Forum Minireview

Transcriptional Regulation of Neuronal Genes and Its Effect on Neural Functions: NAD-Dependent Histone Deacetylase SIRT1 (Sir2α)

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Abstract. Sir2 (silent information regulator 2) is an NAD-dependent deacetylase that is broadly conserved from bacteria to humans. It catalyzes a unique deacetylation reaction using NAD, and specific inhibitors and activators of its activity have been discovered. In yeast, Sir2 deacetylates histones and participates in transcription silencing and the suppression of recombination. Sir2 is also implicated in the regulation of aging, because its increased expression extends the lifespan of yeast and nematodes. Mammalian SIRT1 (Sir2α) is a member of the Sir2 family. Recently, SIRT1 was shown to interact with various transcription factors such as p53, forkhead transcription factor (FOXO) family proteins, and MyoD, and to participate in stress tolerance, differentiation, and development.

Keywords: deacetylase, NAD, aging, transcription factor, differentiation

Introduction

The acetylation and deacetylation of lysine residues on proteins are important modifications that can change protein activity. The acetylation of the amino-terminal tails of histones usually increases transcriptional activity in eukaryotic cells. The transcription unit contains histone acetyl transferase, which acetylates histones to increase the DNA accessibility of the transcription complex. Recently, new roles for histone acetylation have been uncovered, not only in transcription but also in DNA replication, repair, and heterochromatin formation (1). Acetylated lysine is restored to its original deacetylated form by histone deacetylases, and deacetylated histones can fold into more compact nucleosomal structures, silencing gene transcription. Histone deacetylases (HDACs) are divided into three classes. Class I members (HDAC1, 2, 3, 8, 11) are transcriptional co-repressors homologous to yeast RPD3 (2). Class II members (HDAC 4, 5, 6, 7, 9, 10) have domains similar to yeast HDA1 (3). Class III histone deacetylases are distinct from class I and II HDACs and are homologues of the yeast silent information regulator 2 (Sir2).

Sir2, a heterochromatin component in yeast, silences transcription at silent mating loci, telomeres, and ribosomal DNA (rDNA), and this also suppresses recombination in rDNA. In early experiments, the over-expression of Sir2 in yeast induced the global deacetylation of histones, indicating that Sir2 was a histone deacetylase (4). Later, cobB, a bacterial homologue of Sir2, was found to have ribosyltransferase activity (5), leading to experiments showing that Sir2 was also able to transfer ADP-ribose from NAD (6). Subsequently, Sir2 was found to be an NAD-dependent histone deacetylase (7). The ADP-ribosylation of an acetylated lysine residue is an intermediate state of the enzymatic reaction catalyzed by Sir2. Only class III enzymes use NAD as a cofactor. Thus, these enzymes are called NAD-dependent histone deacetylases.

Recently, Sir2 has attracted much attention, because it is related to longevity. The over-expression of Sir2 extends the lifespan of budding yeast, while its knockout shortens the lifespan by about 50% (8). Deletion of the Sir2 gene increases homologous recombination at the rDNA loci, which results in the formation of extrachromosomal rDNA circles. Although extrachromosomal rDNA circles are not found in human cells, they are a
cause of aging in budding yeast; these rDNA circles dose-dependently shorten the lifespan of yeast (9). On the other hand, calorie restriction extends the lifespan in a broad range of organisms from yeasts to mammals. In yeast, lifespan extension by calorie restriction requires Sir2 (10). Sir2 is the defining member of a broadly conserved family found in organisms ranging from bacteria to humans; the members of this family are also called sirtuins. In the nematode, the gene most homologous to the yeast Sir2 gene is sir-2.1. A duplication containing the sir-2.1 gene confers a lifespan that is extended by up to 50% (11). The mammalian homologues consist of seven members, SIRT1 – SIRT7. SIRT1 (also known as Sir2α) is the closest homologue of yeast Sir2 and has been extensively studied.

### Enzymatic reaction of an NAD-dependent histone deacetylase

Sir2 and SIRT1 deacetylate an acetylated protein in the presence of NAD to generate the deacetylated protein, 2'-O-acetyl-ADP-ribose, and nicotinamide (Fig. 1) (12, 13); thus, the enzyme breaks NAD into 2'-O-acetyl-ADP-ribose and nicotinamide. 2'-O-Acetyl-ADP-ribose is a newly identified compound. The crystal structure of a Sir2 homologue suggests the NAD-dependent deacetylation occurs via a two-step mechanism, nicotinamide cleavage followed by ADP-ribose transfer to acetate (14, 15). Because Sir2 catalyzes a unique deacetylation reaction using NAD, specific inhibitors and activators of its activity have been discovered. Nicotinamide strongly inhibits yeast Sir2 and mammalian SIRT1 (IC\textsubscript{50} < 50 µM) (16). In addition, yeast-based phenotypic screening shows that α-substituted β-naphthol compounds, such as sirtinol and splitomicin, inhibit Sir2 and SIRT1 activities (17, 18). Small molecules that activate Sir2 have also been reported. Resveratrol, a polyphenol found in red wine, lowers the Michaelis constant of SIRT1 for both the acetylated substrate and NAD and increases cell survival by stimulating the SIRT1-dependent deacetylation of p53 (see below) (19). In yeast, resveratrol mimics calorie restriction by stimulating Sir2, increasing DNA stability, and extending the lifespan by 70% (19). The effect of resveratrol on \textit{Caenorhabditis elegans}, \textit{Droso-

