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Sirtuins and Neurodegeneration
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ABSTRACT
Sirtuins are highly conserved NAD+-dependent enzymes connected to an increasing set of biological processes. These enzymes have attracted major interest because of their roles in age-related diseases. Sirtuins are implicated in various biological pathways related to stress response, mitochondrial dysfunction, oxidative stress, protein aggregation and inflammatory processes that are intertwined with age-related neurodegenerative diseases.

Introduction
The silencer information regulator (Sir) family of proteins has attracted much attention during the past decade due to its prominent role in many cellular processes. The first sirtuin (Sir2) was discovered in 1984 in yeast; however, interest did not begin until an effect on life span was observed, in yeast (Saccharomyces cerevisiae). Subsequent studies in C. elegans and then the fruit fly, D. melanogaster proved successful in corroborating the effect of increased lifespan. More specifically, the effect of increased lifespan in the fruit fly was correlated with calorie restriction (CR). Afterwards, it was shown that Sir2 extends longevity minimally at best in C. elegans and the role of Sir2 in D. melanogaster is controversial. Sirtuins were recognized as NAD+-dependent deacetylases, and then implicated in chromatin silencing and the metabolic pathways. Once the enzymatic functions of sirtuins were elucidated, sirtuins ability to extend life span were shown to involve similar pathways as those of CR. However, the literature regarding the role of yeast, fly and worm sirtuins in CR is controversial. On the other hand, mammalian sirtuins were found to have various functions in the central nervous system, liver, pancreas, skeletal muscle, and adipose tissue.

There are seven homologs (SIRT1-7) with different enzymatic activities and localizations in the mammalian cell. SIRT1, -6, and -7 reside primarily in the nucleus and have direct effects on nuclear transcription of genes involved in metabolism; however SIRT1 shuttles to the cytoplasm when required to act on cytoplasmic targets. SIRT1 is highly expressed in the adult brain with high levels in the cortex, hippocampus, cerebellum and hypothalamus. There are lower levels of SIRT1 in white matter. SIRT1 is primarily expressed in neurons and considered a nuclear protein. However, studies have shown that it is also located in cytoplasm. SIRT2 is cytoplasmic and deacetylates tubulin microtubules and transcription factors that shuttle from the cytoplasm to the nucleus. SIRT2 is expressed primarily in oligodendrocytes and plays a role in myelin sheath formation as well as oligodendrogial
translocates to the mitochondria upon cellular stress. Diseases might be through its effects in mitochondria. Protective roles of SIRT1 against neurodegenerative diseases are well-established, and it is expected that some of the protective roles of SIRT1 might be through its effects in mitochondria.

SIRT1’s role in Neurodegeneration

SIRT1 is the most extensively studied sirtuin among all sirtuins. SIRT1 was subsequently studied as a mediator of CR, a dietary regimen aimed at reducing caloric intake by 20-30% without malnutrition. CR was previously shown to slow down the aging process, increasing health and lifespan in several animal models. A recent study demonstrated that hypothalamic overexpression of SIRT1 extends longevity and delays aging in mice. These phenotypes of mice are achieved by increased neural activity observed in the dorsomedial and lateral hypothalamic nuclei.

Aging is the major risk factor for the development of neurodegenerative diseases. SIRT1 orchestrates different stress response pathways. More explicitly, SIRT1 targets multiple transcriptional regulators, such as p53, FOXO3a, and NFκB. Since SIRT1 is also important in regulating mitochondrial function, it is expected that some of the protective roles of SIRT1 against neurodegenerative diseases might be through its effects in mitochondria.

SIRT1’s role in Alzheimer’s Disease

Aging population carries a significant amount of neurodegenerative disease, due to increased life expectancy. The most common serious neurodegenerative disorder is Alzheimer’s disease (AD) causing severe cognitive and behavioral deficits. AD affects 35 million people worldwide and is the leading cause of dementia among the elderly. Aβ is the product of the proteolytic cleavage of the amyloid-β protein precursor (AβPP) in 38, 40, or 42 amino acid peptides and is viewed as the toxic mediator of AD. Aβ oligomerization is toxic to neurons in vitro and in vivo and produces synaptic dysfunction, calcium dysregulation, oxidative stress, and neuroinflammation. Moreover, Aβ (specifically Aβ42) is the primary component of the senile plaques that develop in the brains of AD.

