

Role of SIRT1 in autoimmune demyelination and neurodegeneration

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Abstract Multiple sclerosis (MS) is a demyelinating disease characterized by chronic inflammation of the central nervous system, in which many factors can act together to influence disease susceptibility and progression. SIRT1 is a member of the histone deacetylase class III family of proteins and is an NAD⁺-dependent histone and protein deacetylase. SIRT1 can induce chromatin silencing through the deacetylation of histones and plays an important role as a key regulator of a wide variety of cellular and physiological processes including DNA damage, cell survival, metabolism, aging, and neurodegeneration. It has gained a lot of attention recently because many studies in animal models of demyelinating and neurodegenerative diseases have shown that SIRT1 induction can ameliorate the course of the disease. SIRT1 expression was found to be decreased in the peripheral blood mononuclear cells of MS patients during relapses. SIRT1 represents a possible biomarker of

relapses and a potential new target for therapeutic intervention in MS. Modulation of SIRT1 may be a valuable strategy for treating or preventing MS and neurodegenerative central nervous system disorders.

Keywords SIRT1 · Multiple sclerosis · Experimental allergic encephalomyelitis · Acetylation · RGC-32 · Peripheral blood mononuclear cells

Introduction

Posttranslational modifications (PTMs) play an important role in the regulation of many physiological processes in organisms. These modifications include acetylation and deacetylation of histone and nonhistone proteins associated with DNA [1]. These reactions are mainly mediated by two opposing types of enzyme: histone acetyltransferase (HAT) and histone deacetylase (HDAC) [2]. PTMs of histone proteins have the ability to affect the architectural organization of chromatin, which is critical to the regulation of gene expression [3]. These reactions take place without altering the DNA sequence [4]. The state of chromatin at any given moment is controlled by the degree of acetylation and deacetylation of lysine residues in the amino-terminal tails of the histones, including histones H1, H3, and H4 [5]. Histones' termini have many positively charged lysine amino acids, which interact favorably with the negative charges in DNA [6]. Acetylation of these lysine residues on the histones removes the positive charge, thereby decreasing the interaction of the N-termini of the histones with the negatively charged phosphate groups of DNA. As a consequence of this hyperacetylation process, the condensed chromatin is transformed into a more relaxed structure and permits the binding of transcription

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factors to the DNA. Hence, greater levels of gene transcription are present [7]. HDACs remove acetyl groups, which are negatively charged, and allow the interaction between the positively charged lysine residues and DNA. Therefore, the chromatin becomes more compacted in its structure, and genes become silenced [8]. Thus, the acetylation and deacetylation of histone proteins play a pivotal role in epigenetic regulation in many cell types.

Eighteen different mammalian HDACs have been identified, and they are divided into four classes on the basis of their function and DNA sequence similarity. They have different locations inside the cell and possess a high diversity of intracellular targets [9]. Classes I, II, and IV are Zn²⁺-dependent HDACs, whereas class III is the only NAD⁺-dependent HDAC (Table 1) [10, 11]. The biological relevance of the HDACs as deacetylases rests on the fact that deacetylation is now known to be an extremely important and widespread regulatory mechanism. Nevertheless, it is important to emphasize that deacetylases have many other nonhistone targets. More than 2,000 proteins are acetylated in mammalian cells [12, 13]. In this review, we will discuss the biological activities of SIRT1, a class III HDAC, and its potential implications in autoimmune demyelination, neurodegeneration, and multiple sclerosis (MS).

An overview of sirtuins

The founding member of the sirtuin (SIRT) family, silent mating-type information regulator 2 (Sir2) from yeast, was

identified in 1984 as a gene that is required for maintaining silent chromatin [14]. The role of sirtuins in aging was first described by Kaerberlein et al. [15]. These proteins received their name as a result of their homology to Sir2; although first identified in yeast (*Saccharomyces cerevisiae*) [16, 17], Sir2 was later found in the fruit fly (*Drosophila melanogaster*) [18] and roundworm (*Caenorhabditis elegans*) [19]. It was originally characterized as a chromatin-silencing component that repressed gene transcription at selected loci [20]. Overexpression of Sir2 has clearly been shown to extend the replicative life span in yeast [15]. Sirtuins belong to the class III HDAC family and are present in numerous species, including humans [21]. In humans as well as in rodents, seven structurally and functionally different sirtuins have been identified (SIRT1 to SIRT7). They are located in different subcellular compartments, including the nucleus, cytosol, and mitochondria (Table 2) [22, 23]. Three of them (SIRT1, SIRT6, and SIRT7) are clearly localized to the nuclear compartment; SIRT7 is mostly restricted to the nucleolar region. SIRT1 is known to shuttle to the cytoplasm [24, 25]. SIRT2 is the only one found exclusively in the cytoplasm. SIRT3, SIRT4, and SIRT5 are mitochondrial proteins, although SIRT3 is also found in the nucleus under normal conditions. Their structure is defined by the presence of a central catalytic domain, formed by approximately 250 residues, which functions as an enzymatic core. This core is flanked by variable amino (N-) and carboxy (C-) terminals that differ among the various sirtuins [3, 26]. This variety of terminal regions helps explain the diversity of sirtuin functions [27].

