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SIRT1 regulation modulates stroke outcome

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Abstract

Silent Information Regulator 1 (SIRT1) is a NAD+ -dependent histone deacetylase that represses gene expression and plays a role in longevity. SIRT1 responds to diverse stress conditions and regulates metabolism in nutrient deficiency conditions, therefore it is involved in adaptive pathways to better fulfill tissue needs in a disturbed environment. SIRT1 overexpression or activation is protective in neurodegenerative diseases. Its role in acute nervous system injury, such as brain ischemia, is emerging but whether SIRT1 activation improves stroke outcome is still a matter of controversy. In the present review, we will document present knowledge about the contribution of SIRT1 in death/survival in cell and animal models of brain ischemia and discuss whether SIRT1 could be a valuable target for therapeutic intervention in human stroke.

Keywords

Sirtuin, histone deacetylase, endogenous neuroprotection, stress protein, cerebral ischemia.

Introduction

Stroke is a cerebrovascular disease leading to death or disability and it has high prevalence among the elderly population. The occlusion of a cerebral artery can cause devastating effects in brain function since neurons are deprived of oxygen and energy resources in minutes. As a consequence of energy depletion, the cells become unable to maintain the ionic gradient across the membrane and excitatory neurotransmitters are released causing neuronal death by excitotoxicity. Following this early phase of necrosis, the formation of free radicals, changes in gene expression, apoptosis and inflammation contribute to the delayed phase of tissue damage. This means that sooner or later, the vulnerable neurons are committed to death. Different degrees of regional blood flow reduction cause the formation of two main territories: the core in which cells die very fast and the penumbra encompassing areas in which neurons cannot sustain their electrical activity but maintain their membrane ionic gradient. Neurons in the latter situation can be rescued if appropriate blood reperfusion occurs.

Yet, spontaneous recovery is not unusual and may be related to epigenetic mechanisms. The phenomenon of protection after sublethal ischemia is known as ischemic preconditioning (IPC) and develops brain tolerance to a second severe ischemic event. In animal models, preconditioning studies provided clues to identify emergency pathways that are turned on to save the damaged tissue [1]. Also, some studies in humans support that transient ischemic attacks in patients can offer some protection against severe stroke [2, 3]. Therefore, it is worth investigating the endogenous defense pathways and generating tools to arouse them in order to develop efficient therapeutic strategies. Among the potential cell rescuers, sirtuins in general and SIRT1 in particular warrant a special interest, as they can modulate gene expression and adapt cell metabolism to new requirements in response to the tremendous cellular stress produced by cerebral ischemia.

SIRT1 is the mammalian homolog of yeast Sir2 and its role in expanding lifespan was first described in this organism, then in *Caenorhabditis elegans* and in *Drosophila*. Though, SIRT1 did not increase lifespan in mammals, transgenic mice with moderate increased levels of SIRT1 age healthier than wild-type counterparts with a lower risk to develop cancer and metabolic syndrome, and they present reduced levels of aging markers [4].

SIRT1 function in peripheral organs has been extensively studied and can be consulted in other reviews [5].

Severe brain nutrient depletion after stroke makes the SIRT1 system an excellent candidate for modulation in view of therapeutic intervention. Here, we will briefly outline the functions of SIRT1 that may be more relevant to stroke and then sum up the studies that tried to unveil the putative contribution of SIRT1 in post-ischemic neuronal fate.