The mounting evidence for the implication of the effects of SIRT1 in AD initially came from research primarily focused on the effects of SIRT1 overexpression mediated by small molecule modulators, such as NAD+ or resveratrol. For example, in vitro, both were found to reduce Aβ oligomers in a concentration dependent manner. In addition, both small molecule modulators were found to ameliorate oxidative stress and inflammation. Another study with SIRT1 overexpression found that Aβ toxicity in microglia was reduced via inhibition of NFκB signaling, which induces inflammation.

CR was shown to attenuate Alzheimer’s disease type brain amyloidosis in Squirrel monkeys and was subsequently studied in the APPswe/PSEN1dE9 mouse line. The research suggested that attenuation of beta-amyloid content in the brain during CR can be reproduced in mice. CR may influence AD amyloid neuropathology. In an in vitro work using N2a cells expressing human Swedish APP mutation (N2aSwe), it was shown that overexpression of SIRT1 gene resulted in a significant increase in ADAM10 and sAPPa levels in this cell type.

In N2aSwe cells, increased Ab accumulation was accompanied by increased Ac-tau and P-tau levels together with elevated P300 and GSK3b P-Tyr216 expression; their expressions were significantly reduced by cilostazol and resveratrol treatments. This study showed that increased cyclic AMP (cAMP) levels via inhibiting type III phosphodiesterase enzyme. In this study, cilostazol was demonstrated to enhance the protein expressions of RARb and ADAM10. In addition, ADAM10 elevation induced by cilostazol was significantly attenuated by LE135 (a RARb inhibitor), sirtinol (SIRT1 inhibitor), and RARb-gene silencing. The authors conclude that the increase in the levels of ADAM10/a-secretase activity and the reduction in the intracellular Ab accumulation, which are based on the cilostazol-stimulated SIRT1-linked RARb activation, ameliorate AD-associated neurodegeneration.

Another study demonstrated that apolipoprotein E4 allele (ApoE) significantly reduces the ratio of soluble amyloid precursor protein alpha (sAPPa) to Ab and also decreases SirT1 expression. It was also shown that Ab reduces SIRT1 expression, triggers Tau phosphorylation and APP phosphorylation. Additionally, programmed cell death is induced as a result of Ab accumulation. Therapeutic up-regulation of SIRT1 might provide opportunities for the amelioration of Alzheimer’s-disease-type neuropathology through inhibition of amyloidogenesis. Ultimately, further analysis into this aspect is necessary if any progress is to be made.
Other transgenic mice studies include a p25 transgenic mouse, which overexpresses the cdk5-activating human p25 protein thereby exhibiting tau hyperphosphorylation and neurodegeneration with features similar to AD. Tau is a microtubule-binding protein found in high content in neurons and is responsible for the assembly and stability of microtubules. Hyperphosphorylation of the tau protein leads toward tangles in an ordered fashion such as paired helices and straight filaments. These self-assembled tangles are implicated in the pathogenesis of AD as well as other tauopathies. Cyclin-dependent kinase 5 (cdk5) and its regulatory subunit p35 have important roles in the development of the mammalian central nervous system. Proteolytic cleavage of p35 yields p25, which then activates cdk5. Accumulation of p25 is a hallmark of neurodegenerative disease. In a mouse model (cdk5-activating p25), injection of the SIRT1-activating polyphenol resveratrol resulted in less hippocampal degeneration, less cognitive deficit, and reduced acetylation of SIRT1 substrates PGC-1α and p53. Additionally, when SIRT1 was directly overexpressed in the hippocampus of these mice using a lentiviral vector, the effects of resveratrol injections were corroborated.

Tau and SIRT1 have previously shown a relationship in that tau is acetylated at multiple lysine residues and SIRT1 regulates the level of phosphorylated tau via deacetylation. When tau is acetylated by the histone acetyltransferase, p300, the breakdown of tau is inhibited. SIRT1 inhibition was shown to increase tau levels thereby increasing phosphorylated tau. In summary, degradation of phosphorylated tau improved cognitive function and reduced neuronal cell death.

