Zhai P et al (2007) Sirt1 regulates 14-3-3 proteins to activate DAF-16 and extend life span. Cell 125, 1165-1177

78. Lee SH and Min KJ (2013) Caloric restriction and its mimetics. BMB Rep 46, 181-187

79. Canto C and Auwerx J (2009) Caloric restriction, SIRT1 and longevity. Trends Endocrinol Metab 20, 325-331

80. Sinclair DA (2005) Toward a unified theory of caloric restriction and longevity regulation. Mech Ageing Dev 126, 987-1002

81. Cohen HY, Millar C, Bitterman KJ et al (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305, 390-392

82. Robin J, Helfand SL and Frankel S (2002) Longevity regulation by Drosophila Rpd3 deacetylase and caloric restriction. Science 298, 1745

83. Chen D, Bruno J, Easlon E et al (2008) Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev 22, 1753-1757

84. Firestein R, Blander G, Michan S et al (2008) The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. PLoS One 3, e2020

85. Kim HS, Xiao C, Wang RH et al (2010) Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. Cell Metab 12, 224-236

86. Palacios OM, Carmona JJ, Michan S et al (2009) Diet and exercise signals regulate SIRT3 and activate AMPK and PGC-1alpha in skeletal muscle. Aging (Albany NY) 1, 771-783

87. Someya S, Yu W, Hallows WC et al (2010) Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. Cell 143, 802-812

88. Lin SJ, Defossez PA and Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science 289, 2126-2128
Sirtuin and its activators: promising targets for longevity

Shin-Hae Lee, et al.

89. Anderson RM, Bitterman KJ, Wood JG, Medvedik O and Sinclair DA (2003) Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. Nature 423, 181-185

90. Hansen M, Taubert S, Crawford D, Libina N, Lee SJ and Kenyon C (2007) Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. Aging Cell 6, 95-110

91. Boily G, Seiffert EL, Bevilacqua L et al (2008) SirT1 regulates energy metabolism and response to caloric restriction in mice. PLoS One 3, e1759

92. Qiu X, Brown K, Hirschy MD, Verdin E and Chen D (2010) Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metab 12, 662-667

93. Kaeberlein M, Kirkland KT, Fields S and Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol 2, E296

94. Smith DL Jr, McClure JM, Matecc M and Smith JS (2007) Calorie restriction extends the chronological lifespan of Saccharomyces cerevisiae independently of the Sirtuins. Aging Cell 6, 649-662

95. Fabrizio P, Gattazzo C, Battistella L et al (2005) Sir2 blocks extreme life-span extension. Cell 123, 655-667

96. Tsuchiya M, Dang N, Kerr EO et al (2006) Sirtuin-independent effects of nicotinamide on lifespan extension from calorie restriction in yeast. Aging Cell 5, 505-514

97. Kaeberlein TL, Smith ED, Tsuchiya M et al (2006) Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell 5, 487-494

98. Lee GD, Wilson MA, Zhu M et al (2006) Dietary deprivation extends lifespan in Caenorhabditis elegans. Aging Cell 5, 515-524

99. Greer EL and Brunet A (2009) Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in C. elegans. Aging Cell 8, 113-127

100. Guarente L (2005) Calorie restriction and SIR2 genes—towards a mechanism. Mech Ageing Dev 126, 923-928

101. Lamming DW, Latorre-Esteves M, Medvedik O et al (2005) HST2 mediates Sir2-independent life-span extension by calorie restriction. Science 309, 1861-1864

102. Wood JG, Rognina B, Lavo S et al (2004) SirT1 activators mimic caloric restriction and delay aging in metazoans. Nature 430, 686-689

103. Hubbard BP, Gomes AP, Dai H et al (2012) Evidence for a common mechanism of SirT1 regulation by allostERIC activators. Science 339, 1216-1219

104. Valenzano DR, Terzibasi E, Genade T, Cattaneo A, Domenici L and Cellerino A (2006) Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate. Curr Biol 16, 296-300

105. Rascon B, Hubbard BP, Sinclair DA and Andram GV (2012) The lifespan extension effects of resveratrol are conserved in the honey bee and may be driven by a mechanism related to calorid restriction. Aging (Albany NY) 4, 499-508

106. Ota H, Eto M, Kano MR et al (2008) Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of SirT1 in human endothelial cells. Arterioscler Thromb Vasc Biol 28, 1634-1639

107. Jamal J, Mustafa MR and Wong PF (2014) Paeonol protects against premature senescence in endothelial cells by modulating Sir2tin 1 pathway. J Ethnopharmacol 154, 428-436

108. Ota H, Eto M, Kano MR et al (2010) Induction of endothelial nitric oxide synthase, SirT1, and catalase by statins inhibits endothelial senescence through the Akt pathway. Arterioscler Thromb Vasc Biol 30, 2205-2211

109. Suo R, Zhao ZZ, Tang ZH et al (2013) Hydrogen sulfide prevents H2O2-induced senescence in human umbilical vein endothelial cells through SirT1 activation. Mol Med Rep 7, 1865-1870

110. Lee MK, Choi YJ, Sung SH, Shin DI, Kim JW and Kim YC (1995) Antihypotensive activity of icariin, a major constituent of Epimedium koreanum. Planta Med 61, 523-526

111. Lee YA, Cho EJ and Yokozawa T (2008) Protective effect of persimmon (Diospyros kaki) peel proanthocyanidin against oxidative damage under H2O2-induced cellular senescence. Biol Pharm Bull 31, 1265-1269