Description/enzymatic activity

SIRT1 is a member of the sirtuin enzyme family comprising 7 proteins in mammals, SIRT1-7, and it belongs to the class III histone deacetylase (HDAC). The sirtuins are distributed in different cellular compartments: nucleus, cytoplasm and mitochondria according to their substrates and functions [6]. Excepting SIRT4, which is an ADP-ribosyltransferase, all sirtuins are deacetylases. Conversely to class I and class II HDAC, the sirtuins require NAD+ as a

co-substrate. SIRT1 hydrolyzes NAD+ to remove the acetyl group of substrates with acetyl-lysine to generate the deacetylated substrate, nicotinamide (NAM) and 2'-*O*-Acetyl-ADP-Ribose (Figure 1). Nicotinamide phosphoribosyltransferase (Nampt) then converts NAM to nicotinamide mononucleotide (NMN), which in turn generates NAD, a reaction catalyzed by NMN adenylyl transferase (Nmnat). NAM is an inhibitor of all sirtuins and trichostatin A (TSA) is a general inhibitor of class I and class II HDAC [5, 7]. Of special note, Nampt is the limiting enzyme in the salvage pathway to generate NAD, therefore changes in NAD+ availability due to imbalance in the NAD/NADH ratio, Nampt dysfunction or alteration in the energetic state of the cell will condition SIRT1 activity.

Subcellular localization

SIRT1 is mainly localized in the nucleus, where it deacetylates histones and transcription factors, but it can also be present in the cytoplasm under certain conditions. For instance, differentiation leads to changes in SIRT1 distribution. In embryonic cardiomyoblasts, SIRT1 is predominantly nuclear, whereas in differentiated cardiomyocytes, SIRT1 is present in the cytoplasm. Studies in normal and transformed fibroblast, lung and prostate cells showed that while SIRT1 is localized in the nucleus of normal cells, it is predominantly cytoplasmic in cancer cells [8]. The activation of the IGF1/PI3K pathway leads to the accumulation of SIRT1 in the cytoplasm.

Oxidative stress-induced apoptosis in myocytes leads to the nucleo-cytoplasmic shuttling of SIRT1 and overexpression of nuclear but not cytoplasmic SIRT1 is anti-apoptotic [9, 10]. Nuclear targeting of SIRT1 is dependent of Akt [9] and SIRT1 activity seems to be affected by its phosphorylation state. Site-directed mutagenesis demonstrated that SIRT1 contains two nuclear localization signal and two nuclear export signal sequences [9].

Function

Acetylation is a post-translational modification that similarly to phosphorylation, SUMOylation or ubiquitination regulates protein function [11]. Histones, but also transcription factors and cytoplasmic proteins undergo lysine acetylation. SIRT1 was initially described as a histone deacetylase, that could promote the formation of repressive chromatin [12]. Histone acetylation loosens the chromatin structure and facilitates transcription, whereas histone deacetylation by SIRT1 and other HDACs promotes chromatin compaction hampering the accession of transcription factors to some promoter binding sites, thereby conveying a general gene silencing. SIRT1 also deacetylates non-histone proteins, mainly transcription factors such as forkhead box (FOXO), p53, peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and p65 [13]. Acetylation of the tumor repressor transcription factor p53 stabilizes it, whereas deacetylation of the human Lys382 residue of p53 by SIRT1 precludes p53 nuclear translocation and the transcription-dependent pro-apoptotic effect of p53 [14].

SIRT1 is ubiquitous and its pivotal role in the regulation of lipids and glucose metabolism through deacetylation of PGC-1α, as well as its involvement in apoptosis, embryogenesis and inflammation has been extensively studied in peripheral organs. However, the role of SIRT1 in the brain requires deep exploration. Below, we recapitulate some of the SIRT1 functions (Figure 2) that may have a significant impact in the infarcted brain.

