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Graphical Abstract

Redox-regulating sirtuins in aging, caloric restriction, and exercise

Zsolt Radak [a,b], Erika Koltai [a], Albert W. Taylor [a], Mitsuru Higuchi [a,b], Shuzo Kumagai [c], Hideki Ohno [d], Sataro Goto [a], Istvan Boldogh [e]

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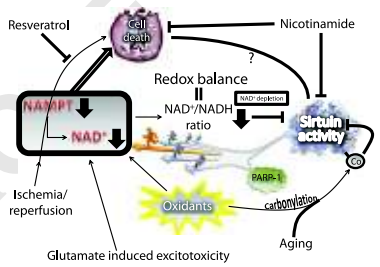

Highlights

► Sirtuins deacetylate important transcription factors that adjust the redox balance. ► Sirtuin-mediated protein stabilization was readily related to housekeeping and redox regulation. ► Sirtuins are able to alter the progress of aging. ► Adaptation to caloric restriction and physical exercise involves sirtuin-regulated pathways.

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Review Article

Redox-regulating sirtuins in aging, caloric restriction, and exercise

Zsolt Radak[a,b,*], Erika Koltai[a], Albert W. Taylor[a], Mitsuru Higuchi[a,b], Shuzo Kumagai[c], Hideki Ohno[d], Sataro Goto[a], Istvan Boldogh[e]

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ABSTRACT

The consequence of decreased nicotinamide adenine dinucleotide (NAD+) levels as a result of oxidative challenge is altered activity of sirtuins, which, in turn, brings about a wide range of modifications in mammalian cellular metabolism. Sirtuins, especially SIRT1, deacetylate important transcription factors such as p53, forkhead homeobox type O proteins, nuclear factor κB, or peroxisome proliferator-activated receptor γ coactivator 1α (which controls the transcription of pro- and antioxidant enzymes, by which the cellular redox state is affected). The role of SIRT1 in DNA repair is enigmatic, because it activates Ku70 to cope with double-strand breaks, but deacetylation of apurinic/apyrimidinic endonuclease 1 and probably of 8-oxoguanine-DNA glycosylase 1 decreases the activity of these DNA repair enzymes. The protein-stabilizing effects of the NAD+-dependent lysine deacetylases are readily related to housekeeping and redox regulation. The role of sirtuins in caloric restriction (CR)-related longevity in yeast is currently under debate. However, in mammals, it seems certain that sirtuins are involved in many cellular processes that mediate longevity and disease prevention via the effects of CR through the vascular, neuronal, and muscular systems. Regular physical exercise-mediated health promotion also involves sirtuin-regulated pathways including the antioxidant-, macromolecular damage repair-, energy-, mitochondrial function-, and neuronal plasticity-associated pathways. This review critically evaluates these findings and points out the age-associated role of sirtuins.

Contents

Abbreviations: 8-oxoG, 8-oxo-7, 8-dihydroguanine; APE1, apurinic/apyrimidinic endonuclease 1; BER, DNA base excision repair; CR, caloric restriction; DSB, DNA double-strand break; FOXO, forkhead homeobox type O protein; eIF2α, eukaryotic initiation factor 2α; eNOS, endothelial nitric oxide synthase; IL-1β, interleukin 1β; OGG1, 8-oxoguanine-DNA glycosylase 1; PIG3, p53-induced gene 3; PARP1, poly(ADP-ribose) polymerase 1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; MST1, mammalian sterile 20-like kinase 1; NAD+, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NOXA, NADPH activator A; SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1 or NAD+-dependent deacetylase sirtuin 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; Sir2, silent information regulator 2; TNF-α, tumor necrosis factor α; Trx, thioredoxin; UCP2, uncoupling protein 2

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Introduction

Sirtuins (silent information regulator 2 (Sir2)1 proteins) belong to an ancient family of evolutionarily conserved NAD+-dependent enzymes with deacetylase and/or mono-ADP-ribosyltransferase activity and are implicated in diverse cellular processes. The sirtuin family is ubiquitously distributed in mammals, with seven homologs (SIRTs 1–7), and their expression/activity shows organ and organelle specificity. Powerful protein deacetylase activity of SIRT1, SIRT2, SIRT3, and SIRT5 has been reported toward histones, whereas SIRT4, SIRT6, and SIRT7 have no such detectable enzymatic activity on histone peptide substrate [1,2]. SIRTs 3–5 are predominantly localized to the mitochondria. Mammalian sirtuins are closely involved in metabolism [2–4], which is linked to the mitochondrial generation of reactive oxygen species (ROS) [5,6]. SIRT1 is downstream in ROS signaling because of a dependence on the availability of NAD, but it can be important upstream in cellular regulators, including forkhead homeobox type O factor 3 (FOXO3) [7], muscle-specific RING finger protein 1 [8], and the v-Akt murine thymoma viral oncogene homolog 1 (Akt1) [9].

The crystal structure of human SIRT1 (a homolog of yeast Sir2) reveals a large groove intersected by a pocket lined with hydrophobic residues, conserved with class-specific protein-binding sites of each Sir2 class [10]. Activity of most of the sirtuins is controlled by posttranslational modifications, as well as the availability of NAD+. It has been shown that they are phosphorylated at N- and C-terminals, which play a role in substrate binding [11]. Moreover, in addition to phosphorylation, it appears that S-nitrosylation of SIRT1 impairs the catalytic activity of enzymes via a nitrosylated glyceraldehyde-3-phosphate dehydrogenase-mediated process [12]. Additionally, thioredoxin (Trx) regulates cellular redox balance through reversible oxidization of its redox-active cysteine residues (-Cys-Gly-Pro-Cys-), which can mediate protein S-denitrosylation [13–15] and hence the activity of sirtuins. The deacetylase domain of sirtuins consists of approximately 250 amino acids, differentiated by divergent N- and C-terminal extensions [16]. In the budding yeast Saccharomyces cerevisiae, the Sir proteins are involved in a wide array of cellular processes, including the nonhomologous end-joining repair of DNA [17], the stabilization of the replication forks in the ribosomal (r) DNA to prevent DNA breaks, recombination, and the generation of extrachromosomal rDNA circles [18], which leads to aging of this organism [19].

One of the first studies that linked sirtuins to aging was based on the observation that proteins encoded by SIR genes are responsible for silencing the rDNA of S. cerevisiae [20]. The same group of investigators also demonstrated that Sir2 is redox sensitive because of its NAD+ dependence. Moreover, they showed that sirtuins have deacetylase activity from eubacteria to humans [21]. Guarente and his group then demonstrated that redistribution of the Sir2 complex from a telomere to the nucleolus is associated with aging in yeast [19,22], and overexpression of this gene extends their life span [23]. Sinclair and co-workers showed that life extension in yeast could be done via the salvage pathway of NAD+ [24], and life-span extension during caloric restriction (CR) is associated with activation of Sir2 genes in yeast and the mammalian homolog SIRT1 of human cells [25,26]. Now, mounting data suggest an active role for sirtuins in aging and age-associated diseases. However, as with most phenomena in science, the convincing effects of sirtuins in mammalian aging are not without debate [27].

This paper reviews the redox sensitivity of sirtuins and the role of these lysine deacetylases, especially SIRT1, in the aging process. The authors also crucially review the data of knockout and overexpression models, as well as the effects of CR and physical exercise. The effects of resveratrol, which is a potent stimulator of SIRT1, have been reviewed elsewhere [28–31].

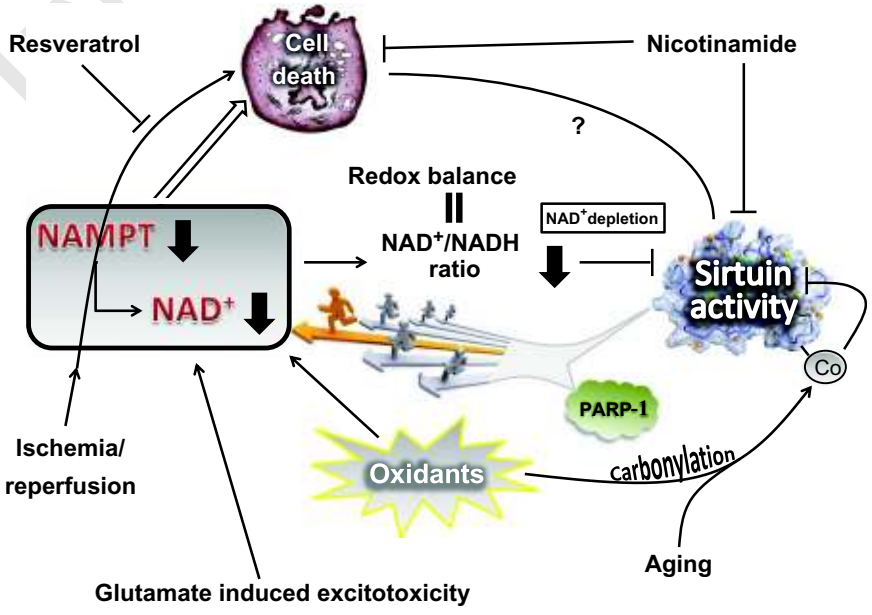

Fig. 1. The suggested mechanisms between the availability of NAD and the activities of sirtuins and PARP is shown. Metabolic challenges modulate NAD levels, NAD:NADH ratio, and cell fate by shifting to either pro- or antiapoptotic pathways.

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Role of sirtuins in cellular redox balance

Redox balance and sirtuins: up- and downstream regulators

The activity of sirtuins can be readily modified by the availability of NAD+, expressed as a function of NAD:NADH ratio, which is a marker of cellular redox balance. Metabolic challenges, such as hypoxemia–ischemia and reoxygenation, result in deceased NAD and nicotinamide phosphoribosyltransferase (NAMPT) levels and decreased activity of sirtuins [32–35]. It was recently shown that treatment with glycated low-density lipoprotein resulted in increased ROS production in vascular endothelial cells and in lowered mitochondrial membrane potential, the NAD:NADH ratio [36]. It has been shown that glutamate-induced excitotoxicity in neurons and ischemia in mouse brains result in marked decreases in NAD+ levels, which is a precausative step before cell death [32]. ROS can readily induce DNA damage, and in response to DNA strand breaks poly (ADP-ribose) polymerase 1 (PARP1) consumes significant amounts of NAD+ to produce poly(ADP-ribose) polymers on target proteins in a process called poly-ADP-ribosylation [37]. Significant DNA damage-associated PARP activation can lead to NAD+ depletion resulting in energy crisis [38].

Hence, administration of nicotinamide, which is a NAD+ precursor and a noncompetitive inhibitor of SIRT1, has rescued cells from apoptosis and necrosis [32]. On the other hand resveratrol supplementation did not increase cell survival. In contrast, in ischemic–reperfused cardiomyocytes resveratrol supplementation mediated cell survival via mitogen-activated protein kinase pathways [39].