SIRT1’s role in Parkinson’s disease

Parkinson’s disease (PD) is another progressive neurodegenerative disorder affecting the central nervous system. The neuropathology involves loss of dopaminergic neurons in the substantia nigra pars compacta, and its symptoms include muscle rigidity, bradykinesia, resting tremor and postural instability, amongst others. Cytoplasmic inclusions called Lewy bodies (LB) containing the protein α-synuclein, some proteasomal and lysosomal subunits have been found on histological analysis of PD. Although the reason for the neuronal cell loss still remains elusive, misfolding, oligomerization and α-synuclein aggregation have been implicated in the neuropathology of PD and parkinsonian plus disorders such as multiple systems atrophy (MSA). Studies of PD models and the functions of genes implicated in inherited forms of PD have looked at two different possibilities leading toward dopaminergic neuronal cell death: (i) mitochondrial dysfunction and oxidative stress, and (ii) misfolding and aggregation of proteins.

In other cell culture studies, dietary resveratrol was shown to attenuate oxidative stress induced by the parkinsonian mimetic 6-hydroxydopamine as well as the controversial treatment for PD, L-3,4-dihydroxyphenylalanine (L-dopa). The authors of these studies suggest that the antioxidant properties of resveratrol are involved in its attenuation of oxidative stress as the neuroprotective effect, rather than investigating SIRT1 activation. Moreover, resveratrol exhibited a neuroprotective effect on dopaminergic neurons in midbrain slice culture from multiple insults. In this study, it was determined that the neuroprotective effect was indeed, void of SIRT1 activation because SIRT1 inhibitors did not attenuate the protective resveratrol effects.

Studies performed so far suggest that SIRT1 may be a pharmacological target in the treatment of neurodegenerative diseases by deacetylating different target proteins. However, additional studies are essential to substantially prove SIRT1 provides a molecular basis of protection. For example, one study, which used the well-established 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD in SIRT1 transgenic mice, resulted in no protection. Neuron-specific enolase (NSE) SIRT1 transgenic mice were generated to overexpress human SIRT1 in neurons. Overexpression of SIRT1 did not have any neuroprotective effects against the neuronal damage induced by ischemia or MPTP.

SIRT1’s role in Huntington’s Disease

Huntington’s disease (HD) is a neurodegenerative disease that affects four to seven individuals per 100,000. HD is a fatal neurodegenerative disorder caused by an expanded polyglutamine repeat in huntingtin (HTT) protein.

In a mouse model of HD, brain-specific knockout of SIRT1 results in exacerbation of brain pathology, whereas overexpression of SIRT1 improved survival, neuropathology and the expression of BDNF. SIRT1 deacetylase activity was shown to directly target neurons to mediate neuroprotection from mutant HTT. The neuroprotective effect of SIRT1 requires the presence of CREB-regulated transcription coactivator 1 (CRTC1), a brain-specific modulator of CREB activity. Under normal conditions, SIRT1 deacetylates and activates CRTC1 by promoting its dephosphorylation and its interaction with CREB. BDNF was identified as a key target of SIRT1 and CRTC1 transcriptional activity in both normal and HD neurons. Mutant HTT interferes with the CRTC1-CREB interaction to repress BDNF transcription, and SIRT1 rescues this defect in vitro and in vivo. Together, these findings show a neuroprotective role for SIRT1 in mammalian HD models.

SIRT1’s role in ALS

Loss of neurons with age is also seen in amyotrophic
lateral sclerosis (ALS). The underlying causes of
deneration of the neuromuscular junction and eventual
motor neuron death in ALS have not been resolved.

The superoxide dismutase 1 (SOD1)(G93A) mutant mouse is a frequently used animal model of ALS. As
mentioned earlier, resveratrol, a polyphenolic molecule that enhances SIRT1 activity, improved motor function and
survival in the SOD1 mouse model via modulation of p53
acetylation\textsuperscript{58}. Manipulation of SIRT1 deacetylase activity had effects at the protein level in healthy aging organisms; however, resveratrol treatment did not lead to functional
improvement or increased longevity in a mouse model of
ALS\textsuperscript{53}. In a similar mouse model, region specific changes
in the immunoreactivity of SIRT1 expression in the central
nervous system was observed. SIRT1 increases in cerebral
cortical pyramidal cells, hippocampal pyramidal cells of
area CA1-3 and dentate gyrus cells, thalamus, and spinal
cord\textsuperscript{54}.

