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ABSTRACT

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 prolongevity effect of Sirtuin is dependent with the level of and with the tissue expression of Sirtuin. Since Sirtuin is also believed to mediate the prolongevity 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.

INTRODUCTION

The Sirtuin family is nicotinamide dinucleotide (NAD+)-dependent deacylases 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 deproprionylation (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*, *sir2-2*, *sir2-3*, 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 and/or cytoplasm of neurons and fat bodies (10). Although recently reported that fly *Sirt4* contains a mitochondrial targeting sequence (11), the *Sirt4* 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 heterochromatic 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 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* 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. cereviseasae*, worms *C. elegans*, fruit flies *D. melanogaster*, and mice (4, 10, 50, 51). The first investigation for the prolongevity 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/α co-expression; *SIR2* alone also extended the lifespan by repressing the recombination and generation of toxic rDNA circles (4). The prolongevity effect of *SIR2* has been confirmed in higher organisms, while there are different mechanisms of exerting prolongevity 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 had 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 on *daf-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 Sirtuin. 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 Sirtuin has also been well investigated. SIRT1 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 Sirtuin-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 (66). 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 Sirtuin 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 *ftt-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 SIR-2.1-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. elegant* (69). Furthermore, mitochondrial regulators such as *cts-1* and *fzo-1,* and the mitochondrial unfolded protein response (UPR^{mt}) gene *hsp-6*, were reported to increase by *sir-2.1* overexpression, and the knock-down of UPR^{mt} 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 UPR^{mt} (70).

Deliberations on the role of Sirtuin on lifespan extension

Although numerous evidences indicate that overexpression of Sirtuin 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 *geIn3* worm strains, used for overexpressing Sirtuin 10-30 fold, indicating that the longevity effect of this Sirtuin overexpressing strain is due to a lack of genetic background standardization (73). In addition, *geIn3* strain also has an unlinked *dyf* mutation which attributes to the longevity effect of the strain (73), indicating that the lifespan extension of *geIn3* is also due to incorrectly matched controls. The report also showed that overexpression of *dSir2* using outcrossed $dSir2^{EP2300}$ and the two newly constructed lines containing inducible *UAS-dSir2* under ubiquitously expressing *Tubulin-Gal4* 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 RNAi* in the fat body did not affect the lifespan of flies (56). Later, this argument was also refuted. Viswanathan and Guarente showed that *geIn3* worms still have long lifespan after outcrossing compared to outcrossed control lines (74).

These contradictory results concerning the effect of Sirtuin overexpression on lifespan might be explained by the extent of overexpression of Sirtuin. 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 Sirtuin 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 Sirtuins (79, 80). In many animal models, the expression and activity of Sirtuin 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 stain (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 *fob1Δ* (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 $dSir2^{4.5}$ and $dSir2^{17}$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 *Sir2* mutant (102), suggesting that resveratrol extends the lifespan in a Sir2-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), paeonol (107), statins (108), hydrogen sulfide (109), Icariin (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 potency, solubility, and bioavailability as compared to resveratrol (117). Especially, SRT1720, a synthetic STACs

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 oxazolo(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 *S. cerevisiae* and *D. melanogaster* (127, 129), and only slightly extends 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 mice 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 *anmt-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 *anmt-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 dNAAM

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 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.

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413 **CONFLICTS OF INTEREST**

414 The authors declare no conflict of interest.

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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 ADP-ribosyl-transferase	Lifespan extension DNA repair Cell cycle arrest Cellular senescence
	SIRT2	Cytoplasm	Deacetylase	Cell cycle regulation
	SIRT3	Mitochondria	Deacetylase	Mitochondrial function Oxidative stress Centenarian-linked SNPs
	SIRT4	Mitochondria	ADP-ribosyl-transferase Deacetylase	Fatty acid oxidation Apoptosis
	SIRT5	Mitochondria	Demalonylase Desuccinylase Deacetylase	Fatty acid oxidation Oxidative stress
	SIRT6	Nucleus (chromatin)	ADP-ribosyl-transferase Deacetylase Deacetylase	Lifespan extension DNA repair Genome stability Telomere maintenance
	SIRT7	Nucleolus	Deacetylase	Epigenetic regulation Stress resistance Apoptosis

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