The concentration of NAD+ seems to be crucial, because when PARP1 was knocked out the phenotype of mice resembled the SIRT1 of overexpressed phenotypes, and similar phenomena have occurred when PARP1 was inhibited by pharmacological agents [40]. These data strongly point to a competition between PARP1 and SIRT1 for the common cofactor NAD+ (Fig. 1). On the other hand, when the NAD+ level was depleted first by oxidants, such as hydrogen peroxide or cigarette smoke, then the PARP1 inhibition was not effective in gaining back SIRT1 activity. Nonetheless, the NAD+ content was restored [35]. One of the causative factors could be that oxidants resulted in carbonylation of amino acid residues of SIRT1, which, in turn, could curb the activity of the enzyme [35,41]. This could happen with aging also. Koltai and colleagues [42] observed that with aging there is an increase in the level of SIRT1, which is associated with decreased activity of the enzyme and appears to be due to increased carbonylation. In this study, carbonylation of SIRT1 was not studied specifically; however, the overall carbonylation of the cytosolic proteins has been found to significantly increase in older animals. In a recent study in which pelvic human skin samples were used, DNA damage was positively correlated with aging and negatively correlated with NAD+ levels [43]. Therefore, it seems that oxidants could affect sirtuins via depletion of NAD+ and oxidative modification of amino acid residues, leading to decreased activity or level of the enzyme [40,44]. On the other hand, it has also been shown that the overexpression of SIRT1 has beneficial effects in resistance to oxidative stress [39,44,45].

The effects of sirtuin-regulated transcription factors on redox balance

Sirtuin-mediated deacetylation of key transcription factors results in altered gene expression of key antioxidant enzymes or those producing ROS. Here we focus on redox regulation by sirtuins via p53, FOXOs, nuclear factor κB (NF-κB), and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) as examples.

SIRT1 is involved in stress responses, cellular metabolism, and aging through deacetylation of a variety of substrates including p53. p53 is acetylated by CBP/p300 acetyltransferases at lysine residues, including Lys 370, 372, 382, and 386 at the carboxy-terminal region [46,47]. Activated p53 then enhance ROS production through mitochondrial dysfunction and/or increased expression of genes that are involved in redox modulation, such as the p53-upregulated modulator of apoptosis (PUMA), NADPH activator A (NOXA), and p53-induced gene 3 (PIG3) [48,49]. In addition, it seems that NADPH oxidase 1-generated ROS inhibit the acetylation of p53 at Lys 382 via SIRT1. Deacetylation at Lys 382 is important in the control of gene expression from PUMA, NOXA, and PIG3, i.e., the proapoptotic function of p53 [50]. Interestingly, p53 via p21 indirectly activates nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a central transcription factor in the antioxidant response. Nrf2 binds to the antioxidant-response element 2, a cis-acting enhancer found in promoters, and, via increased antioxidant gene expression, counteracts oxidative challenge [51].

In mammals there are four FOXO transcription factors, and, among them, FOXO1, FOXO3a, and FOXO4 are involved in redox regulation [52,53]. FOXOs are negatively regulated by the phosphatidylinositol 3-kinase–Akt signaling pathway. When cells were treated with hydrogen peroxide, the FOXO3 acetylation was increased by CBP/p300 and, as a result, the resistance against oxidative stress was enhanced [54]. Moreover, oxidants caused translocation of FOXO3 into the nucleus, where it can be deacetylated by SIRT1, which would enhance the activation of GADD45. This is important for stress resistance by the induction of DNA repair, because GADD45 is required for efficient DNA base excision repair (BER) and nucleotide excision repair (NER) [54,55]. In addition, FOXO3 directly activates the transcription of Mn-superoxide dismutase (SOD) and catalase [56] to attenuate the toxicity of mitochondrial-derived superoxide anion and hydrogen peroxide, thereby preventing damage to genomic and mitochondrial (mt) DNA, lipids, and proteins. In addition to FOXO3, FOXO1 can also alter redox states. For instance, Akt-mediated phosphorylation of PGC-1α at serine 570 results in inactivation, and this conformational change leads to reversible disruption of the FOXO1 response element of the catalase promoter, therefore curbing the expression of catalase [57]. In addition, it appears that FOXO4 inhibits NF-κB and thereby the proinflammatory chemokine/cytokine expression and ROS production associated with the activation of inflammatory cells [58].

NF-κB-dependent signaling plays a role in the aging process, with a wide range of effects in addition to inflammation [59]. NF-κB is a family of inducible transcription factors that plays a central role in controlling expression of networks responsible for proinflammatory gene expression, cell proliferation, differentiation, or apoptosis [60]. NF-κB release from cytoplasmic inhibition is necessary, but not sufficient, for target gene induction, e.g., that induced by ROS RelA/p65 phosphorylated at serine 276, which is coincident with its release from IκBα in the canonical pathway. Upon phosphorylation, RelA/p65 translocates into the nucleus and mediates the promoter-specific recruitment of p300/CBP, which acetylates it at lysine 510 to enhance transcription. It has also been shown that SIRT1 deacetylates the RelA/p65 on lysine 310 and thus blocks the transcriptional activity of NF-κB [61], resulting in active silencing of the chromatin (Fig. 2). SIRT1 has been shown to be capable of decreasing NF-κB-induced inflammation by deacetylation and prevents the tumor necrosis factor α (TNF-α)-mediated activation of matrix metalloproteinase 9, interleukin 1β (IL-1β), IL-6, and inducible nitric oxide synthase [62].In addition, it has been suggested that SIRT1 binds to the promoter regions of IL-1β and TNF-α in a cell model of sepsis and plays a role in epigenetic regulation during endotoxin tolerance [63]. However, it is clear that suppression of NF-κB activity by SIRT1 modulates redox signaling, not just by regulating inflammation but also through the expression of Mn-SOD [64,65].

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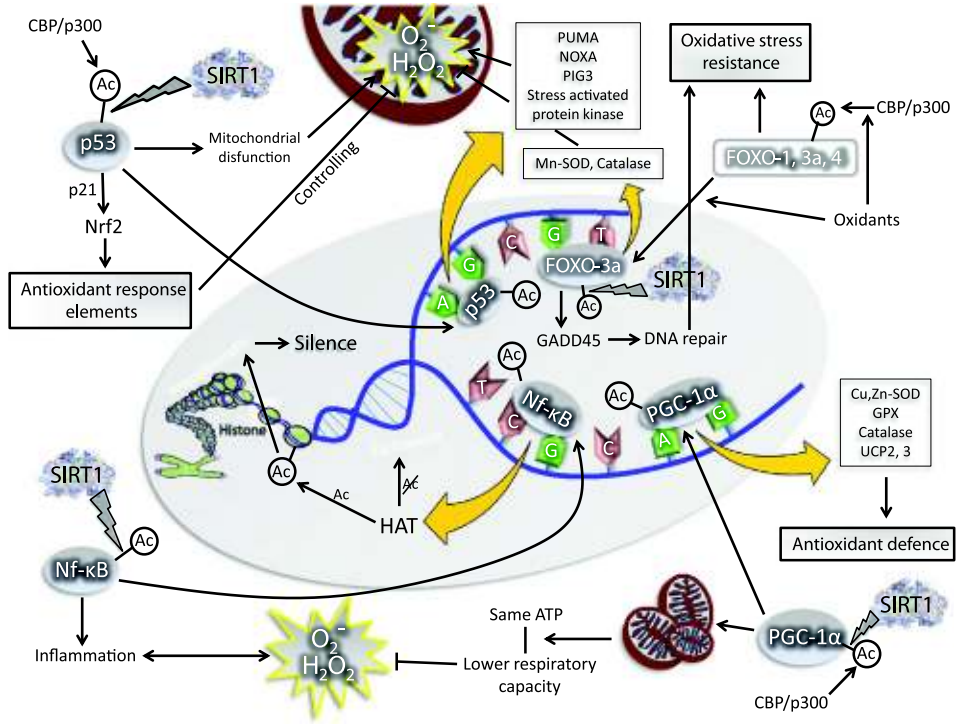

Fig. 2. Sirtuins are involved in a complex way in redox regulation. By the deacetylation of transcription factors, such as p53, FOXOs, NF-κB, and PGC-1α, they modulate the transcription of pro- and antioxidant genes, mitochondrial biogenesis, activity of oxidative damage-repairing enzymes, and chromatin structure.

PGC-1-related proteins are expressed ubiquitously, but PGC-1α and -1β are enriched in mitochondria-rich tissues such as cardiac and skeletal muscles. PGC-1α is a master transcriptional coactivator that modulates the gene expression involved in energy metabolism and energy expenditure. PGC-1α is a direct link between external physiological stimuli and the regulation of mitochondrial biogenesis. PGC-1α regulates the activity of a large number of transcription factors, including peroxisome proliferator-activated receptors c and a, estrogen receptor-related a, FOXO1, hepatocyte nuclear factor 4a, and nuclear respiratory factor 1. Moreover, PGC-1α also regulates gene transcription encoding mitochondrial electron transport chain proteins, uncoupling protein 2 (UCP2) and UCP3, and key antioxidant enzymes, such as Cu,Zn-SOD, glutathione peroxidase, and catalase [66,67]. Its transcriptional coactivator function is primarily regulated by acetylation via p300/CBP and deacetylation by SIRT1 [68]. Mitochondrial biogenesis itself can be an important cog in redox homeostasis, because for the same ATP production more mitochondria can work at a lower respiratory capacity, and then fewer ROS are produced. Contrarily, decreased mitochondrial capacity at metabolic stress, such as exercise or ischemia, would result in enhanced ROS production. These observations support PGC-1α's active involvement in antioxidant defense [69]. Inflammation is associated with increased ROS production and oxidative damage, and the antioxidant role of PGC-1α is further supported by the anti-inflammatory effects of this coactivator [70].

Sirtuins in regulation of genome integrity

One of the first studies on sirtuins and DNA repair as well as telomere silencing was done in yeast cells (S. cerevisiae). These studies revealed that Ku protein interacts with sirtuins during DNA double-strand break (DSB) repair of telomere regions [71,72]. Studies have also shown that SIRT1 deacetylates Ku70 in mammalian cells, revealing antiapoptotic effects [26,73]. Another sirtuin family member, SIRT6, interacts with PARP1 to enhance the efficiency of repair of DSBs [74]. SIRT6 is also important for other DNA repair pathways, because in SIRT6-ablated cells, overexpression of a single-strand break gap-filling protein, DNA polymerase β, rescued cells, implying that SIRT6-mediated deacetylation has a crucial role in BER processes [75]. Consequently, a lack of SIRT6 in knockout mice decreased the life span [76]. Moreover, many human cancers show decreased levels of SIRT1, and SIRT1 mutant mice exhibit an increased incidence of cancer [77].