The role that SIRT1 activation plays in the pathogenesis
of ALS remains unclear; however, via activating SIRT1,
resveratrol was shown to protect against neurodegeneration
in a cell model of ALS\textsuperscript{55}. SIRT1 expression was found to be
much lower in the mutant (SOD1)(G93A)-bearing VSC4.1
cells compared to (SOD1)(wt) cells when both were
cultured in low-serum medium, indicating the involvement
of SIRT1 activation defects in the pathogenesis of ALS under
energetic stress. Further investigation revealed that
resveratrol had a dose-dependent protective effect on this
ALS cell model. This further demonstrated a role for
SIRT1 activation in the protection of neuronal cells from
degeneration. These findings suggest that resveratrol can
protect the ALS cell model from mutant SOD1-mediated
toxicity through up-regulating the expression of SIRT1,
which might represent a potential therapeutic target for
preventing the motor neuron degeneration in ALS patients.

**SIRT2’s role in Neurodegeneration**

SIRT2 is an oligodendroglial cytoplasmic protein and
localized to the outer and juxtanodal loops in the
myelin sheath. Among cytoplasmic proteins of OLN-93
oligodendrocytes, alpha-tubulin is the main substrate of
SIRT2 deacetylase\textsuperscript{56}. There exists a counterbalancing role
of SIRT2 against a facilitator effect of tubulin acetylation
on oligodendroglial differentiation. SIRT2 availability to
oligodendroglia has important implications for myelination, myelin-axon interaction, and brain aging. In addition to \(\gamma\)-tubulin and histone H4 substrates, SIRT2
deacetylates forhead transcription factors of class O,
FOXO1 and FOXO3\textsuperscript{14}. Since FOXO transcription factors are
involved in several cellular processes, including yet not
limited to DNA repair; cell cycle, apoptosis, metabolism
and aging, SIRT2 has been initially investigated in these
processes\textsuperscript{57}. The effective control of FOXO activity in
response to environmental stimuli is likely to be critical
to prevent aging and age-dependent diseases, including
cancer, neurodegenerative diseases and diabetes.

**SIRT2’s role in Parkinson’s Disease**

SIRT2 inhibition, pharmacologically or genetically, was
found to be beneficial in the rescue of alpha-synuclein
toxicity in both in vitro and in vivo models of Parkinson’s
Disease (PD). A potent inhibitor of SIRT2 was shown to
inhibit alpha-synuclein toxicity and modified inclusion
morphology in a cellular model of PD\textsuperscript{58}. Genetic inhibition
of SIRT2 via small interfering RNA similarly rescued alpha-
synuclein toxicity. In addition, the inhibitors protected
against dopaminergic cell death both in vitro and in a
Drosophila model of PD. In addition, the interaction between
oligomeric alpha-synuclein and acetylated microtubules
was investigated as a source of neurodegeneration. SIRT2
inhibition was shown to increase microtubule-dependent
transport of neurotoxic alpha-synuclein oligomers to the
nucleation aggregation site, facilitating formation of
inclusions called LB\textsuperscript{59}.

It was recently shown that SIRT2 in the brain enhanced
MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-
induced nigrostriatal damage via deacetylating FOXO3a and
activating Bim\textsuperscript{59}. MPTP is a dopaminergic neurotoxin that
replicates most of the clinical features of PD and produces
a reliable and reproducible lesion of the nigrostriatal
dopaminergic pathway and neurodegeneration after its
systemic administration\textsuperscript{57}. Chronic administration of MPTP
induces lesion via apoptosis\textsuperscript{57}. It was shown that SIRT2
deacetylates Foxo3a, increases RNA and protein levels
of Bim, a proapoptotic factor, and as a result, enhances
apoptosis in the MPTP model of PD. Neurodegeneration
induced by chronic MPTP regimen is prevented by genetic
deletion of SIRT2 in mouse. Deletion of SIRT2 lead to the
reduction of apoptosis due to an increase in acetylation of
Foxo3a and a decrease in Bim levels\textsuperscript{59}.