112. Ramis MR, Esteban S, Miralles A, Tan DX and Reiter RJ (2015) Caloric restriction, resveratrol and melatonin: Role of SirT1 and implications for aging and related diseases. Mech Ageing Dev 146-148:28-41

113. Sun Q, Jia N, Wang W, Jin H, Xu J and Hu H (2014) Activation of SirT1 by curcumin blocks the neurotoxicity of amyloid-beta25-35 in rat cortical neurons. Biochem Biophys Res Commun 448, 89-94

114. Kayashima Y, Katayanagi Y, Tanaka K, Fukutomi R, Hiramoto S and Imai S (2017) Alkylresorcinols activate SirT1 and delay aging in Drosophila melanogaster. Sci Rep 7, 43679

115. Lee KS, Lee BS, Semnani S et al (2010) Curcumin extends life span, improves health span, and modulates the expression of age-associated aging genes in Drosophila melanogaster. Rejuvenation Res 13, 561-570

116. Bonkowski MS and Sinclair DA (2016) Slowing ageing by design: the rise of NAD(+) and sirtuin-activating compounds. Nat Rev Mol Cell Biol 17, 679-690

117. Hubbard BP and Sinclair DA (2014) Small molecule SIRT1 activators for the treatment of aging and age-related diseases. Trends Pharmacol Sci 35, 146-154

118. Milne JC, Lambert PD, Schenk S et al (2007) Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 450, 712-716

119. Minor RK, Baur JA, Gomes AP et al (2011) SRT1720 improves survival and healthspan of obese mice. Sci Rep 1, 70

120. Mercken EM, Mitchell SJ, Martin-Montalvo A et al (2014) SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass. Aging Cell 13, 787-796

121. Bemis JE, Vu CB, Xie R et al (2009) Discovery of oxazolol[4,5-b]pyridines and related heterocyclic analogs as novel SIRT1 activators. Bioorg Med Chem Lett 19, 2350-2353

122. Vu CB, Bemis JE, Disch JS et al (2009) Discovery of imidazo[1,2-b]thiazole derivatives as novel SIRT1 activators. J Med Chem 52, 2350-2353
Sirtuin and its activators: promising targets for longevity
Shin-Hae Lee, et al.

activators. J Med Chem 52, 1275-1283

123. Mai A, Valente S, Meade S et al (2009) Study of 1,4-dihydropyridine structural scaffold: discovery of novel sirtuin activators and inhibitors. J Med Chem 52, 5496-5504

124. Saave AA, Moir RD, Schramm VL and Willis IM (2005) Chemical activation of Sir2-dependent silencing by relief of nicotinamide inhibition. Mol Cell 17, 595-601

125. Pacholec M, Bealesale JE, Chrunk B B et al (2010) SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J Biol Chem 285, 8340-8351

126. Borra MT, Smith BC and Denu JM (2005) Mechanism of human SIRT1 activation by resveratrol. J Biol Chem 280, 17187-17195

127. Kadnerlein M, McDonagh T, Heitweg B B et al (2005) Substrate-specific activation of sirtuins by resveratrol. J Biol Chem 280, 17038-17045

128. Chung JH (2012) Using PDE inhibitors to harness the benefits of calorie restriction: lessons from resveratrol. Aging (Albany NY) 4, 144-145

129. Bass TM, Weininkove D, Houthooft K, Gems D and Partridge L (2007) Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. Mech Ageing Dev 128, 546-552

130. Hashimoto T, Horikawa M, Nomura T and Sakamoto K (2010) Nicotinamide adenine dinucleotide extends the lifespan of Caenorhabditis elegans mediated by sir-2.1 and daf-16. Biogerontology 11, 31-43

131. Scheibyme-Knudsen M, Mitchell SJ, Fang EF et al (2014) A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. Cell Metab 20, 840-855

132. Zhang H, Ryu D, Wu Y et al (2016) NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. Science 352, 1436-1443

133. Yoshino J, Mills KF, Yoon MJ and Imai S (2011) Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 14, 528-536

134. Canto C, Houtkooper RH, Pirinen E et al (2012) The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 15, 838-847

135. Schneissker M, Mansfeld J, Kuhlow D et al (2013) Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide. Nat Chem Biol 9, 693-700

136. Rongvaux A, Shea RJ, Mulks MH et al (2002) Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol 32, 3225-3234

137. Revollo JR, Grimm AA and Imai S (2004) The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. J Biol Chem 279, 50754-50763

138. Balan V, Miller GS, Kaplun L et al (2008) Life span extension and neuronal cell protection by Drosophila nicotinamidase. J Biol Chem 283, 27810-27819

139. Gomes AP, Price NL, Ling AJ et al (2013) Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell 155, 1624-1638

140. Barbosa MT, Soares SM, Novak CM et al (2007) The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. FASEB J 21, 3629-3639

141. Canto C, Jiang LQ, Deshmukh AS et al (2010) Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab 11, 213-219

142. Fulco M, Cen Y, Zhao P et al (2008) Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev Cell 14, 661-673

143. Canto C, Gerhart-Hines Z, Feige JN et al (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458, 1056-1060

144. Moroz N, Carmona JJ, Anderson E, Hart AC, Sinclair DA and Blackwell TK (2014) Dietary restriction involves NAD(+) -dependent mechanisms and a shift toward oxidative metabolism. Aging Cell 13, 1075-1085

145. Lin SJ, Ford E, Haigis M, Liszt G and Guarente L (2004) Calorie restriction extends yeast life span by lowering the level of NADH. Genes Dev 18, 12-16