As mentioned earlier, histone deacetylation was first recognized as the main function of sirtuins. Nevertheless, over the years, many authors have gradually discovered that these regulatory proteins possess a high diversity of biological functions, just like the rest of the HDACs [28–31]. In addition to acting as important regulators of the aging process and metabolism, sirtuins also play a key role in cell differentiation and senescence [32] (Fig. 1). Sirtuins protect cells against the stress response and multiple pathologic conditions, including cancer [33–35] and many neurodegenerative diseases, which will be discussed further below [36]. Their ability to counteract oxidative damage associated with certain diseases by regulating the systems that control redox reactions has been studied in detail [37]. As mentioned above, the effects of sirtuins are the result of their capacity to enzymatically regulate the activity of a wide variety of cellular pathways and several types of substrates. In the past two decades, the range of deacetylase targets has broadened beyond the histones to include many nonhistone substrates. Nonhistone targets of SIRT1 are mainly transcription factors, transcriptional coactivators, DNA repair enzymes, protein kinases, and

Table 1 Histone deacetylase classification

HDAC	Depends on	Isoforms	Protein domain (aa)
Class I	Zn ²⁺	HDAC 1	482
		HDAC 2	488
		HDAC 3	428
		HDAC 8	377
Class IIa	Zn ²⁺	HDAC 4	1,084
		HDAC 5	1,122
		HDAC 7	912
Class IIb	Zn ²⁺	HDAC 9	1,609
		HDAC 6	1,215
Class III	NAD ⁺	HDAC 10	669
		SIRT1	747
		SIRT2	389
		SIRT3	399
		SIRT4	314
		SIRT5	310
		SIRT6	355
Class IV	Zn ²⁺	SIRT7	400
		HDAC 11	347

Table 2 Sirtuins: location, their targets and biological implications

Sirtuin	Activity	Cellular location	Targets	Biological implications
SIRT1	Deacetylation	Nucleus, cytoplasm	FOXO, PGC-1 α , p53, NF- κ B, Notch, HIF1 α , LXR, FXR, SREBp1c, HES1	Cell survival, metabolism, DNA damage, stress resistance, lipid and glucose homeostasis
SIRT2	Deacetylation	Cytoplasm	FOXO1, PEPCK, tubulin, H4, PAR-3	Cell cycle control, cell motility
SIRT3	Deacetylation, ADP-ribosylation	Mitochondria	FOXO3, LCAD, GDH, IDH2, ACADL, PIP2, GLUD1, PGC-1 α	Metabolism, thermogenesis
SIRT4	ADP-ribosylation	Mitochondria	GDH	Insulin secretion, metabolism
SIRT5	Deacetylation, demalonylation, desuccinylation	Mitochondria	CPS1	Unknown
SIRT6	ADP-ribosylation	Nucleus	DNA Pol β , H3K9, H3K56	DNA repair, glucose homeostasis
SIRT7	Deacetylation	Nucleolus	Pol I	rDNA transcription

FOXO Forkhead box, subgroup O, *PGC-1 α* peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *HIF1 α* hypoxia-inducible factor 1-alpha, *LXR* liver X receptor, *SREBp1c* sterol regulatory element-binding protein-1c, *HES1* hairy and enhancer of split-1, *PEPCK* phosphoenolpyruvate carboxykinase, *PAR-3* protease-activated receptors 3, *LCAD* long-chain acyl-CoA dehydrogenase, *GDH* glutamate dehydrogenase, *IDH2* isocitrate dehydrogenase 2, *ACADL* acyl-CoA dehydrogenase, long chain, *PIP2* phosphatidylinositol 4,5-bisphosphate, *GLUD1* glutamate dehydrogenase 1, *PGC-1 α* peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *GDH* glutamate dehydrogenase, *CPS1* carbamoyl-phosphate synthetase 1, *DNA Pol β* DNA polymerase-beta, *H3K9* histone H3 lysine 9, *H3K56* histone H3 lysine 56, *Pol I* polymerase I