The apurinic/apyrimidinic endonuclease 1 (APE1) plays a central role in the repair of oxidized and alkylated bases in mammalian genomes via the BER pathway. APE1 also functions as redox effector factor 1 for several transcription factors, including activator protein-1 (AP-1), HIF1α, and p53 [78]. Importantly, both repair and transcriptional function (coactivator and corepressor) of APE1 are modulated by acetylation in redox-dependent and redox-independent mechanisms [79]. It has also been shown that APE1 is a target of SIRT1-mediated deacetylation, and the administration of resveratrol increases the activity of this important BER enzyme [80]. Along with APE1, human 8-oxoguanine-DNA glycosylase 1 (OGG1) is the major DNA glycosylase for excision of 7,8-dihydro-8-oxoguanine (8-oxoG) and ring-opened fapyguanine. OGG1 has been shown to be acetylated by p300/CBP at lysines 338 and 341, which results in a significant increase in its activity [81]. We have recently demonstrated that Ac-OGG1 is present in human skeletal muscle and rat tissues, and the level of acetylation decreases with aging, whereas a parallel pattern was found for SIRT1 activity [82,83]. Moreover, we have shown that resveratrol administration decreases the acetylation level of OGG1 and ablation of SIRT1 by siRNA-increased levels of Ac-OGG1. This finding suggests that SIRT1 is counteracted by OGG1 (Sarga, Z. Radak, and I. Boldogh, unpublished observation). It has been recently shown that the OGG1–8-oxoG complex interacts with and catalyzes GDP–GTP exchange in the canonical Ras family GTPases H-, K-, and N-Ras. These data imply that in the presence of 8-oxoG base, OGG1 acts as a guanine nucleotide

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exchange factor [84]. In turn, Ras–GTP via Raf1 (v-raf-1 murine leukemia viral oncogene homolog 1) phosphorylates the mitogen-activated kinases MEK1,2/ERK1, which may lead to ROS production. In addition to BER, NER is also modulated by sirtuins, because the xeroderma pigmentosum group A is deacetylated and suppressed by SIRT1 [85,86]. Moreover, it was also shown that human cancers have decreased levels of SIRT1, and SIRT1 mutant mice exhibited an increased incidence of cancer [77].

Sirtuins in protein stability and cellular stress response

Housekeeping of oxidative stress and damage is crucial in redox balance as it affects the fate of the cells. A group of genes involved in preserving cellular homeostasis during stress conditions, called vitagenes, and sirtuins, among others, are involved in a wide spectrum of cellular defense [14,87,88]. Indeed, one of the ways by which sirtuins regulate the stress response is through protein stability, which can readily affect the levels of oxidatively modified/damaged proteins [89]. One of the ways by which SIRT1 protects cells from oxidative stress is its involvement in the integrated stress response pathway via eukaryotic initiation factor 2α (eIF2α). During stress, cells suppress the levels of protein synthesis and mobilize most of the available sources to cope with stress [90], and the reduction in eIF2–GTP levels leads to a general reduction in global protein synthesis. It turns out that SIRT1 interacts with mediators of eIF2α dephosphorylation, suggesting a role for SIRT1 in the eIF2α-related early stress response [90], which partly depends on the NAD:NADH ratio.

Cellular stress response is also mediated by heat shock transcription factor 1 (HSF1), which when acetylated negatively regulates DNA binding. On the other hand, SIRT1-mediated deacetylation of HSF1 enhances its binding to the heat shock protein 70 promoter by maintaining HSF1 in a deacetylated, DNA-binding-competent state [91]. This finding established the role of SIRT1 in the heat shock response and longevity, because HSF1 has been linked to longer life spans [92], as well as in protein stability, because heat shock proteins are important modulators of protein degradation. Indeed, the effect of acetylation on protein stability is another acting point in the cellular stress response by which redox balance is supervised by sirtuins [82,93–96].

The role of acetylation and deacetylation of protein stability has been reviewed by others [97,98]. One of the first proteins for which acetylation was linked to stability was p53 [47,99]. Acetylation of lysine residues prevents ubiquitination of the given residues, thus hampering signaling for proteolytic degradation [98]. Indeed, inhibition of SIRT1 by nicotinamide decreased the polyubiquitination of FOXO3 and hence the protein levels of this transcription factor [96]. The activation of SIRT1 resulted in enhanced degradation of FOXO3 by the proteasome system. Aging results in a loss of the activity of SIRT1, which is associated with increased levels of lysine acetylation in rat brain and skeletal muscle [42,82,100]. The level of acetylated lysine residues correlated well with that of carbonylation, which may mean that acetylated lysine could not have been ubiquitinated and degraded by proteasomes. If this happens, the half-life of proteins increases, which is believed to take place with aging, resulting in the enhanced accumulation of damaged proteins and consequently decreased physiological function [101]. However, acetylation of nonhistone proteins also takes place in a very selective manner [102].

Acetylation of histone residues by sirtuins alters the transcription from genes that are involved in redox homeostasis (Fig. 3). For instance, SIRTs 1–3 and 6 are involved in shaping chromatin structure, and the well-known target of SIRT1 deacetylation is the acetylated histone H3 lysine 9, which is a precausative step toward the methylation of the same lysine residue by the histone methyltransferase suppressor of variegation (Suv) 39h1 [103]. SIRT1 preserves pericentromeric heterochromatin via stabilization of Suv39h1 by preventing the polyubiquitination of lysine 87

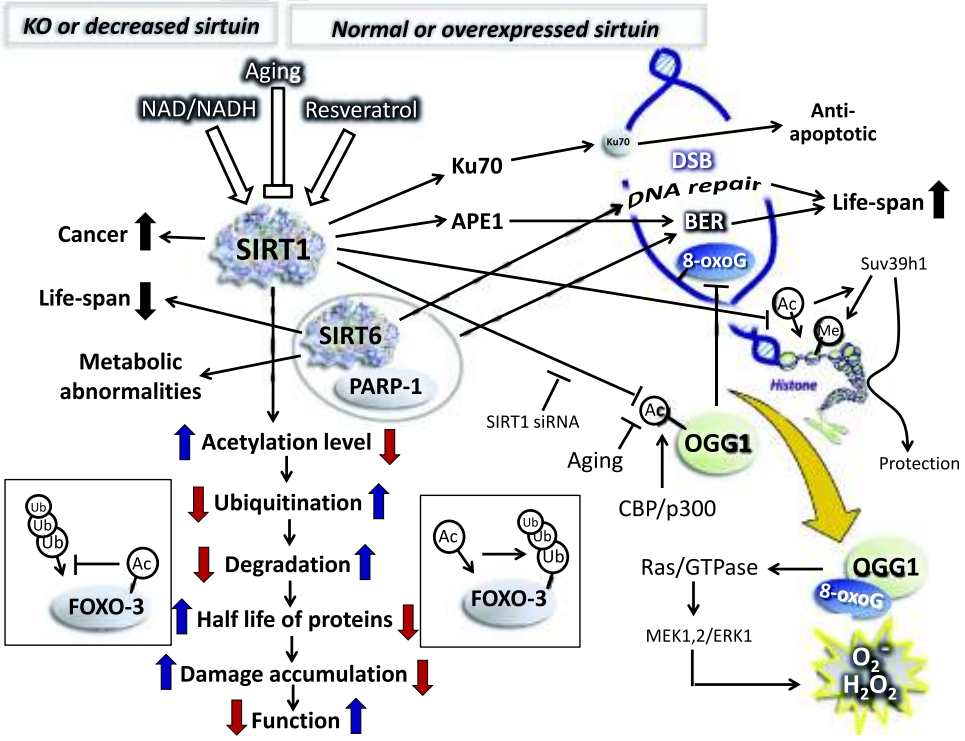

Fig. 3. Sirtuins regulate the stability of proteins, which in turn would affect cell and organ function. Modulation of DNA repair could be associated with longevity, metabolic abnormalities, cancer, and aging.

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of this methyltransferase by E3-ubiquitin ligase [104]. The stabilization of Suv39h1 under stress conditions, such as oxidative stress, results in enhanced protection of chromatin structure.

Sirtuins and mammalian aging

The involvement of sirtuins in the aging of yeast, such as the silencing of rDNA, is quite different and less complex than that occurring in mammalian systems. However, even in yeast, the life extension effects of overexpression of Sir2 have been questioned recently [27]. It appears that SIRT1 regulates mammalian FOXOs more than does the bacterial homolog Sir2, and the inhibition of FOXOs and p53 results in an antiapoptotic process, which is closely related to the resistance to oxidative stress. Deacetylation of p53 by SIRT1 attenuates the DNA-binding and transcriptional activity, whereas deacetylation of FOXOs can result in suppression of transcription from proapoptotic genes and enhance activity of those that are involved in oxidative stress resistance [54]. Therefore, the effects of SIRT1 on these transcription factors are to promote cell survival in mammals. Recently, it was reported that mammalian sterile 20-like kinase (MST1) can inhibit SIRT1 by phosphorylation in response to DNA damage, which consequently leads to apoptosis through the acetylation of p53. This observation further supports the key role of SIRT1 in cellular resistance during oxidative stress [105]. The controlling role of SIRT1 on p53 can alter redox balance, because the proapoptotic role of p53 can induce genes involved in ROS production, and this transcription factor can activate enzymes in the antioxidant system [106,107]. It is suggested that low levels of deacetylated p53 suppress cellular levels of ROS, whereas high levels of acetylated p53 induce ROS production-related genes (see review in [108]).

In terms of aging, it has been shown that overexpression of SIRT1 in primary fibroblasts decreased acetylation of p53 and antagonized PML/p53-induced premature aging of cells in culture [109]. The question is whether this activity occurs at the level of the organism in vivo as well. The finding that there is an increase in the levels of p53, FOXO1, and SIRT1 in the nuclei of cells of old animals [110] may be indicative of a complex setting in mammals. In sporadic inclusion-body myositis, which is an aging-associated muscle disease, it was found that deacetylation of p53 was decreased [111]. Aging results in decreased intracellular levels of NAD+ and NAD:NADH ratio in various organs of old rats, and this finding was associated with decreased activity of SIRT1 and increased acetylation of p53 [33]. Moreover, aging processes resulted in a lower activity of complexes I–IV in the mitochondria and enhanced oxidative DNA damage [33]. In support, an increase in SIRT1 levels has been shown in skeletal muscle of aged animals, and this was associated with decreased activity of the enzyme along with lower levels of NAMPT and increased levels of oxidative damage [42]. During the aging process, the level of noncoding microRNA R-34a and microRNA R-93 is increased in rat liver, and these microRNAs suppress the activity of specificity protein 1 and Nrf2 transcription factors, resulting in decreased SIRT1 and microsomal glutathione S-transferase 1 expression [112]. This posttranslational repression of SIRT1 and microsomal glutathione S-transferase 1 naturally results in decreased oxidative defense during aging in the rat liver.