**SIRT2’s role in Huntington’s Disease**

The molecular pathogenesis of Huntington’s disease
(HD) is complex and many mechanisms and cellular
processes have been proposed as potential sites of
therapeutic intervention. Previous studies in invertebrate
and cell culture HD models have suggested that
inhibition of SIRT2 could have beneficial consequences
on disease progression\textsuperscript{60,61}. SIRT2 has been proposed to
deacetylate \(\alpha\)-tubulin, histone H4 K16 and to regulate
cholesterol biogenesis, a pathway, which is dysregulated
in HD patients and HD mouse models\textsuperscript{59}. The distribution
and function of the SIRT2 microtubule deacetylase in
differentiated, postmitotic neurons were investigated and
it was determined that expression of specific isoforms of
SIRT2 in the mammalian central nervous system exhibits
age-dependent accumulation in the mouse brain and spinal cord. Furthermore, endogenous SIRT2 expression correlates with reduced α-tubulin acetylation in primary mouse cortical neurons; therefore, it is postulated that brain-enriched species of SIRT2 may function as the microtubule deacetylases in mature neurons.

An in vivo study, SIRT2 was reduced or ablated to further explore the function of SIRT2 and to assess whether SIRT2 loss has a beneficial impact on disease progression in the R6/2 mouse model of HD. The reduction or loss of SIRT2 had no effect on the acetylation of α-tubulin or H4K16 or on cholesterol biosynthesis in the brains of wild type mice. In addition, genetic reduction or ablation of SIRT2 had no effect on HD progression as assessed by a battery of physiological and behavioral tests. Therefore, it was concluded that SIRT2 inhibition does not modify disease progression in the R6/2 mouse model of HD and SIRT2 inhibition should not be prioritized as a therapeutic option for HD.

Another in vivo model utilizes the efficacy of a brain-permeable SIRT2 inhibitor in two separate genetic mouse models of HD. It was reported that compound treatment resulted in improved motor function, extended survival, and reduced brain atrophy with reduction of aggregated mutant huntingtin (a hallmark of HD pathology). In addition, genetic or pharmacologic inhibition of SIRT2 in a striatal neuronal model of HD resulted in gene expression changes including significant down-regulation of the RNAs responsible for sterol biosynthesis. Mutant huntingtin fragments increases sterols in neuronal cells; therefore, manipulation of sterol biosynthesis at the transcriptional level would prove beneficial.

Most of the studies showed that the inhibition of SIRT2 is beneficial for HD except the study that used R6/2 as a model. R6/2 mouse model displays aggressive HD phenotype, therefore additional studies should be designed where different HD mouse models are utilized. The outcome of the deletion of SIRT2 in these mouse models should be analyzed in order to assess the role of SIRT2 in HD.

In summary, in all the disease models studied, inhibition of SIRT2 seems to have beneficial effects by deacetylating different targets. Therefore, inhibiting the activity of this enzyme might be effective in designing effective therapies.

**Mitochondrial Sirtuins and Neurodegeneration**

Three of the mammalian sirtuins, SIRT3, SIRT4 and SIRT5 are localized in mitochondria, which are the major organelles responsible for energy production, balance and metabolism. SIRT3 was shown to have strong deacetylase activity. On the other hand, SIRT4 has adenosine diphosphate (ADP)–ribosylation activity, and SIRT5 has desuccinylase and demalonylase activity in addition to weak deacetylation activity. A recent study showed that SIRT3 localizes to the mitochondria, does not exist in nucleus and it is a proteolytically modified deacetylase. The study also emphasizes that SIRT3 does the majority of deacetylation in mitochondria. A number of targets were identified for SIRT3; however, only a handful of substrates were described for SIRT4 and SIRT5. Mitochondrial dysfunction is associated with age-related disorders such as metabolic syndrome, cancer and neurodegeneration. The role of mitochondrial sirtuins in brain and their relation to neurodegeneration is not broadly studied except for a few recent in vivo studies using toxins such as kainic acid and MPTP.