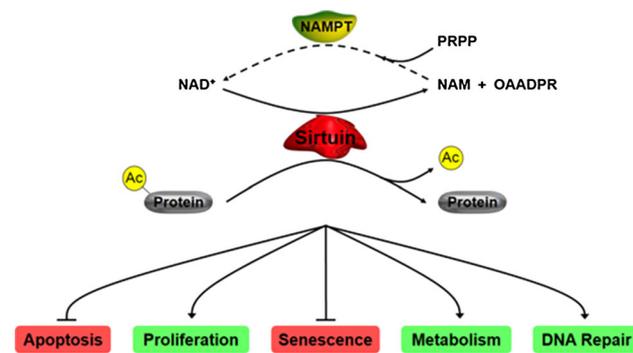


Fig. 1 Role of sirtuins in cell functions and pathways. SIRT1 is involved in multiple cellular pathways related to aging, inflammation, epigenetics, cancer, and a variety of cellular functions including the cell cycle, apoptosis, senescence, DNA repair, and proliferation. SIRT1 functions in the cell via histone deacetylase (HDAC) enzymatic activity. These enzymes target histones as well as a variety of nonhistone substrates, which include transcription regulators, tumor suppressors, structural proteins, DNA repair proteins, cell signaling proteins, transport proteins, and enzymes

phosphatases, among others. In recent times, the number of these targets identified in mammals has grown considerably [38]. Moreover, SIRT 3, 4, 5, and 6 have been reported to have other functions, including ADP-ribosyltransferase activity [39, 40] (Table 2). Some authors have even described the demalonylation activity of SIRT5 [41, 42].

One of the aspects that distinguish sirtuins is their requirement for the metabolic coenzyme NAD⁺ to perform their cellular functions. NAD⁺ serves as substrate of SIRT1. This dependence on NAD⁺ corroborates one of the

links between SIRT1 activity and caloric restriction as well as other stress conditions, including oxidative stress [43, 44]. This relationship was first characterized with respect to calorie restriction-induced life span extension in yeast but has since been expanded to mammalian systems. Such conditions increase the ratio of NAD⁺/NADH, and the relatively high level of NAD⁺ enhances the activity of sirtuins [45]. NAD⁺ not only serves as a precursor and an essential nutrient for cell growth and function, but it also provides specific protective cellular mechanisms that determine neuronal survival [46].

SIRT1

Of the seven sirtuins, SIRT1 has been the most thoroughly studied. SIRT1 is also the most evolutionarily conserved among the homologs; in humans, it contains 747 amino acids, with a measured molecular size of 12 kDa [47]. The role of SIRT1 under normal physiological conditions, as well as during pathologic states, has spawned great interest. Just like other sirtuins, SIRT1 deacetylates histones as well as many nonhistone proteins, including transcription factors. Accumulating evidence has linked SIRT1 to aging, metabolic diseases, neurodegenerative diseases, cancer, and cardiovascular dysfunction [48]. Its activity can be adjusted in response to many different stimuli. It is mainly found in the nucleus and cytoplasm, but it is important to remember that its activation status can cause a shift in its subcellular location, specifically via nucleo-cytoplasmic shuttling. In a recent study of MS brains, it was found that

SIRT1 was present mainly in the cytoplasm, whereas phosphorylated SIRT1 (p-SIRT1) was localized only to the nuclei [49]. In addition, DNA damage causes a global redistribution of SIRT1 away from silent repeat sequences and gene promoters to sites of DNA damage [50]. These changes in intracellular location are likely to play a role in the regulation of SIRT1 function [10].

Regulation of SIRT1 expression and activation

SIRT1 has two coupled enzymatic activities: deacetylating its targets and breaking down NAD^+ . During the second process, an acetyl group of the substrate is transferred to the ADP-ribose (ADPR) moiety of NAD^+ , with the SIRT1 substrate becoming deacetylated. Once NAD^+ gains the acetyl group, it becomes destabilized, breaking down to one molecule of nicotinamide and one molecule of 20-*O*-acetyl-ADP-ribose (OAADPR) (Fig. 1). As an end product of sirtuin deacetylation, nicotinamide is an effective inhibitor of SIRT1 activity *in vivo* and *in vitro* [51]. At normal concentrations, nicotinamide binds to the sirtuins, causing their inhibition [52].