SIRT1 in the stress response

A good evidence demonstrating SIRT1 involvement in the adaptive response to stress conditions relies on calorie restriction experiments, in which starvation leads to a decrease in the NAD+/NADH ratio and SIRT1 activation [15]. Calorie restriction (CR) constitutes a mild stress that conveys several benefits to the whole body. Indeed, CR turns on fatty acid oxidation, regulates glucose homeostasis and induces mitochondrial biogenesis to produce energy in a nitric oxide-dependent manner [5]. Animals deficient in SIRT1 do not bear full adaptive changes when subjected to calorie restriction [15], whereas SIRT1 overexpression mimicks some aspects of the CR response such as the improvement of glucose tolerance, reduced blood levels of insulin, glucose and cholesterol, and enhanced oxygen consumption [16]. Apart from the metabolic aspect, CR induces the expression of chaperones and neuroprotective factors that help cells to cope with lethal injuries, such as stroke. Indeed, a straightforward study demonstrated that intermittent feeding triggers the expression of brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), heat shock protein 70 (Hsp70), the anti-oxidant heme oxygenase-1 (HO-1) and glucose regulated protein (Grp78) and reduces infarct volume in young mice subjected to transient middle cerebral artery occlusion (tMCAO) [17]. Resveratrol (RSV), a polyphenol present in grape skin and red wine was identified as an activator of SIRT1 that turns on energy production and exhibits anti-oxidant, anti-apoptotic and anti-inflammatory properties [18]. Later on Dasgupta et al. [19] among others demonstrated that stimulation of SIRT1 by resveratrol was indirect and occurred through the activation of AMPK [19]. AMPK is an energy sensor that is activated by phosphorylation when the AMP/ATP ratio is low. In these conditions, AMPK promotes the generation of NAD+ to fuel the production of ATP. The resultant NAD+ increase enhances SIRT1 activity [20].

Anti-oxidant and anti-apoptotic properties of SIRT1

The anti-oxidant properties of SIRT1 rely basically on targeting the FOXO transcription factors. FOXO3 and FOXO4 are involved in apoptotic processes but SIRT1 overexpression modifies their activity and subcellular localization. For instance, FOXO3 is deacetylated by SIRT1 in neurons exposed to hydrogen peroxide [21] and deacetylated FOXO3a induces the expression of catalase and MnSOD that reduce radical oxygen species [22].

SIRT1 targets several transcription factors: p53, p65, HIF-1α, HIF-2α, that participate in pro- or anti-apoptosis pathways [6]. Deacetylation of p53 by SIRT1 precludes death in cultured neurons challenged with camptothecin, a DNA damaging agent [23] and pharmacological activation of SIRT1 with RSV increases expression of Bcl-2 and Bcl-$_{XL}$ [24, 25]. In the same line, overexpression of SIRT1 prevents apoptosis induced by low potassium in cerebellar granule neurons [26].

Anti-inflammatory effect of SIRT1

SIRT1 reduces inflammation in many tissues, such as lung and adipose tissue and in autoimmune diseases (reviewed in [27, 28]). Yeung et al. [29] demonstrated for the first time that SIRT1 deacetylated p65 and regulated the transcriptional activity of NFκB. In RAW 264.7 macrophages, RSV inhibits LPS-induced production of nitric oxide, TNF-α and prostaglandin E2 through Akt activation, AMPK phosphorylation and SIRT1 upregulation [30]. In a model of inflammation in mice, activators of SIRT1 abrogated NFκB-mediated transcription and attenuated LPS-induced production of the pro-inflammatory cytokines TNF-α and IL-12 [31]. AP-1 is another substrate of SIRT1 and overexpression of the latter reduced the expression of Cox-2 a target gene of AP-1 [28].

SIRT1 in the normal and aging brain, and in neurodegenerative diseases

In the normal brain, SIRT1 participates to the control of circadian rhythms that can be modified according to changes in the diet [32] and is involved in neuronal differentiation [33]. SIRT1 is abundant in neurons from areas such as hypothalamus that sense fluctuations in nutrients levels and regulate the metabolism, as determined by *in situ* hybridization. In addition, high mRNA expression was detected in the cerebellum, the hippocampus and the piriform cortex [34].

Aging is the major risk factor for neurodegenerative disorders. Since on the one hand SIRT1 levels and activity decrease with aging and on the other hand SIRT1 slows down cellular senescence [35, 5], extensive research has focused on SIRT1 and a great effort was dedicated to design molecules that increase SIRT1 activity. The beneficial effect of sirtuins in neurodegenerative pathologies has been reviewed in several papers [36, 37]. Among others, SIRT1 is able to improve neuronal survival in brain disorders involving protein aggregates and axon dysfunction.