SIRT3 was shown to attenuate MPTP-induced nigrostriatal damage in mice. SIRT3 KO mice displayed increased degeneration compared to wild type mice after MPTP administration. Decreased levels of superoxide dismutase 2 (SOD2), a specific mitochondrial antioxidant enzyme, and glutathione peroxidase expression was detected in MPTP-induced SIRT3 KO mice compared to wild-type controls. Therefore, it was concluded that SIRT3 might be protective in MPTP-induced neurodegeneration via improving antioxidant capacity in mitochondria. A similar study was conducted using SIRT5 KO mice. MPTP-induced nigrostriatal degeneration was elevated in SIRT5 KO mice together with a larger decrease in the expression level of SOD2 compared to wild type controls.

SIRT4 was shown to have different physiological roles in various tissues but its role in brain was unexplored. A recent study showed that SIRT4 is up-regulated in brain in response to treatment with kainic acid, an excitotoxin. Glutamate transport keeps low extracellular levels of glutamate in the brain to prevent excitotoxicity that leads to neurodegeneration and inefficient neurotransmission. SIRT4 KO mice displayed severe seizure phenotypes compared to wild type mice after exposure to kainic acid. Deletion of SIRT4 also leads to reduced glutamate transporter expression in brain after kainic acid administration. This study showed a novel stress response role for SIRT4 in maintaining efficient glutamate transport and preventing excitotoxicity.

**SIRT6, SIRT7 and Neurodegeneration**

SIRT6 is a nuclear enzyme known to deacetylate histone H3-lysine 9 (H3K9). In previous studies, SIRT6 was found to promote longevity. Due to the phenotype observed in SIRT6 knockout mice, this protein is also thought to regulate DNA base excision repair (BER) and metabolic functions. A more recent study showed that SIRT6 regulates the stability of Tau protein and the lack of SIRT6 increases hyperphosphorylated tau levels. Moreover, RNA and protein levels of SIRT6 were found to be decreased in Alzheimer’s disease patients. Therefore, SIRT6 and its
downstream targets could be targeted for therapeutical strategies in neurodegenerative diseases.

SIRT7 is the least characterized sirtuin and found in nucleolus\cite{74}. It interacts with rDNA and regulates RNA Pol I \cite{74}. SIRT7 was shown to be overexpressed in many human tumors\cite{75} and recent studies identified SIRT7 as a proto-oncogene\cite{76}. However, SIRT7 is largely unexplored for neurodegenerative diseases and brain. Similar expression could be modulated in cell and animal models of neurodegenerative diseases, similar to the previous studies conducted for other sirtuins. SIRT7 is also expected to have major functions in neuronal pathways and diseases.

**Conclusion**

The functions of sirtuins in the brain are still unknown; however, published studies show that they have important roles in neurodegenerative diseases. Substrates of sirtuins are molecules that take part in the fundamental cellular pathways and are crucial to the biology of the organism. Investigating the roles of sirtuins in neurodegeneration not only elucidates whether these molecules are of importance therapeutically but also identifies their novel functions in brain (Figure 1).

Although all seven mammalian sirtuins are expressed in the brain, only the roles of SIRT1 and SIRT2 in neurodegeneration were extensively studied (Figure 1). The findings show that SIRT1 activation generally displays a protective role against neurodegenerative diseases; whereas, SIRT2 inhibition or deletion shows a positive outcome. These two enzymes seem to be quite different although they share a common catalytic domain. They have different targets and they are located in different subcellular locations. In light of the studies conducted, we can conclude that activating SIRT1 and inhibiting SIRT2 seem to be beneficial for the organism. On the other hand, deletion of mitochondrial sirtuins in mice display increased degeneration in brain after administration of toxins. Therefore, designing pharmacological activators or inhibitors for sirtuins is a crucial research area since they might be beneficial against neurodegenerative diseases. It is extremely important to develop selective activators or inhibitors that target a specific sirtuin since they have different functions in different cellular compartments. Additionally, the level of sirtuins in specific areas of the brain may hold a prognostic value for the detection or diagnosis of the diseases. However, the best modulators of sirtuins against age-related disorders can only be designed if we understand the molecular mechanisms underlying their effects in neurodegenerative diseases and brain.

**References**

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