Most studies focus on the identification of SIRT1 substrates rather than on the regulation of SIRT1 itself. The regulation of SIRT1 activation is not yet completely understood. However, a variety of cellular pathways have been found to increase SIRT1 expression and activity under conditions such as oxidative stress, exercise, calorie restriction, and starvation [53]. Here, we will briefly present some of the mechanisms that are known to regulate SIRT1 activity.

Expression of SIRT1 can be regulated at the transcriptional level. The basal level of SIRT1 is regulated by the E2F transcription factor 1 (E2F1), through binding to the SIRT1 promoter at a consensus site [54, 55]. Calorie restriction, DNA damage, and certain cellular stresses such as oxidative stress all increase the transcriptional activity of E2F1, leading to an increase in the level of SIRT1 transcription [55]. Furthermore, SIRT1 binds to E2F1 and inhibits E2F1 activity, forming a negative feedback loop [55]. Other important transcription factors that upregulate SIRT1 are the Forkhead box proteins (FOXO) [56]. FOXO1 binds to several sites within the SIRT1 promoter and enables its transcription [57]. FOXO3a is another SIRT1 regulator, and starvation in mammal cells activates FOXO3a and consequently augments SIRT1 transcription, indicating an important role for these proteins in nutrient-sensing signaling [58]. SIRT1 deacetylates FOXO1 and increases its transcriptional activity, forming a positive feedback loop [57]. Such feedback loops may play important roles in the fine regulation of SIRT1 expression.

PTMs are known to impact SIRT1 activation. There is recent evidence concerning the importance of various

phosphorylation sites that are mainly found in the terminal regions of SIRT1. Mass spectrometry experiments have detected at least 13 serine/threonine phosphorylation sites in the N- and C-terminal domains of SIRT1, and it is likely that many more exist [59]. C-Jun N-terminal kinase (JNK 1), for instance, is capable of phosphorylating SIRT1 at Ser 27 and 47 as well as Thr 530. In addition, the cyclin B/cyclin-dependent kinase 1 (CDK1) complex phosphorylates SIRT1 at Thr 530 and Ser 540. Another serine residue located in the highly conserved core domain of SIRT1, Ser 434, has been recently revealed to be a phosphorylation target as well. These phosphorylations appear to increase the deacetylase activity of SIRT1 toward one of its substrates, histone H3 [60]. Phosphorylation of SIRT1 occurs under oxidative stress and increases the nuclear translocation and enzymatic activity of SIRT1 [41, 61, 62]. There is strong evidence that under oxidative stress, a nucleo-cytoplasmic shuttling of SIRT1 occurs [25, 63]. It has also been demonstrated that the cytoplasmic accumulation of SIRT1 keeps it away from the nucleus, making it unable to exert its inactivating function on some of its substrates. Consequently, the cytoplasmic localization of SIRT1 may be representative of its presence, but not of its degree of functional activity [64, 65].

A group of proteins known as small ubiquitin-like modifiers (SUMO), also interact with SIRT1, increasing its activity through sumoylation. Sumoylation of Lys 734, for example, significantly increases the enzymatic activity of SIRT1. There is evidence from animal models that the mutation of certain sumoylation sites in SIRT1 impairs its deacetylase activity toward p53 and histones. The desumoylation of SIRT1 occurs after genotoxic stress, leading to increased cell death. Sumoylation and desumoylation of SIRT1 can therefore function as a molecular switch to regulate SIRT1 activity in response to cellular stresses. This kind of modification can decrease apoptosis, and it is therefore hypothesized to correlate strongly with cell survival [66]. Two regulatory proteins have also been found to alter SIRT1 activity. The mechanism by which these proteins modify the activity of SIRT1 is not completely understood; however, in response to cellular stresses, both the active regulator of SIRT1 (AROS) and the deleted in breast cancer-1 (DBC-1) protein form complexes with SIRT1. AROS increases the deacetylating activity of SIRT1 on p53 after DNA damage, inhibiting p53-mediated transcription of pro-apoptotic genes [67]. SIRT1 is the only sirtuin regulated by DBC-1, which functions as a negative regulator, inhibiting the activity of SIRT1 on p53 [68].

Resveratrol (3,5,4'-trihydroxystilbene) is the first-identified and most studied SIRT1 activator. Although the underlying mechanisms are not fully understood, it has been shown to activate SIRT1 directly via an allosteric site adjacent to the catalytic domain. Furthermore, resveratrol

has proven to have biological and protective functions other than activating SIRT1 [69, 70]. Resveratrol is a polyphenol stilbene found in grape skins, berries, and nuts. It is also found in low doses in wine. It was originally described as an antifungal resistance factor of plants [71]. There is strong evidence that it has an effect when consistently consumed by animals in significant amounts; *in vivo* assays have proven to extend the life span in worms, flies, and yeast. *In vitro* assays have demonstrated that it not only increases the life span but also reduces inflammation and protects against cancer and certain neurodegenerative diseases [69, 70].