There is a paucity of these studies on human healthy aging and sirtuins. SIRT1 plays a role in telomere length, which is used as a marker to appraise the aging process [113,114]. A single-nucleotide polymorphism in the SIRT1 gene and another in the 3′ flanking region of XRCC6 interfered with the length of the telomere in human leukocytes and aging [115]. Moreover, it was also reported that the frequency of the minor allele of SIRT1 was enhanced in older subjects. Data from human and animal studies revealed that SIRT1 could modulate and deacetylate endothelial nitric oxide synthase (eNOS), which in turn resulted in altered NO production. The acetylation level of aortic eNOS of 30-month-old rats was many times higher than that of young rats [116]. Sirtinol was used to inhibit SIRT1 in this study, and it abolished the age-associated difference in endothelium-dependent dilatation, and the results gained from the samples of endothelial cells, obtained from the brachial artery of young and old subjects, confirmed that in healthy aging SIRT1 could play a role in endothelial dysfunction [116].

mRNA analysis revealed no age-associated changes in SIRT1 and SIRT3 expression in skeletal muscle in young vs old subjects [117]. Similar results were obtained in the rat myocardium of young and old rats [118]. It seems that, as a result of aging, SIRT1 and SIRT6 levels increase in rat skeletal muscle, whereas the activity of OGG1 is decreased [42]. Interestingly, the SIRT1 levels in the hippocampus of the same animals decreased as a result of aging, whereas we found elevated levels of SIRT3 [82]. An increased level of 8-oxoG in the hippocampus with elevated levels of OGG1 was observed in aged subjects. However, when we checked the acetylation level of OGG1, it was found that aging significantly decreased the Ac-OGG1 content. Therefore, it was suggested that the drop in OGG1 acetylation could account for the increased 8-oxoG levels in cellular DNA of hippocampus [82]. We further studied the acetylation pattern of OGG1, and our unpublished data suggested that SIRT1 interacts with and deacetylates OGG1 as in case of APE1.

Analysis of specimens from human brain regions of healthy and Alzheimer disease (AD) subjects showed that SIRT1 expression at both the RNA and the protein level is significantly decreased in the parietal cortex of AD patients, whereas cortical expression was not affected [119]. Global cognition scores obtained before death would suggest that the decrease in SIRT1 levels is related to the accumulation of amyloid-β and τ in the cerebral cortex of persons with AD [119]. On the other hand, it was also reported that in age-related cataracts in healthy older humans there was a lower level of SIRT1 expression, and the levels of SIRT1, p53, FOXO3, and FOXO4 were comparable to data from young subjects [120]. It is also clear that the antiapoptotic role of SIRT1 can contribute to increased tumor growth [121], which can be attenuated by SIRT1 inhibitors. The complexity of SIRT1 in senescence involves stem cell differentiation, which emphasizes the role of SIRT1 in controversial processes during aging [122].

Sirtuin knockout and transgenic animal models

Despite drawbacks, knockout animal models offer one of the most powerful means for studying gene function. There are powerful data showing that knockdown or overexpression of sirtuins results in shorter or longer life spans, respectively, in model organisms from yeast to mice [4,76], although the information in the literature is not unequivocal.

It has been reported that in SIRT1 knockout mice, although the oxidative damage was decreased in the brain, the life span was shorter, and CR was not effective [123]. In addition, inhibition of SIRT1 decreased insulin-like growth factor 1 (IGF-1) signaling and increased resistance against oxidative challenge. According to that report, SIRT1, under certain physiological conditions, enhances IGF-1 signaling by deacetylating insulin receptor substrate 2 (IRS2), resulting in Ras/ERK activation, increasing ROS production and altering redox signaling [123]. Knocking out SIRT1 in mice with A53T α-synuclein mutation decreased, while overexpression increased, the life span of transgenic mice [124].

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Moreover, in another disease model, which was developed to mimic Huntington disease, it was found that ablation of SIRT1 caused exacerbation of brain pathology, whereas brain-specific overexpression of SIRT1 attenuated the consequences of Huntington disease, partly by the upregulation of brain-derived neurotrophic factors leading to enhanced survival [125].

On the other hand, data from another research group indicated that ablation of SIRT1 resulted in impaired memory, cognitive function, and spatial learning, and the expression of genes important for synaptic plasticity and metabolism was decreased [126]. In contrast, SIRT1-overexpressing mice showed normal cognitive functions and learning. It should be pointed out that overexpression of SIRT1 in striatum and hippocampus, under the control of the α subunit of Ca2+–calmodulin-dependent protein kinase II promoter, decreased glucose transporter type 4 level in skeletal muscle and increased body fat. This genetic manipulation altered the performance in an open-field test, through the deacetylation of IRS2, which induced phosphorylation of IRS2 and ERK1/2 in the striatum of these mice [127]. SIRT1 knockout mice revealed that SIRT1 is important to development, and the hyperacetylation of p53 was observed in SIRT1-ablated models, which may indicate that SIRT1 is crucial for p53-mediated apoptosis [128]. Indeed, it has been shown that SIRT1 mutant and p53 mutant mice develop tumors in multiple tissues, which also emphasizes the possible role of SIRT1 in the regulation of p53 [77].

It has been reported that SIRT1 knockout mice have greater mobility; when they were exposed to treadmill running and the rotarod test, their performance was better than that of wild-type mice [129]. It would be important to understand how the ablation of SIRT1 in this model could result in better physical performance. On the other hand, overexpression of SIRT1 in mice also resulted in better performance on the rotarod test, and their phenotype resembled those of mice that are exposed to CR [130]. Therefore, overall, data from knockout models clearly showed how complex the role of SIRT1 is in mammalian physiology and how carefully the knockout data need to be evaluated in terms of SIRT1's function. A major problem of these studies is that the proteins that are targets of SIRT1 are not apparent, in most cases, nor is how deacetylation of those proteins is mechanistically related to the outcomes observed.

Regulation of sirtuins by CR and exercise

CR is known to increase mean and maximum life spans, and regular physical exercise can also enhance mean life span; moreover, sirtuins are indicated as being involved in both cases. For example, a number of studies have shown that sirtuins, especially SIRT1, mediate CR-induced longevity. However, the role of Sir2 in CR was questioned by Kaeberlein and co-workers [131,132], but their model used yeast, which differs from mammalian models. When 12-month-old rats were subjected to CR, as 60% of the daily food allotment, the result was a downregulation of the IGF-1 pathway and SIRT1-mediated regulation of p53, Ku70, and FOXO3 [26]. This was one of the first demonstrations of the powerful effect of CR on SIRT1-associated signaling. In another rat study, in which the dietary amino acids, except methionine, were restricted to 40%, increased SIRT1 levels were found along with unchanged levels of PGC-1α and of mtDNA damage, but with decreased levels of mitochondrial protein oxidation [133].

Thioredoxin-interacting protein (TXNIP) has been suggested to elevate sensitivity to oxidative stress through inhibition of Trx [134], having an effect opposite to that of sirtuins on life span [135,136]. Consistently, it has been shown that limited calorie availability inhibited TXNIP and slightly induced SIRT1, whereas resveratrol had biphasic effects [137], and its inhibitory role on TXNIP was mediated through AMPK pathways.

Csiszar and co-workers [138] demonstrated that life-long CR significantly decreased age-associated vascular dysfunction, oxidative stress, and NF-κB activity. They also showed from a relating cell culture that inhibition of SIRT1 by silencing RNA eliminated the beneficial effects of CR on inflammation and antioxidants, possibly indicating that SIRT1 is actively involved in the CR-mediated signaling process. Aging results in decreased protein levels of SIRT1 in the hippocampus [82], and this decrease can be attenuated by CR [139], which may mean that CR could act via SIRT1. Studies on cAMP responsive-element binding 1 (CREB1) knockout mice revealed that CR (given as 60% of the ad libitum normal diet) decreased SIRT1 levels in the hippocampus and cortex, whereas in wild-type animals the SIRT1 was upregulated by CR [140]. That report presented evidence that CR in the brain was mediated by CREB1, which in turn induced SIRT1 expression, and suggested that SIRT1-associated metabolic processes played a role in neuronal plasticity. In addition, SIRT1 is also involved in the effects of CR in skeletal muscle, because it has been shown that the transcription factor signal transducer and activator of transcription 3 is deacetylated and therefore inactivated by SIRT1 during CR, leading to a downregulation of insulin-stimulated phosphoinositide 3-kinase, resulting in enhanced insulin sensitivity [141]. Moreover, another study reported that SIRT1, via its interaction with AP-1, promotes macrophage function during CR [142]. Studies on CR have resulted in some controversial issues on mitochondrial biogenesis, at least those using experimental rodents [143,144]. Data from human skeletal muscle provide evidence that CR can result in enhanced mitochondrial mass and increased SIRT1 levels [145]. Therefore, it seems to be supported by a number of papers that CR indeed acts through SIRT1-controlled molecular processes in mammalian systems.

The effects of exercise, similar to those of CR, appear to be systemic and complex [146], as it has been shown that both a single bout of exercise and endurance training result in elevated levels of SIRT1 protein in the skeletal muscle of rats [147]. Prolonged moderate exercise training resulted in increased SIRT1 levels in rat hearts. Moreover, the results demonstrated an enhanced content and expression of FOXO3, along with elevated expression of GADD45 [148]. In addition, this study concluded that exercise normalizes the aging-associated alteration in heart and adipose tissue. Similar conclusions were drawn with skeletal muscle, whereby exercise counterbalanced the age-associated decline in NAMPT content and decrease in SIRT1 activity [42]. Data indicate that exercise can attenuate the age-associated decrease in mitochondrial protein synthesis in skeletal muscle of rats, which included the loss of SIRT1 and PGC-1α levels as well [149]. Similar data have been reported for mouse liver [150].

Studies using human skeletal muscle have also shown exercise-related upregulation of NAMPT content [151], an important adaptive response to exercise-induced metabolic challenge, which can help to regulate redox balance through the NAD:NADH ratio, thus modulating the activities of sirtuins, PARP, and lactate dehydrogenase. Two weeks of high-intensity interval training in humans resulted in significant increases in mitochondrial biogenesis, increased nuclear PGC-1α content, and increased GLUT4 content, all of which appear indicative of improved insulin sensitivity of these subjects [152]. A single bout of exercise increased the SIRT1 expression in young individuals, whereas it failed to have similar effects on the aged, and, in general, young subjects responded with greater levels of mRNA expression of mitochondrial biogenesis-related genes [117].

Exercise appears to increase SIRT1 levels in the hippocampus [153], but not in the cerebellum [100]. However, data from other studies have shown that age-associated decreases in brain

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function can be alleviated by regular exercise [82,154]. Moreover, recent data indicate that the age-induced increases in Ku70 levels can be attenuated by the combined effects of IGF-1 and exercise [155]. The protein content of SIRT1 decreased, whereas SIRT3 increased, with aging. Exercise-induced redox and metabolic challenges and adaptive responses to regular activity lead us to suggest that sirtuin-mediated metabolic pathways could be important to mitochondrial biogenesis, fuel availability, antioxidant response, and DNA repair.