![Fig. 1. The enzymatic activity of Sir2. Sir2 deacetylates an acetylated protein in the presence of NAD. For each deacetylation of an acetyl lysine residue, one NAD molecule is decomposed into 2'-O-acetyl-ADP-ribose and nicotinamide. Nicotinamide, sirtinol, and splitomicin are potent inhibitors of the reaction, while resveratrol, a polyphenol compound found in red wine, activates Sir2. Resveratrol lowers the K_m values for the substrate and NAD by 35 and 5 times, respectively, in vitro.](image-url)
Sir-2.1 extends the lifespan of animal. As previously mentioned, the over-expression of increase in DAF-16 activity extends the lifespan of the in adult longevity. Consistent with this finding, an FOXO4, which promote cell-cycle arrest by inducing activity (26, 27). Thus, SIRT1 activates FOXO1 and activity. SIRT1 binds acetylated FOXO4 and restores its acetylation of FOXO4 and inhibits its transcription and activates it (25). Hydrogen peroxide induces the SIRT1 deacetylates FOXO1 at lysines 242, 245, and 262 inhibits FOXO3’s function of inducing cell death (24). SIRT1 deacetylates FOXO1 at lysines 242, 245, and 262 and activates it (25). Hydrogen peroxide induces the acetylation of FOXO4 and inhibits its transcription activity. SIRT1 binds acetylated FOXO4 and restores its activity (26, 27). Thus, SIRT1 activates FOXO1 and FOXO4, which promote cell-cycle arrest by inducing p27\textsuperscript{kip1}; SIRT1 also induces cellular resistance to oxidative stress by increasing the levels of manganese superoxide dismutase (25, 26) and GADD45 (growth arrest and DNA damage-inducible protein 45) (27). However, SIRT1 was reported to repress the activity of FOXO3 and other FOXOs in a luciferase assay (28). Therefore, the function of SIRT1 may be different depending on the target gene of the FOXOs.

Basic helix-loop-helix (bHLH) transcription factors play important roles as activators or repressors in development. Drosophila Sir2 interacts with members of the Hairy/Enhancer of Split/Deadpan (HES) family of bHLH transcription factors and affects development and sex ratios (29). Mammalian SIRT1 is reported to bind HES1 and HEY2, which are human Hairy homologues; it binds to the bHLH domain of HES1 (30). MyoD is a bHLH transcription factor that induces muscular differentiation. SIRT1 binds MyoD and suppresses its activity (31). The over-expression of SIRT1 retards the differentiation of muscle precursor cells. The \([NAD] / [NADH] \text{ ratio}\) decreases as muscle cells differentiate, while an increased \([NAD] / [NADH] \text{ ratio}\) inhibits muscle gene expression. Because the SIRT1 activity is augmented by an increase in NAD, SIRT1 may regulate skeletal muscle differentiation as a potential sensor of the redox state, which is reflected in the \([NAD] / [NADH] \text{ ratio}\) (31).

Several other transcription factors appear to be regulated by SIRT1. SIRT1 represses PPAR-\(\gamma\), a key regulator of adipogenesis, by docking with its cofactors NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors) (32). The upregulation of SIRT1 triggers lipolysis and loss of fat. SIRT1 inhibits the transcriptional activity of NF-\(\kappa\)B by deacetylating NF-\(\kappa\)B’s subunit, RelA/p65, at lysine 310 (33). Thus, although SIRT1 is capable of protecting cells from p53-induced-apoptosis, it may augment apoptosis by repressing NF-\(\kappa\)B. CTIP2 represses the transcription of its target genes and is implicated in hematopoietic cell development. SIRT1 is reported to bind CTIP2 and accelerate the transcriptional repression by this molecule (34). Finally, yeast Sir2 silences transcription at rDNA. Acetylation of TAF\textsubscript{68}, a subunit of the RNA polymerase I complex, enhances the binding of TAF\textsubscript{68} to the rDNA promoter and induces rDNA transcription in mammals. SIRT1 was shown to deacetylate TAF\textsubscript{68} and repress rDNA transcription (35).

**SIRT1 and brain**

SIRT1-deficient mice are small at birth, and most die during the early postnatal period (36, 37). They have

*phila melanogaster,* and mammalian cells is now under investigation.