SIRT1 in the central nervous system (CNS)

Recently, SIRT1 has gained a lot of attention in neurology, and some of its important roles in the CNS have been elucidated. It has been shown to be beneficial in multiple models of neuropathology, primarily by regulating deacetylation pathways of different protein targets that protect against neurodegeneration [72, 73]. SIRT1 is expressed in several regions of the adult human brain, with high levels in the cortex, hippocampus, cerebellum, and hypothalamus and low levels in the white matter [74]. In mice, SIRT1 is also abundantly expressed in several regions of the hypothalamus, particularly in the arcuate, paraventricular, and ventro- and dorsomedial nuclei. In the adult rat brain, SIRT1 can be found in the hippocampus, cerebellum, and cerebral cortex [75]. SIRT1 is also highly expressed in mouse embryos, especially in the brain, spinal cord, and dorsal root ganglion [76]. Among the various cell types in the brain, SIRT1 is expressed in neurons [72, 77]. SIRT1 regulates the long-term survival of neurons through direct interactions with various transcription factors. For example, SIRT1 represses p53 and FOXO-mediated apoptosis [78]. Although NAD⁺ has been described as being capable of protecting neurons without the involvement of SIRT1 activity, others have suggested that SIRT1 is the downstream effector of nicotinamide mononucleotide adenyltransferase (NMNAT) activation that leads to axonal protection. For example, Araki et al. [79] have shown that only SIRT1 is involved in mediating the protective effects of NMNAT1 and NAD⁺. SIRT1 sensitizes neurons to oxidative damage but also has neuroprotective effects. It has also been shown to reduce neurodegeneration by preventing axonal degeneration, polyglutamine toxicity, and microglia-mediated amyloid β toxicity [80]. SIRT1 activation regulates important genes that exert neuroprotective actions, such as p53, nuclear factor- κ B (NF κ B), peroxisome proliferator-activated receptors alpha and gamma (PPAR α and γ), PPAR γ coactivator-1 α (PGC-1 α), liver X receptor, and FOXO (Table 1) [81–84].

Role of SIRT1 in neurodegenerative diseases

A large number of studies have shown that overexpression and activation of SIRT1 are neuroprotective in both acute CNS injuries and chronic neurodegenerative diseases [36]. Three major mechanisms through which SIRT1 affects neurodegenerative disorders have been described: deacetylation of transcription factors, which improves neural plasticity; restoration of protein homeostasis by reducing accumulation of toxic protein aggregates, which is accomplished by direct deacetylation and direct interaction with proteotoxic species [85]; and enhancement of mitochondrial function by reducing oxidative stress and suppressing sustained chronic inflammation. Many neurodegenerative diseases have distinctive clinical manifestations, with specific areas of the CNS being impaired in the various conditions. However, certain features are common to most of them: Many are characterized by irreversibility associated with a progressive clinical course and by an idiopathic degeneration of distinct neuronal populations. Many share key features such as the loss of protein homeostasis in the form of intra- or extracellular accumulation of soluble and insoluble protein aggregates [including amyloid β (A β), hyperphosphorylated tau protein, α -synuclein, and huntingtin]. It has been postulated that genetic factors, epigenetic aberrations, environment, diet, and lifestyle all play significant roles in these disorders.

Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder in humans. It is also considered the leading cause of dementia among the elderly, affecting nearly half of all people over the age of 85 [86, 87]. The pathologic hallmark of the disease is the formation of amyloid plaques. According to the amyloid hypothesis, beta-amyloid (A β) aggregates and forms these extracellular plaques [88]. The plaques are generated from the proteolytic cleavage of the amyloid precursor protein (APP) and considered to be the etiological agent of AD pathology. Both intracellular and extracellular soluble oligomeric forms of A β can in fact initiate synaptic malfunctions and the onset of AD symptoms [89, 90]. In AD, hyperphosphorylated tau protein accumulates in neurofibrillary tangles, which are the pathologic hallmarks of the human disease.