Conclusion

Sirtuins are master regulators of a wide range of metabolic processes in mammalian cells, and aging affects sirtuin-associated molecular pathways. Sirtuins could be on both sides of redox regulation, because the oxidative stress-related loss of NAD content would affect their activity. However, by their interactions with a number of transcription factors that target pro-oxidant and antioxidant genes, such as p53, NF-κB, PGC-1α, and FOXOs, sirtuins are not just dependent but also regulatory factors of the redox state. In addition, the role of sirtuins in oxidative damage repair is another pathway that could significantly alter the redox state. Accumulating data suggest that sirtuins, especially SIRT1, are potential targets for natural and pharmaceutical interventions into aging processes. Among natural interventions, it seems that CR and regular physical exercise act through sirtuin-associated processes, which has promise to improve health.

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References

[1] North, B. J.; Marshall, B. L.; Borra, M. T.; Denu, J. M.; Verdin, E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol. Cell* **11:**437–444; 2003.
[2] Houtkooper, R. H.; Pirinen, E.; Auwerx, J. Sirtuins as regulators of metabolism and healthspan. *Nat. Rev. Mol. Cell Biol.* **13:**225–238; 2012.
[3] Ralser, M.; Michel, S.; Breitenbach, M. Sirtuins as regulators of the yeast metabolic network. *Front. Pharmacol* **3:**32; 2012.
[4] Kanfi, Y.; Naiman, S.; Amir, G.; Peshti, V.; Zinman, G.; Nahum, L.; Bar-Joseph, Z.; Cohen, H. Y. The sirtuin SIRT6 regulates lifespan in male mice. *Nature* **483:**218–221; 2012.
[5] Vendelbo, M. H.; Nair, K. S. Mitochondrial longevity pathways. *Biochim. Biophys. Acta* **1813:**634–644; 2011.
[6] Lenaz, G. Mitochondria and reactive oxygen species:. which role in physiology and pathology? *Adv. Exp. Med. Biol* **942:**93–136; 2012.
[7] Jiang, M.; Wang, J.; Fu, J.; Du, L.; Jeong, H.; West, T.; Xiang, L.; Peng, Q.; Hou, Z.; Cai, H.; Seredenina, T.; Arbez, N.; Zhu, S.; Sommers, K.; Qian, J.; Zhang, J.; Mori, S.; Yang, X. W.; Tamashiro, K. L.; Aja, S.; Moran, T. H.; Luthi-Carter, R.; Martin, B.; Maudsley, S.; Mattson, M. P.; Cichewicz, R. H.; Ross, C. A.; Holtzman, D. M.; Krainc, D.; Duan, W. Neuroprotective role of Sirt1 in mammalian models of Huntington's disease through activation of multiple Sirt1 targets. *Nat. Med* **18:**153–158; 2012.
[8] Alamdari, N.; Aversa, Z.; Castillero, E.; Gurav, A.; Petkova, V.; Tizio, S.; Hasselgren, P. O. Resveratrol prevents dexamethasone-induced expression of the muscle atrophy-related ubiquitin ligases atrogin-1 and MuRF1 in cultured myotubes through a SIRT1-dependent mechanism. *Biochem. Biophys. Res. Commun.* **417:**528–533; 2012.
[9] Sundaresan, N. R.; Pillai, V. B.; Wolfgeher, D.; Samant, S.; Vasudevan, P.; Parekh, V.; Raghuraman, H.; Cunningham, J. M.; Gupta, M.; Gupta, M. P. The deacetylase SIRT1 promotes membrane localization and activation of Akt and PDK1 during tumorigenesis and cardiac hypertrophy. *Sci. Signaling* **4:**ra46; 2011.
[10] Finnin, M. S.; Donigian, J. R.; Pavletich, N. P. Structure of the histone deacetylase SIRT2. *Nat. Struct. Biol* **8:**621–625; 2001.
[11] Sasaki, T.; Maier, B.; Koclega, K. D.; Chruszcz, M.; Gluba, W.; Stukenberg, P. T.; Minor, W.; Scrable, H. Phosphorylation regulates SIRT1 function. PLoS One. *e4020* ; 2008.
[12] Kornberg, M. D.; Sen, N.; Hara, M. R.; Juluri, K. R.; Nguyen, J. V.; Snowman, A. M.; Law, L.; Hester, L. D.; Snyder, S. H GAPDH mediates nitrosylation of nuclear proteins. *Nat. Cell Biol.* **12:**1094–1100; 2010.
[13] Sengupta, R.; Holmgren, A. Thioredoxin and thioredoxin reductase in relation to reversible s-nitrosylation. *Antioxid. Redox Signaling* **18:**259–269; 2013.
[14] Calabrese, V.; Cornelius, C.; Dinkova-Kostova, A. T.; Calabrese, E. J.; Mattson, M. P. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxid. Redox Signaling* **13:**1763–1811; 2010.
[15] Calabrese, V.; Cornelius, C.; Dinkova-Kostova, A. T.; Calabrese, E. J. Vitagenes, cellular stress response, and acetylcarnitine: relevance to hormesis. *Biofactors* **35:**146–160; 2009.
[16] Zschoernig, B.; Mahlknecht, U. Carboxy-terminal phosphorylation of SIRT1 by protein kinase CK2. *Biochem. Biophys. Res. Commun.* **381:**372–377; 2009.
[17] Tsukamoto, Y.; Kato, J.; Ikeda, H. Silencing factors participate in DNA repair and recombination in Saccharomyces cerevisiae. *Nature* **388:**900–903; 1997.
[18] Kaeberlein, M.; McVey, M.; Guarente, L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev* **13:**2570–2580; 1999.
[19] Sinclair, D. A.; Guarente, L. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* **91:**1033–1042; 1997.
[20] Smith, J. S.; Boeke, J. D. An unusual form of transcriptional silencing in yeast ribosomal DNA. *Genes Dev.* **11:**241–254; 1997.
[21] Smith, J. S.; Brachmann, C. B.; Celic, I.; Kenna, M. A.; Muhammad, S.; Starai, V. J.; Avalos, J. L.; Escalante-Semerena, J. C.; Grubmeyer, C.; Wolberger, C.; Boeke, J. D. A phylogenetically conserved NAD+-dependent protein deacetylase activity in the Sir2 protein family. *Proc. Natl. Acad. Sci. USA* **97:**6658–6663; 2000.
[22] Kennedy, B. K.; Gotta, M.; Sinclair, D. A.; Mills, K.; McNabb, D. S.; Murthy, M.; Pak, S. M.; Laroche, T.; Gasser, S. M.; Guarente, L. Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in S. cerevisiae. *Cell* **89:**381–391; 1997.
[23] Tissenbaum, H. A.; Guarente, L. Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature* **410:**227–230; 2001.
[24] Anderson, R. M.; Bitterman, K. J.; Wood, J. G.; Medvedik, O.; Cohen, H.; Lin, S. S.; Manchester, J. K.; Gordon, J. I.; Sinclair, D. A. Manipulation of a nuclear NAD+ salvage pathway delays aging without altering steady-state NAD+ levels. *J. Biol. Chem.* **277:**18881–18890; 2002.
[25] Anderson, R. M.; Bitterman, K. J.; Wood, J. G.; Medvedik, O.; Sinclair, D. A. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae. *Nature* **423:**181–185; 2003.
[26] Cohen, H. Y.; Miller, C.; Bitterman, K. J.; Wall, N. R.; Hekking, B.; Kessler, B.; Howitz, K. T.; Gorospe, M.; de Cabo, R.; Sinclair, D. A. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* **305:**390–392; 2004.
[27] Burnett, C.; Valentini, S.; Cabreiro, F.; Goss, M.; Somogyvari, M.; Piper, M. D.; Hoddinott, M.; Sutphin, G. L.; Leko, V.; McElwee, J. J.; Vazquez-Manrique, R. P.; Orfila, A. M.; Ackerman, D.; Au, C.; Vinti, G.; Riesen, M.; Howard, K.; Neri, C.; Bedalov, A.; Kaeberlein, M.; Soti, C.; Partridge, L.; Gems, D. Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. *Nature* **477:**482–485; 2011.
[28] Fernandez, A. F.; Fraga, M. F. The effects of the dietary polyphenol resveratrol on human healthy aging and lifespan. *Epigenetics* **6:**870–874; 2011.
[29] Hu, Y.; Liu, J.; Wang, J.; Liu, Q. The controversial links among calorie restriction, SIRT1, and resveratrol. *Free Radic. Biol. Med.* **51:**250–256; 2011.
[30] Ungvari, Z.; Sonntag, W. E.; de Cabo, R.; Baur, J. A.; Csiszar, A. Mitochondrial protection by resveratrol. *Exerc. Sport Sci. Rev.* **39:**128–132; 2011.
[31] Das, M.; Das, D. K. Resveratrol and cardiovascular health. *Mol. Aspects Med.* **31:**503–512; 2010.
[32] Liu, D.; Gharavi, R.; Pitta, M.; Gleichmann, M.; Mattson, M. P. Nicotinamide prevents NAD+ depletion and protects neurons against excitotoxicity and cerebral ischemia: NAD+ consumption by SIRT1 may endanger energetically compromised neurons. *Neuromol. Med.* **11:**28–42; 2009.
[33] Braidy, N.; Guillemin, G. J.; Mansour, H.; Chan-Ling, T.; Poljak, A.; Grant, R. Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in Wistar rats. *PLoS One* **6:**e19194; 2011.
[34] Hsu, C. P.; Oka, S.; Shao, D.; Hariharan, N.; Sadoshima, J. Nicotinamide phosphoribosyltransferase regulates cell survival through NAD+ synthesis in cardiac myocytes. *Circ. Res.* **105:**481–491; 2009.
[35] Caito, S.; Hwang, J. W.; Chung, S.; Yao, H.; Sundar, I. K.; Rahman, I. PARP-1 inhibition does not restore oxidant-mediated reduction in SIRT1 activity. *Biochem. Biophys. Res. Commun.* **392:**264–270; 2010.
[36] Sangle, G. V.; Chowdhury, S. K.; Xie, X.; Stelmack, G. L.; Halayko, A. J.; Shen, G. X. Impairment of mitochondrial respiratory chain activity in aortic endothelial cells induced by glycated low-density lipoprotein. *Free Radic. Biol. Med.* **48:**781–790; 2010.
[37] Xia, W.; Wang, Z.; Wang, Q.; Han, J.; Zhao, C.; Hong, Y.; Zeng, L.; Tang, L.; Ying, W. Roles of NAD+/NADH and NADP+/NADPH in cell death. *Curr. Pharm. Des* **15:**12–19; 2009.
[38] Kolthur-Seetharam, U.; Dantzer, F.; McBurney, M. W.; de Murcia, G.; Sassone-Corsi, P. Control of AIF-mediated cell death by the functional interplay of SIRT1 and PARP-1 in response to DNA damage. *Cell Cycle* **5:**873–877; 2006.