**SIRT1 and transcription factors**

Lysines 9 and 14 in the amino-terminal tail of histone H3 and lysine 16 of histone H4 are deacetylated by yeast Sir2 and mammalian SIRT1 (Sir2\(\alpha\)) (7). Mammalian SIRT1 interacts with and regulates not only histones but also various transcription factors, such as the tumor suppressor p53, forkhead transcription factors (FOXOs), and a fat regulator, peroxisome proliferator-activated receptor-\(\gamma\) (PPAR-\(\gamma\)). The acetylation of lysine residues in the C-terminal regulatory domain of p53 enhances the sequence-specific binding of p53. SIRT1 deacetylates p53 at lysine 382 in the C-terminal domain and represses its transcriptional activity (20, 21). SIRT1 represses p53-dependent apoptosis in response to DNA damage and oxidative stress and promotes cell survival under cellular stress induced by etoposide treatment or irradiation (20, 21).

Genetic studies in *C. elegans* revealed that insulin /IGF signal transduction regulates longevity (22). The insulin/IGF signal triggers a kinase cascade that includes phosphatidylinositol-3-OH kinase and results in the phosphorylation of DAF-16, a FOXO transcription factor in the nematode, and its exclusion from nuclei. Mutations that attenuate insulin/IGF signaling in *C. elegans* and *Drosophila* produce a 2- to 3-fold increase in adult longevity. Consistent with this finding, an increase in DAF-16 activity extends the lifespan of the animal. As previously mentioned, the over-expression of sir-2.1 extends the lifespan of *C. elegans*. Genetic analysis indicated that sir-2.1 functions upstream of DAF-16 (11). The interaction between Sir2 and insulin/IGF signal transduction has attracted a great deal of attention; in particular, the possibility that Sir2 regulates FOXO transcription factors directly has been of interest.

Mammalian FOXO transcription factors consist of FOXO1 (FKHR), FOXO3 (FKHRL1), FOXO4 (AFX), and FOXO6. FOXOs have been implicated in regulating metabolism, cell-cycle progression, stress tolerance, repair of DNA damage, and apoptosis (23). In mammals, SIRT1 deacetylates FOXO3, and shows a dual effect on FOXO3 function; it increases FOXO3’s ability to induce cell-cycle arrest and resistance to oxidative stress, and it inhibits FOXO3’s function of inducing cell death (24). SIRT1 deacetylates FOXO1 at lysines 242, 245, and 262 and activates it (25). Hydrogen peroxide induces the acetylation of FOXO4 and inhibits its transcription activity. SIRT1 binds acetylated FOXO4 and restores its activity (26, 27). Thus, SIRT1 activates FOXO1 and FOXO4, which promote cell-cycle arrest by inducing
multiple developmental defects of the retina, heart, lung, and pancreas, and sometimes exhibit exencephaly. Accordingly, SIRT1 expression is high during embryogenesis (38). The highest SIRT1 mRNA expression in mice is detected as early as E4.5. In embryos, SIRT1 is expressed at high levels in the brain, heart, spinal cord, and dorsal root ganglions. These results suggest new roles for SIRT1, not only in early embryogenesis but also in neurogenesis and cardiogenesis, that are stage-specific. We have found that ependymal cells and some cells of the subventricular zone express SIRT1 in the adult brain. Neurospheres cultured from E14.5 mouse brain highly express SIRT1 (S. Hisahara et al., submitted manuscript). The functional role played by SIRT1 in cells express SIRT1 (Y. Horio et al., unpublished observation). For example, large neurons in the striatum and cortex showed high and moderate immunoreactivity against SIRT1, respectively. The physiological function of SIRT1 in neuronal cells is unknown. SIRT1 binds FOXO1 (25). Because activation of FOXO1 contributes to delayed neuronal death after ischemic injury (39), SIRT1 may participate in the mechanism of neuronal cell death. Neuronal gene expression may be affected by SIRT1. SIRT1 promotes gluconeogenic/glycolytic pathways through deacetylation and activation of PGC-1α, a key transcription factor of glucose production (40). SIRT1 reduces white adipocytes by repressing PPAR-γ (32). Thus SIRT1 modulates metabolic rate in peripheral organs. Blood glucose and hormonal signals such as anorexigenic leptin and orexigenic ghrelin regulate appetite expression through synthesis and secretion of neuropeptide-Y in the hypothalamus (41). It is tempting that SIRT1 also affects energy balance by regulating the transcription of neuropeptides and their receptors in satiety centers.

Conclusion

Although SIRT1 participates in cellular survival and resistance to stress, a direct relationship between SIRT1 and aging has not been shown. Sir2 and sir2.1 extend the lifespan of yeast and nematode, respectively. Recent experiments have revealed diverse functions for SIRT1 in mammals. Future studies of the SIRTs and their interactions should help unravel some of the entangled mechanisms that underlie aging.

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