The initial clues about the link between SIRT1 and AD came from studies reporting that resveratrol was able to attenuate A β -induced cell death *in vitro* [91, 92]. Resveratrol reduced A β -induced toxicity in certain cell lines [92] and hippocampal neurons [93], thereby ameliorating AD

pathology. Other studies *in vivo* have shown that resveratrol can also reduce plaque formation in transgenic mouse models of AD [13, 94]. In addition, overexpression of SIRT1 has been described to lower A β levels by reducing its production from APP [95]. It has been documented that SIRT1 deacetylates tau protein in multiple residues, leaving more lysine residues free and enhancing tau polyubiquitination for posterior proteosomal degradation [96]. SIRT1 levels are negatively correlated with the amount of neurofibrillary tangles in AD [97].

Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 1 % of the population over 60 years of age in industrialized countries [98]. The pathologic hallmark of the disease in PD is a significant loss of dopaminergic neurons in the substantia nigra and the accumulation of both α -synuclein and ubiquitin in the form of intracytoplasmic Lewy bodies [99]. Evidence *in vitro* and from animal models indicates that SIRT1 overexpression by genetic means or SIRT1 activation by resveratrol treatment may be protective against PD [100, 101]. SIRT1 deacetylates heat shock factor 1 (HSF1) and PGC-1 α . HSF1 increases the transcription of molecular chaperones, promoting protein folding and decreasing their aggregation, and PGC-1 α protects dopaminergic neurons from cell degeneration [102]. In addition, SIRT1 may also regulate autophagy and mitophagy [103].

Huntington's disease

Huntington's disease (HD) is an autosomal-dominant neurological disorder characterized by cognitive dysfunction, personality changes, and loss of coordination and motor functions [104]. Its etiology involves the expansion of a CAG repeat that affects the conformation and aggregation propensity of the huntingtin protein [105]. In the nervous system, PGC-1 α is required for the induction of enzymes responsible for detoxifying reactive oxygen species (ROS). In HD, mutant huntingtin represses PGC-1 α , which can lead to an exacerbation of neurodegeneration [106]. Genetic models of SIRT1 overexpression have shown that sirtuins can protect against mutant huntingtin toxicity in three different mouse models of HD [107, 108]. SIRT1 deacetylates and activates PGC-1 α directly and enhances the transcription of the PGC-1 α gene via an interaction with MyoD. PGC-1 α is then able to ameliorate the mitochondrial dysfunction and neuronal toxicity induced by mutant huntingtin. Thus, SIRT1 can protect against HD-related neurodegeneration by activating

PGC-1 α and partially preventing mitochondrial impairment. Jeong et al. [107] have demonstrated that deletion of the catalytic exon of SIRT1 exacerbates the disease, whereas overexpression of SIRT1 improves survival and reduces protein aggregation. FOXO3a deacetylation is another SIRT1 target implicated in promoting cell survival in HD models. Parker et al. [109] have suggested that integration of β -catenin, sirtuin, and FOXO signaling provides protection from the early phases of mutant huntingtin toxicity.

SIRT1 in MS and EAE

Multiple sclerosis is the most common neurological disease of the CNS affecting young adults [110]. It is a chronic inflammatory, demyelinating, and neurodegenerative disease of the central nervous system. Although the cause of this disease remains unknown, it is generally accepted that it is the result of immune system activation [111]. Within the past few decades, the large quantity of research done on experimental autoimmune encephalitis (EAE), the most commonly used animal model of MS, has strengthened the hypothesis that MS immunopathology is largely a result of the action of myelin-specific pro-inflammatory T cells on the CNS. There is evidence that B cells, macrophages, antibodies and complement system activation also play a role in the pathogenesis of this complex disorder [112]. Just as in MS, EAE manifests as an autoimmune response against myelin antigens in the CNS that is driven by autoreactive T cells [113], which play a critical role in disease pathogenesis.

Several recent studies using EAE models have provided strong evidence supporting the beneficial effects of the modulation of sirtuins, especially of SIRT1, and its potential usefulness in demyelinating and inflammatory diseases affecting the CNS such as MS [114]. Shindler et al. [115] have tested whether the activators of SIRT1, SRT647, and SRT501 protect mice from optic neuritis in a SJL/J model of EAE. Their study showed that intravitreal injection of SRT647 and SRT501 prevents the loss of retinal ganglion cells but does not prevent inflammation or the clinical signs of EAE. In addition, they showed in this EAE model that activation of SIRT1 can prevent neuronal damage and associated long-term neurological dysfunction [115]. In addition, Prozorovski et al. [116] have examined the influence of redox state on neural precursor cell (NPC) differentiation and found that SIRT1 levels increase in GFAP-positive cells near EAE lesions. Resveratrol has been also used to increase the expression of SIRT1 in EAE models and has been shown to have protective effects. A recent study has shown how SIRT1 activation with resveratrol reduces disease severity in a chronic model of EAE [117]. In a chronic EAE model, resveratrol was shown to delay symptom onset and decrease neuronal loss and