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[39] Becatti, M.; Taddei, N.; Cecchi, C.; Nassi, N.; Nassi, P. A.; Fiorillo, C. SIRT1 modulates MAPK pathways in ischemic–reperfused cardiomyocytes. *Cell. Mol. Life Sci.* **69:**2245–2260; 2012.

[40] Bai, P.; Canto, C.; Oudart, H.; Brunyanszki, A.; Cen, Y.; Thomas, C.; Yamamoto, H.; Huber, A.; Kiss, B.; Houtkooper, R. H.; Schoonjans, K.; Schreiber, V.; Sauve, A. A.; Menissier-de Murcia, J.; Auwerx, J. PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* **13:**461–468; 2011.

[41] Stadtman, E. R. Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences. *Free Radic. Biol. Med.* **9:**315–325; 1990.

[42] Koltai, E.; Szabo, Z.; Atalay, M.; Boldogh, I.; Naito, H.; Goto, S.; Nyakas, C.; Radak, Z. Exercise alters SIRT1, SIRT6, NAD and NAMPT levels in skeletal muscle of aged rats. *Mech. Ageing Dev.* **131:**21–28; 2010.

[43] Massudi, H.; Grant, R.; Braidy, N.; Guest, J.; Farnsworth, B.; Guillemin, G. J. Age-associated changes in oxidative stress and NAD+ metabolism in human tissue. PLoS One. *e42357* ; 2012.

[44] Hong, E. H.; Lee, S. J.; Kim, J. S.; Lee, K. H.; Um, H. D.; Kim, J. H.; Kim, S. J.; Kim, J. I.; Hwang, S. G. Ionizing radiation induces cellular senescence of articular chondrocytes via negative regulation of SIRT1 by p38 kinase. *J. Biol. Chem.* **285:**1283–1295; 2010.

[45] Hsu, C. P.; Zhai, P.; Yamamoto, T.; Maejima, Y.; Matsushima, S.; Hariharan, N.; Shao, D.; Takagi, H.; Oka, S.; Sadoshima, J. Silent information regulator 1 protects the heart from ischemia/reperfusion. *Circulation* **122:**2170–2182; 2010.

[46] Brooks, C. L.; Gu, W. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr. Opin. Cell Biol.* **15:**164–171; 2003.

[47] Yi, J.; Luo, J. SIRT1 and p53, effect on cancer, senescence and beyond. *Biochim. Biophys. Acta* **1804:**1684–1689; 2010.

[48] Ladelfa, M. F.; Toledo, M. F.; Laiseca, J. E.; Monte, M. Interaction of p53 with tumor suppressive and oncogenic signaling pathways to control cellular reactive oxygen species production. *Antioxid. Redox Signaling* **15:**1749–1761; 2011.

[49] Gottlieb, E.; Vousden, K. H. p53 regulation of metabolic pathways. *Cold Spring Harbor Perspect. Biol* **2:**a001040; 2010.

[50] Puca, R.; Nardinocchi, L.; Starace, G.; Rechavi, G.; Sacchi, A.; Givol, D.; D'Orazi, G. Nox1 is involved in p53 deacetylation and suppression of its transcriptional activity and apoptosis. *Free Radic. Biol. Med.* **48:**1338–1346; 2010.

[51] Jaiswal, A. K. Nrf2 signaling in coordinated activation of antioxidant gene expression. *Free Radic. Biol. Med.* **36:**1199–1207; 2004.

[52] Storz, P. Forkhead homeobox type O transcription factors in the responses to oxidative stress. *Antioxid. Redox Signaling* **14:**593–605; 2011.

[53] Tothova, Z.; Kollipara, R.; Huntly, B. J.; Lee, B. H.; Castrillon, D. H.; Cullen, D. E.; McDowell, E. P.; Lazo-Kallanian, S.; Williams, I. R.; Sears, C.; Armstrong, S. A.; Passegue, E.; DePinho, R. A.; Gilliland, D. G. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* **128:**325–339; 2007.

[54] Brunet, A.; Sweeney, L. B.; Sturgill, J. F.; Chua, K. F.; Greer, P. L.; Lin, Y.; Tran, H.; Ross, S. E.; Mostoslavsky, R.; Cohen, H. Y.; Hu, L. S.; Cheng, H. L.; Jedrychowski, M. P.; Gygi, S. P.; Sinclair, D. A.; Alt, F. W.; Greenberg, M. E. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303:**2011–2015; 2004.

[55] Niehrs, C.; Schafer, A. Active DNA demethylation by Gadd45 and DNA repair. *Trends Cell Biol.* **22:**220–227; 2012.

[56] Kops, G. J.; Dansen, T. B.; Polderman, P. E.; Saarloos, I.; Wirtz, K. W.; Coffer, P. J.; Huang, T. T.; Bos, J. L.; Medema, R. H.; Burgering, B. M. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* **419:**316–321; 2002.

[57] Xiong, S.; Salazar, G.; San Martin, A.; Ahmad, M.; Patrushev, N.; Hilenski, L.; Nazarewicz, R. R.; Ma, M.; Ushio-Fukai, M.; Alexander, R. W. PGC-1 alpha serine 570 phosphorylation and GCN5-mediated acetylation by angiotensin II drive catalase down-regulation and vascular hypertrophy. *J. Biol. Chem.* **285:**2474–2487; 2010.

[58] Zhou, W.; Cao, Q.; Peng, Y.; Zhang, Q. J.; Castrillon, D. H.; DePinho, R. A.; Liu, Z. P. FoxO4 inhibits NF-kappaB and protects mice against colonic injury and inflammation. *Gastroenterology* **137:**1403–1414; 2009.

[59] Salminen, A.; Kaarniranta, K. NF-kappaB signaling in the aging process. *J. Clin. Immunol.* **29:**397–405; 2009.

[60] Radak, Z.; Chung, H. Y.; Naito, H.; Takahashi, R.; Jung, K. J.; Kim, H. J.; Goto, S. Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. *FASEB J.* **18:**749–750; 2004.

[61] Yeung, F.; Hoberg, J. E.; Ramsey, C. S.; Keller, M. D.; Jones, D. R.; Frye, R. A.; Mayo, M. W. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* **23:**2369–2380; 2004.

[62] Zhu, X.; Liu, Q.; Wang, M.; Liang, M.; Yang, X.; Xu, X.; Zou, H.; Qiu, J. Activation of Sirt1 by resveratrol inhibits TNF-alpha induced inflammation in fibroblasts. PLoS One. *e27081* ; 2011.

[63] Liu, T. F.; Yoza, B. K.; El Gazzar, M.; Vachharajani, V. T.; McCall, C. E. NAD+-dependent SIRT1 deacetylase participates in epigenetic reprogramming during endotoxin tolerance. *J. Biol. Chem.* **286:**9856–9864; 2011.

[64] Jones, P. L.; Kucera, G.; Gordon, H.; Boss, J. M. Cloning and characterization of the murine manganous superoxide dismutase-encoding gene. *Gene* **153:**155–161; 1995.

[65] Hollander, J.; Fiebig, R.; Gore, M.; Ookawara, T.; Ohno, H.; Ji, L. L. Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. *Pflugers Arch.* **442:**426–434; 2001.

[66] St-Pierre, J.; Drori, S.; Uldry, M.; Silvaggi, J. M.; Rhee, J.; Jager, S.; Handschin, C.; Zheng, K.; Lin, J.; Yang, W.; Simon, D. K.; Bachoo, R.; Spiegelman, B. M. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* **127:**397–408; 2006.

[67] Leick, L.; Lyngby, S. S.; Wojtaszewski, J. F.; Pilegaard, H. PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. *Exp. Gerontol.* **45:**336–342; 2010.

[68] Nemoto, S.; Fergusson, M. M.; Finkel, T. SIRT1 functionally interacts with the metabolic regulator and transcriptional coactivator PGC-1α. *J. Biol. Chem.* **280:**16456–16460; 2005.

[69] Olesen, J.; Kiilerich, K.; Pilegaard, H. PGC-1alpha-mediated adaptations in skeletal muscle. *Pflugers Arch.* **460:**153–162; 2010.

[70] Pallas, M.; Casadesus, G.; Smith, M. A.; Coto-Montes, A.; Pelegri, C.; Vilaplana, J.; Camins, A. Resveratrol and neurodegenerative diseases: activation of SIRT1 as the potential pathway towards neuroprotection. *Curr. Neurovasc. Res.* **6:**70–81; 2009.

[71] Boulton, S. J.; Jackson, S. P. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* **17:**1819–1828; 1998.

[72] Mills, K. D.; Sinclair, D. A.; Guarente, L. MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell* **97:**609–620; 1999.

[73] Jeong, J.; Juhn, K.; Lee, H.; Kim, S. H.; Min, B. H.; Lee, K. M.; Cho, M. H.; Park, G. H.; Lee, K. H. SIRT1 promotes DNA repair activity and deacetylation of Ku70. *Exp. Mol. Med.* **39:**8–13; 2007.

[74] Mao, Z.; Hine, C.; Tian, X.; Van Meter, M.; Au, M.; Vaidya, A.; Seluanov, A.; Gorbunova, V. SIRT6 promotes DNA repair under stress by activating PARP1. *Science* **332:**1443–1446; 2011.

[75] Lombard, D. B.; Schwer, B.; Alt, F. W.; Mostoslavsky, R. SIRT6 in DNA repair, metabolism and ageing. *J. Intern. Med.* **263:**128–141; 2008.

[76] Mostoslavsky, R.; Chua, K. F.; Lombard, D. B.; Pang, W. W.; Fischer, M. R.; Gellon, L.; Liu, P.; Mostoslavsky, G.; Franco, S.; Murphy, M. M.; Mills, K. D.; Patel, P.; Hsu, J. T.; Hong, A. L.; Ford, E.; Cheng, H. L.; Kennedy, C.; Nunez, N.; Bronson, R.; Frendewey, D.; Auerbach, W.; Valenzuela, D.; Karow, M.; Hottiger, M. O.; Hursting, S.; Barrett, J. C.; Guarente, L.; Mulligan, R.; Demple, B.; Yancopoulos, G. D.; Alt, F. W. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124:**315–329; 2006.

[77] Wang, R. H.; Sengupta, K.; Li, C.; Kim, H. S.; Cao, L.; Xiao, C.; Kim, S.; Xu, X.; Zheng, Y.; Chilton, B.; Jia, R.; Zheng, Z. M.; Appella, E.; Wang, X. W.; Ried, T.; Deng, C. X. Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. *Cancer Cell* **14:**312–323; 2008.