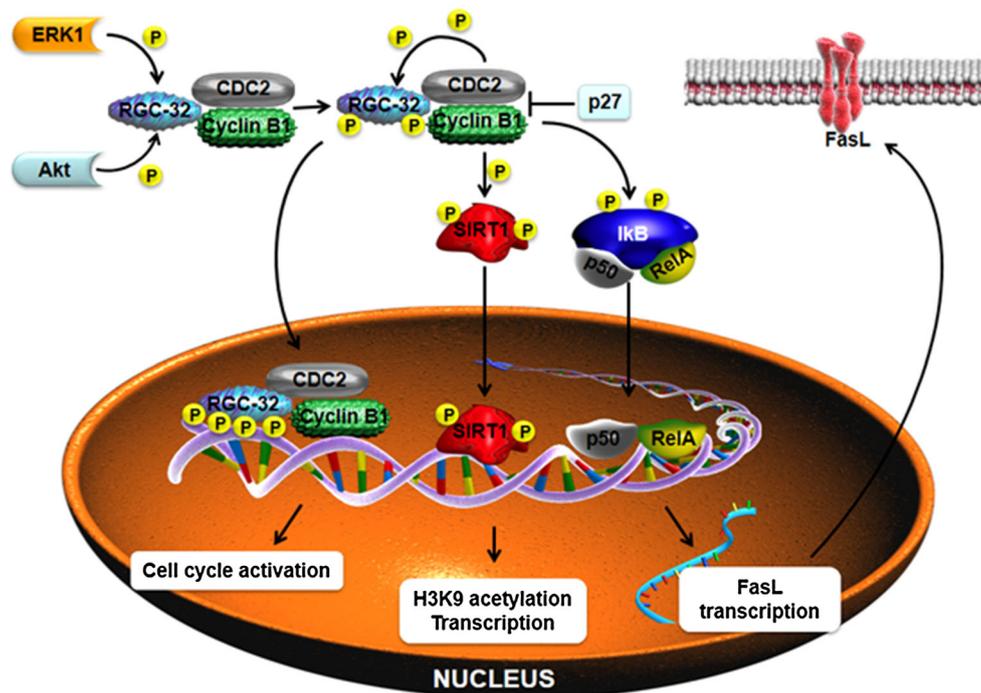


Fig. 2 SIRT1 expression is regulated by RGC-32. Low levels of RGC-32 and SIRT1 have been reported in MS patients with active relapses. SIRT1 expression was significantly reduced after RGC-32 silencing, indicating an important role for RGC-32 in SIRT1 expression. SIRT1, by regulating H3K9 acetylation, also regulates the transcription of target genes. These findings are important because RGC-32 has been found to promote cell cycle activation and survival

paralysis [118]. Other studies have shown the ability of resveratrol to reduce the expression and release of several pro-inflammatory cytokines. Indeed, a study of mice with EAE indicated that induction of SIRT1 activity with resveratrol can decrease the IL-6 and IL-12/23 p40 expression by macrophages [119] but not necessarily by decreasing the development of Th17 or the infiltration of Th17 into the brain. [119]. Resveratrol can also trigger apoptosis in activated T cells and induce a decrease in spinal cord inflammation during EAE [120]. By using SIRT1-overexpressing mice versus wild-type mice, Nimmagadda et al. were able to document the neuroprotective role of SIRT1 in EAE; they showed that its overexpression results in a significant improvement in the clinical score, axon preservation, and neuronal survival, as well as a decrease in inflammation and myelin loss. This protective phenotype appeared to be associated with increased NAD⁺ and brain-derived neurotropic factor (BDNF) levels [121].

To gain additional insight into the role of SIRT1 in MS, a recent study examined its expression in MS brains and in peripheral blood mononuclear cells (PBMCs) obtained from patients with relapsing–remitting MS and compared them to the expression in healthy controls [49]. Since effector T cells migrate into the brain at the time of an MS relapse, the expression of SIRT1 in MS brains in relation to

through the activation of the cdc2/cyclinB1 complex, which also phosphorylates and activates SIRT1. Therefore, these data indicate that regulation of SIRT1 expression is an additional mechanism by which RGC-32 promotes survival. RGC-32 also regulates the expression of FasL, another important mechanism by which RGC-32 regulates survival in PBMCs

that of T cells and macrophages was investigated. SIRT1 was found to be expressed in MS brains by a significant number of perivascular inflammatory cells in both acute and chronic active lesions [49]. In addition, SIRT1 was present on cells in parenchymal areas. These inflammatory cells were present in the entire lesion of acute active cases, whereas the inflammation was restricted to the lesion margins in chronic active lesions. Using double staining, SIRT1 was found to be co-localized with CD4⁺, CD68⁺, oligodendrocytes (OLG), and glial fibrillar acidic protein (GFAP)-positive cells in MS brains, indicating that in addition to inflammatory cells, astrocytes and OLG also express SIRT1. The SIRT1 deposition was not only confined to the MS plaques but was also present in areas of normal adjacent white matter (NAWM) and normal adjacent gray matter (NAGM), indicating a widespread distribution of cells expressing SIRT1. Nevertheless, SIRT1 expression was higher in the MS plaques than in the area adjacent to the MS plaques. SIRT1 was not found to be expressed in normal brain, with the exception of rare neurons in the cortex [49]. These data clearly show that most of the OLG in the MS brain express SIRT1, including those surviving in areas of significant demyelination, indicating that SIRT1 is an important mediator of oligodendrocyte survival in an inflammatory milieu.

SIRT1 mRNA and protein expression in PBMCs were found to be significantly decreased during relapses when compared to the levels in controls and stable MS patients [49]. These data suggest that low levels of SIRT1 can be used a possible biomarker of disease activity in MS patients. SIRT1 is a known regulator of acetylation, methylation, and gene silencing [122]. Acetylation (ac) and methylation (me2) of histone H3 at lysine 9 (H3K9) were assessed in PBMCs by Western blotting. Statistically significantly higher levels of H3K9ac were found during relapses. These changes are in agreement with the described role of SIRT1 in H3K9 acetylation, with increased acetylation being a consequence of the reduced levels of SIRT1 during relapses. The changes in SIRT1 protein expression levels that occurred in the PBMCs from MS patients were positively correlated with the expression of histone H3K9ac and histone H3K9me2 [49]. These changes are consistent with the described role of SIRT1 in H3K9 acetylation, with increased acetylation being a consequence of the reduced levels of SIRT1 during relapses.

Recently, the role of response gene to complement (RGC)-32 in SIRT1 expression was investigated [49]. To investigate whether RGC-32 is required for the expression of SIRT1 mRNA in PBMCs, shRNAs targeting RGC-32 were used to silence its expression. PBMCs were transfected with RGC-32 shRNA lentiviral particles, and the effect on SIRT1 expression was examined. SIRT1 expression was reduced by 50 % after RGC-32 silencing, indicating an important role for RGC-32 in SIRT1 expression. This finding suggests that the expression of SIRT1 is regulated by RGC-32 and confirms previous observations in the SW480 tumor cell line [12, 123]. These data are also consistent with the reported low levels of RGC-32 in MS patients with relapses [124] and have led us to propose that RGC-32 regulates SIRT1 expression and transcriptional activation through H3K9 acetylation (Fig. 2). These findings are important because RGC-32 was found to promote cell cycle activation and survival through the activation of the cdc2/cyclinB1 complex [26, 125], which also phosphorylates and activates SIRT1 [59] (Fig. 2). Therefore, these new data indicate that the regulation of SIRT1 expression is an additional mechanism by which RGC-32 promotes survival. It is also entirely possible that the low levels of RGC-32 expression seen in the PBMCs of MS patients with relapses are responsible for the reduction in SIRT1 expression seen during the active phase of the disease.

To further investigate the relationship between SIRT1 and T cell survival, the effect of the SIRT1 inhibitor sirtinol on apoptosis and FasL expression has been examined. It was found that the expression of FasL was significantly increased, indicating that FasL expression is, at least in part, SIRT1 dependent. In addition, inhibition of SIRT1 led to significant apoptosis in Jurkat cells and in CD4⁺ and CD8⁺ cells from patients with MS.

Conclusions

Experimental studies have demonstrated a protective role for SIRT1 in autoimmune demyelination and neurodegenerative diseases. SIRT1 represents a possible biomarker of MS relapses and a potential new target for therapeutic intervention in MS and neurodegenerative diseases. However, we still have much to learn about the safety and efficacy of sirtuin activators. The use of resveratrol or other activators of SIRT1 should be considered in future clinical trials in order to investigate their potential in preventing MS relapses and promoting neuroprotection and oligodendrocyte survival.

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