[78] Busso, C. S.; Lake, M. W.; Izumi, T. Posttranslational modification of mammalian AP endonuclease (APE1). *Cell Mol. Life Sci.* **67:**3609–3620; 2010.

[79] Bhakat, K. K.; Mantha, A. K.; Mitra, S. Transcriptional regulatory functions of mammalian AP-endonuclease (APE1/Ref-1), an essential multifunctional protein. *Antioxid. Redox Signaling* **11:**621–638; 2009.

[80] Yamamori, T.; DeRicco, J.; Naqvi, A.; Hoffman, T. A.; Mattagajasingh, I.; Kasuno, K.; Jung, S. B.; Kim, C. S.; Irani, K. SIRT1 deacetylates APE1 and regulates cellular base excision repair. *Nucleic Acids Res.* **38:**832–845; 2010.

[81] Bhakat, K. K.; Mokkapati, S. K.; Boldogh, I.; Hazra, T. K.; Mitra, S. Acetylation of human 8-oxoguanine-DNA glycosylase by p300 and its role in 8-oxoguanine repair in vivo. *Mol. Cell. Biol.* **26:**1654–1665; 2006.

[82] Koltai, E.; Zhao, Z.; Lacza, Z.; Cselenyak, A.; Vacz, G.; Nyakas, C.; Boldogh, I.; Ichinoseki-Sekine, N.; Radak, Z. Combined exercise and insulin-like growth factor-1 supplementation induces neurogenesis in old rats, but do not attenuate age-associated DNA damage. *Rejuvenation Res* **14:**585–596; 2011.

[83] Radak, Z.; Bori, Z.; Koltai, E.; Fatouros, I. G.; Jamurtas, A. Z.; Douroudos, I. I.; Terzis, G.; Nikolaidis, M. G.; Chatzinikolaou, A.; Sovatzidis, A.; Kumagai, S.; Naito, H.; Boldogh, I. Age-dependent changes in 8-oxoguanine-DNA glycosylase activity are modulated by adaptive responses to physical exercise in human skeletal muscle. *Free Radic. Biol. Med.* **51:**417–423; 2011.

[84] Boldogh, I.; Hajas, G.; Aguilera-Aguirre, L.; Hegde, M. L.; Radak, Z.; Bacsi, A.; Sur, S.; Hazra, T. K.; Mitra, S. Activation of ras signaling pathway by 8-oxoguanine DNA glycosylase bound to its excision product, 8-oxoguanine. *J. Biol. Chem.* **287:**20769–20773; 2012.

[85] Fan, W.; Luo, J. SIRT1 regulates UV-induced DNA repair through deacetylating XPA. *Mol. Cell* **39:**247–258; 2010.

[86] Ming, M.; Shea, C. R.; Guo, X.; Li, X.; Soltani, K.; Han, W.; He, Y. Y. Regulation of global genome nucleotide excision repair by SIRT1 through xeroderma pigmentosum C. *Proc. Natl. Acad. Sci. USA* **107:**22623–22628; 2010.

[87] Calabrese, V.; Cornelius, C.; Cuzzocrea, S.; Iavicoli, I.; Rizzarelli, E.; Calabrese, E. J. Hormesis, cellular stress response and vitagenes as critical determinants in aging and longevity. *Mol. Aspects Med.* **32:**279–304; 2011.

[88] Calabrese, V.; Cornelius, C.; Dinkova-Kostova, A. T.; Iavicoli, I.; Di Paola, R.; Koverech, A.; Cuzzocrea, S.; Rizzarelli, E.; Calabrese, E. J. Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. *Biochim. Biophys. Acta* **1822:**753–783; 2012.

[89] Radak, Z.; Zhao, Z.; Goto, S.; Koltai, E. Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA. *Mol. Aspects Med.* **32:**305–315; 2011.

[90] Ghosh, H. S.; Reizis, B.; Robbins, P. D. SIRT1 associates with eIF2-alpha and regulates the cellular stress response. *Sci. Rep* **1:**150; 2011.

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[91] Westerheide, S. D.; Anckar, J.; Stevens Jr S. M.; Sistonen, L.; Morimoto, R. I. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science* **323:**1063–1066; 2009.
[92] Hsu, A. L.; Murphy, C. T.; Kenyon, C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300:**1142–1145; 2003.
[93] Jia, G.; Su, L.; Singhal, S.; Liu, X. Emerging roles of SIRT6 on telomere maintenance, DNA repair, metabolism and mammalian aging. *Mol. Cell. Biochem.* **364:**345–350; 2012.
[94] Palacios, J. A.; Herranz, D.; De Bonis, M. L.; Velasco, S.; Serrano, M.; Blasco, M. A. SIRT1 contributes to telomere maintenance and augments global homologous recombination. *J. Cell Biol.* **191:**1299–1313; 2010.
[95] Wang, F.; Chan, C. H.; Chen, K.; Guan, X.; Lin, H. K.; Tong, Q. Deacetylation of FOXO3 by SIRT1 or SIRT2 leads to Skp2-mediated FOXO3 ubiquitination and degradation. *Oncogene* **31:**1546–1557; 2012.
[96] van Loosdregt, J.; Brunen, D.; Fleskens, V.; Pals, C. E.; Lam, E. W.; Coffer, P. J. Rapid temporal control of Foxp3 protein degradation by sirtuin-1. PLoS One. *e19047* ; 2011.
[97] Brandl, A.; Heinzel, T.; Kramer, O. H. Histone deacetylases: salesmen and customers in the post-translational modification market. *Biol. Cell* **101:**193–205; 2009.
[98] Spange, S.; Wagner, T.; Heinzel, T.; Kramer, O. H. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int. J. Biochem. Cell Biol.* **41:**185–198; 2009.
[99] Benkirane, M.; Sardet, C.; Coux, O. Lessons from interconnected ubiquitylation and acetylation of p53: think metastable networks. *Biochem. Soc. Trans.* **38:**98–103; 2010.
[100] Marton, O.; Koltai, E.; Nyakas, C.; Bakonyi, T.; Zenteno-Savin, T.; Kumagai, S.; Goto, S.; Radak, Z. Aging and exercise affect the level of protein acetylation and SIRT1 activity in cerebellum of male rats. *Biogerontology* **11:**679–686; 2010.
[101] Nakamura, A.; Kawakami, K.; Kametani, F.; Nakamoto, H.; Goto, S. Biological significance of protein modifications in aging and calorie restriction. *Ann. N. Y. Acad. Sci* **1197:**33–39; 2010.
[102] Brooks, C. L.; Gu, W. The impact of acetylation and deacetylation on the p53 pathway. *Protein Cell* **2:**456–462; 2011.
[103] Vaquero, A.; Scher, M.; Erdjument-Bromage, H.; Tempst, P.; Serrano, L.; Reinberg, D. SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. *Nature* **450:**440–444; 2007.
[104] Bosch-Presegue, L.; Raurell-Vila, H.; Marazuela-Duque, A.; Kane-Goldsmith, N.; Valle, A.; Oliver, J.; Serrano, L.; Vaquero, A. Stabilization of Suv39H1 by SirT1 is part of oxidative stress response and ensures genome protection. *Mol. Cell* **42:**210–223; 2011.
[105] Yuan, F.; Xie, Q.; Wu, J.; Bai, Y.; Mao, B.; Dong, Y.; Bi, W.; Ji, G.; Tao, W.; Wang, Y.; Yuan, Z. MST1 promotes apoptosis through regulating Sirt1-dependent p53 deacetylation. *J. Biol. Chem.* **286:**6940–6945; 2011.
[106] Polyak, K.; Xia, Y.; Zweier, J. L.; Kinzler, K. W.; Vogelstein, B. A model for p53-induced apoptosis. *Nature* **389:**300–305; 1997.
[107] Hussain, S. P.; Amstad, P.; He, P.; Robles, A.; Lupold, S.; Kaneko, I.; Ichimiya, M.; Sengupta, S.; Mechanic, L.; Okamura, S.; Hofseth, L. J.; Moake, M.; Nagashima, M.; Forrester, K. S.; Harris, C. C. p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. *Cancer Res.* **64:**2350–2356; 2004.
[108] Vurusaner, B.; Poli, G.; Basaga, H. Tumor suppressor genes and ROS: complex networks of interactions. *Free Radic. Biol. Med.* **52:**7–18; 2012.
[109] Langley, E.; Pearson, M.; Faretta, M.; Bauer, U. M.; Frye, R. A.; Minucci, S.; Pelicci, P. G.; Kouzarides, T. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. *EMBO J.* **21:**2383–2396; 2002.
[110] Machida, S.; Booth, F. W. Increased nuclear proteins in muscle satellite cells in aged animals as compared to young growing animals. *Exp. Gerontol* **39:**1521–1525; 2004.
[111] Nogalska, A.; D'Agostino, C.; Engel, W. K.; Davies, K. J.; Askanas, V. Decreased SIRT1 deacetylase activity in sporadic inclusion-body myositis muscle fibers. *Neurobiol. Aging* **31:**1637–1648; 2010.
[112] Li, N.; Muthusamy, S.; Liang, R.; Sarojini, H.; Wang, E. Increased expression of miR-34a and miR-93 in rat liver during aging, and their impact on the expression of Mgst1 and Sirt1. *Mech. Ageing Dev* **132:**75–85; 2011.
[113] Blackburn, E. H. Telomere states and cell fates. *Nature* **408:**53–56; 2000.
[114] Harley, C. B.; Futcher, A. B.; Greider, C. W. Telomeres shorten during ageing of human fibroblasts. *Nature* **345:**458–460; 1990.
[115] Kim, S.; Bi, X.; Czarny-Ratajczak, M.; Dai, J.; Welsh, D. A.; Myers, L.; Welsch, M. A.; Cherry, K. E.; Arnold, J.; Poon, L. W.; Jazwinski, S. M. Telomere maintenance genes SIRT1 and XRCC6 impact age-related decline in telomere length but only SIRT1 is associated with human longevity. *Biogerontology* **13:**119–131; 2012.
[116] Donato, A. J.; Magerko, K. A.; Lawson, B. R.; Durrant, J. R.; Lesniewski, L. A.; Seals, D. R. SIRT-1 and vascular endothelial dysfunction with ageing in mice and humans. *J. Physiol.* **589:**4545–4554; 2011.
[117] Bori, Z.; Zhao, Z.; Koltai, E.; Fatouros, I. G.; Jamurtas, A. Z.; Douroudos, I. I.; Terzis, G.; Chatzinikolaou, A.; Sovatzidis, A.; Draganidis, D.; Boldogh, I.; Radak, Z. The effects of aging, physical training, and a single bout of exercise on mitochondrial protein expression in human skeletal muscle. *Exp. Gerontol.* **47:**417–424; 2012.
[118] LeMoine, C. M.; McClelland, G. B.; Lyons, C. N.; Mathieu-Costello, O.; Moyes, C. D. Control of mitochondrial gene expression in the aging rat myocardium. *Biochem. Cell Biol.* **84:**191–198; 2006.
[119] Julien, C.; Tremblay, C.; Emond, V.; Lebbadi, M.; Salem Jr N.; Bennett, D. A.; Calon, F. Sirtuin 1 reduction parallels the accumulation of tau in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **68:**48–58; 2009.
[120] Zheng, T.; Lu, Y. Changes in SIRT1 expression and its downstream pathways in age-related cataract in humans. *Curr. Eye Res* **36:**449–455; 2011.
[121] Chen, J.; Zhang, B.; Wong, N.; Lo, A. W.; To, K. F.; Chan, A. W.; Ng, M. H.; Ho, C. Y.; Cheng, S. H.; Lai, P. B.; Yu, J.; Ng, H. K.; Ling, M. T.; Huang, A. L.; Cai, X. F.; Ko, B. C. Sirtuin 1 is upregulated in a subset of hepatocellular carcinomas where it is essential for telomere maintenance and tumor cell growth. *Cancer Res.* **71:**4138–4149; 2011.
[122] Calvanese, V.; Fraga, M. F. SirT1 brings stemness closer to cancer and aging. *Aging (Albany NY)* **3:**162–167; 2011.
[123] Li, Y.; Xu, W.; McBurney, M. W.; Longo, V. D. SirT1 inhibition reduces IGF-I/IRS-2/Ras/ERK1/2 signaling and protects neurons. *Cell Metab.* **8:**38–48; 2008.
[124] Donmez, G.; Arun, A.; Chung, C. Y.; McLean, P. J.; Lindquist, S.; Guarente, L. SIRT1 protects against alpha-synuclein aggregation by activating molecular chaperones. *J. Neurosci.* **32:**124–132; 2012.
[125] Jeong, H.; Cohen, D. E.; Cui, L.; Supinski, A.; Savas, J. N.; Mazzulli, J. R.; Yates 3rd J. R.; Bordone, L.; Guarente, L.; Krainc, D Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. *Nat. Med* **18:**159–165; 2012.
[126] Michan, S.; Li, Y.; Chou, M. M.; Parrella, E.; Ge, H.; Long, J. M.; Allard, J. S.; Lewis, K.; Miller, M.; Xu, W.; Mervis, R. F.; Chen, J.; Guerin, K. I.; Smith, L. E.; McBurney, M. W.; Sinclair, D. A.; Baudry, M.; de Cabo, R.; Longo, V. D. SIRT1 is essential for normal cognitive function and synaptic plasticity. *J. Neurosci.* **30:**9695–9707; 2010.
[127] Wu, D.; Qiu, Y.; Gao, X.; Yuan, X. B.; Zhai, Q. Overexpression of SIRT1 in mouse forebrain impairs lipid/glucose metabolism and motor function. PLoS One. *e21759* ; 2011.
[128] Cheng, H. L.; Mostoslavsky, R.; Saito, S.; Manis, J. P.; Gu, Y.; Patel, P.; Bronson, R.; Appella, E.; Alt, F. W.; Chua, K. F. Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc. Natl. Acad. Sci. USA* **100:**10794–10799; 2003.
[129] Chen, D.; Steele, A. D.; Lindquist, S.; Guarente, L. Increase in activity during calorie restriction requires Sirt1. *Science* **310:**1641; 2005.
[130] Bordone, L.; Cohen, D.; Robinson, A.; Motta, M. C.; van Veen, E.; Czopik, A.; Steele, A. D.; Crowe, H.; Marmor, S.; Luo, J.; Gu, W.; Guarente, L. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* **6:**759–767; 2007.
[131] Kaeberlein, M.; Kirkland, K. T.; Fields, S.; Kennedy, B. K. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol.* **2:**E296; 2004.
[132] Kaeberlein, M.; Powers 3rd R. W. Sir2 and calorie restriction in yeast: a skeptical perspective. *Ageing Res. Rev.* **6:**128–140; 2007.
[133] Caro, P.; Gomez, J.; Sanchez, I.; Garcia, R.; Lopez-Torres, M.; Naudi, A.; Portero-Otin, M.; Pamplona, R.; Barja, G. Effect of 40% restriction of dietary amino acids (except methionine) on mitochondrial oxidative stress and biogenesis, AIF and SIRT1 in rat liver. *Biogerontology* **10:**579–592; 2009.
[134] Patwari, P.; Higgins, L. J.; Chutkow, W. A.; Yoshioka, J.; Lee, R. T. The interaction of thioredoxin with Txnip:. evidence for formation of a mixed disulfide by disulfide exchange. *J. Biol. Chem.* **281:**21884–21891; 2006.
[135] Chondrogianni, N.; de C. M. Simoes, D.; Franceschi, C.; Gonos, E. S. Cloning of differentially expressed genes in skin fibroblasts from centenarians. *Biogerontology* **5:**401–409; 2004.
[136] Yoshida, T.; Kondo, N.; Oka, S.; Ahsan, M. K.; Hara, T.; Masutani, H.; Nakamura, H.; Yodoi, J. Thioredoxin-binding protein-2 (TBP-2): its potential roles in the aging process. *Biofactors* **27:**47–51; 2006.
[137] Mousa, S. A.; Gallati, C.; Simone, T.; Dier, E.; Yalcin, M.; Dyskin, E.; Thangirala, S.; Hanko, C.; Rebbaa, A. Dual targeting of the antagonistic pathways mediated by Sirt1 and TXNIP as a putative approach to enhance the efficacy of anti-aging interventions. *Aging (Albany NY)* **1:**412–424; 2009.
[138] Csiszar, A.; Labinskyy, N.; Jimenez, R.; Pinto, J. T.; Ballabh, P.; Losonczy, G.; Pearson, K. J.; de Cabo, R.; Ungvari, Z. Anti-oxidative and anti-inflammatory vasoprotective effects of caloric restriction in aging: role of circulating factors and SIRT1. *Mech. Ageing Dev.* **130:**518–527; 2009.
[139] Quintas, A.; de Solis, A. J.; Diez-Guerra, F. J.; Carrascosa, J. M.; Bogonez, E. Age-associated decrease of SIRT1 expression in rat hippocampus: prevention by late onset caloric restriction. *Exp. Gerontol.* **47:**198–201; 2012.
[140] Fusco, S.; Ripoli, C.; Podda, M. V.; Ranieri, S. C.; Leone, L.; Toietta, G.; McBurney, M. W.; Schutz, G.; Riccio, A.; Grassi, C.; Galeotti, T.; Pani, G. A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction. *Proc. Natl. Acad. Sci. USA* **109:**621–626; 2012.
[141] Schenk, S.; McCurdy, C. E.; Philp, A.; Chen, M. Z.; Holliday, M. J.; Bandyopadhyay, G. K.; Osborn, O.; Baar, K.; Olefsky, J. M. Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. *J. Clin. Invest.* **121:**4281–4288; 2011.
[142] Zhang, R.; Chen, H. Z.; Liu, J. J.; Jia, Y. Y.; Zhang, Z. Q.; Yang, R. F.; Zhang, Y.; Xu, J.; Wei, Y. S.; Liu, D. P.; Liang, C. C. SIRT1 suppresses activator protein-1 transcriptional activity and cyclooxygenase-2 expression in macrophages. *J. Biol. Chem.* **285:**7097–7110; 2010.
[143] Hancock, C. R.; Han, D. H.; Higashida, K.; Kim, S. H.; Holloszy, J. O. Does calorie restriction induce mitochondrial biogenesis? A reevaluation *FASEB J.* **25:**785–791; 2011.
[144] Cerqueira, F. M.; Laurindo, F. R.; Kowaltowski, A. J. Mild mitochondrial uncoupling and calorie restriction increase fasting eNOS, akt and mitochondrial biogenesis. *PLoS One* **6:**e18433; 2011.

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[145] Civitarese, A. E.; Carling, S.; Heilbronn, L. K.; Hulver, M. H.; Ukropcova, B.; Deutsch, W. A.; Smith, S. R.; Ravussin, E. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med.* **4:**e76; 2007.

[146] Radak, Z.; Chung, H. Y.; Goto, S. Systemic adaptation to oxidative challenge induced by regular exercise. *Free Radic. Biol. Med.* **44:**153–159; 2008.

[147] Suwa, M.; Nakano, H.; Radak, Z.; Kumagai, S. Endurance exercise increases the SIRT1 and peroxisome proliferator-activated receptor gamma coactivator-1alpha protein expressions in rat skeletal muscle. *Metabolism* **57:**986–998; 2008.

[148] Ferrara, N.; Rinaldi, B.; Corbi, G.; Conti, V.; Stiuso, P.; Boccuti, S.; Rengo, G.; Rossi, F.; Filippelli, A. Exercise training promotes SIRT1 activity in aged rats. *Rejuvenation Res* **11:**139–150; 2008.

[149] Koltai, E.; Hart, N.; Taylor, A. W.; Goto, S.; Ngo, J. K.; Davies, K. J.; Radak, Z. Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. *Am. J. Physiol. Regul. Integr. Comp. Physiol* **303:**R127–134; 2012.

[150] E, L.; Lu, J.; Burns, J. M.; Swerdlow, R. H. Effect of exercise on mouse liver and brain bioenergetic infrastructures. *Exp. Physiol.* **98:**207–219; 2013.

[151] Costford, S. R.; Bajpeyi, S.; Pasarica, M.; Albarado, D. C.; Thomas, S. C.; Xie, H.; Church, T. S.; Jubrias, S. A.; Conley, K. E.; Smith, S. R. Skeletal muscle NAMPT is induced by exercise in humans. *Am. J. Physiol. Endocrinol. Metab.* **298:**E117–126; 2010.

[152] Little, J. P.; Safdar, A.; Wilkin, G. P.; Tarnopolsky, M. A.; Gibala, M. J. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *J. Physiol.* **588:**1011–1022; 2010.

[153] Gomez-Pinilla, F.; Ying, Z. Differential effects of exercise and dietary docosahexaenoic acid on molecular systems associated with control of allostasis in the hypothalamus and hippocampus. *Neuroscience* **168:**130–137; 2010.

[154] Radak, Z.; Kaneko, T.; Tahara, S.; Nakamoto, H.; Pucsok, J.; Sasvari, M.; Nyakas, C.; Goto, S. Regular exercise improves cognitive function and decreases oxidative damage in rat brain. *Neurochem. Int.* **38:**17–23; 2001.

[155] Gubert, S.; Braso, M. A.; Sacristan, A.; Ortiz, J. A. Synthesis and pharmacological screening of new angiotensin converting enzyme inhibitors. *Farmaco* **45:**59–79; 1990.

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