Alzheimer disease (AD).

In transgenic mice that develop Aβ plaques and learning and memory deficits, overexpression of SIRT1 in the brain, results in lower production of toxic Aβ and increased activity of α-secretase indicating that SIRT1 induces a shift of APP processing, thereby preventing the formation of amyloidogenic peptides [38] and also improves behavioural deficits. The deacetylation of the retinoic acid receptor β by SIRT1 underlies the enhanced transcription of the gene encoding for α-secretase. Tau hyperphosphorylation and accumulation is another hallmark of AD. Acetylation of tau was shown to increase its stability and to prime its phosphorylation. SIRT1-mediated tau deacetylation promotes the degradation of phosphorylated tau [39].

Huntington disease

Parker et al. [40] showed for the first time that SIRT1 overexpression decreased the toxicity of a mutant huntingtin with 128 polyQ repeats in C. elegans and RSV reproduced this effect in neurons derived from HdhQ111 expressing mice. Two independent studies [41, 42] using different mice models of HD nicely showed that SIRT1 deficiency brought forward the onset of the disease, whereas SIRT1 overexpression decreased the toxicity of the mutant hungtintin, reduced brain atrophy and compensated the BDNF deficit, a typical feature of HD [41]. SIRT1 deacetylated TORC1, a coactivator of CREB, thereby increasing the binding of the CREB-TORC1 complex to the BDNF promoter and potentiated BDNF transcription [41]. In addition, SIRT-1 neuroprotection is also dependent of FOXO3a deacetylation [42].

Parkinson disease

A transgenic mouse that expresses the human α-synuclein with the A53T mutant responsible for early-onset Parkinson disease is a model of synucleopathy. This animal model develops α-synuclein aggregates but no loss of dopaminergic neurons. When SIRT1 is overexpressed, life span was extended and less aggregates were formed, whereas deficiency of SIRT1 had the opposite effect. In cultured cells, the mutant α-synuclein induced acetylation of the heat shock factor 1 (HSF-1) transcription factor and high levels of SIRT1 deacetylated HSF-1 and induced the expression of the chaperone Hsp70 only in mutant cells. Silencing of either SIRT1 or hsp70 enhanced cell death [43].

Conversely, in MPP+ injected mice, overexpression of neuronal SIRT1 had no effect on nigral neuron loss [44] and in SH-SY5Y challenged with MPP+, SIRT1 silencing reduced cell death [45]. Therefore, results are still contradictory to draw conclusions about the benefit of SIRT1 in Parkinson disease models.

SIRT1 in stroke models (*in vivo* and *in vitro*)

Acetylation/deacetylation after stroke

Several stress stimuli alter the acetylation status of proteins thereby modifying their activation state [6]. Over-deacetylation of histones occurs after brain ischemia mainly due to a global decrease in histone acetyltransferase activity (HAT) because the energy resources are deficient and less acetyl-CoA is produced [46, 47]. Deacetylation has also been described after excitotoxicity in cortical neurons [48]. To circumvent low lysine-acetylation, specific or broad spectrum HDAC inhibitors were employed in cell and animal models of ischemia and most of the studies showed a protective effect of these inhibitors mediated among others by the induction of chaperones [7, 49, 50, 24]. However, Lanzillotta et al. [24] pointed that general lysine acetylation is not a warranty for neuroprotection. For instance, the combined administration of MS-275, an HDAC1-3 inhibitor, with RSV reduced post-ischemic brain injury. In this case, the deacetylation of the lysine residue 310 of NFκB p65 by SIRT1 controlled NFκB transcriptional activity [24, 51].

Changes in SIRT1 expression after stroke

Transient MCAO that causes extended damage in the cortex and the striatum, as well as prolonged oxygen and glucose deprivation (OGD) and excitotoxicity reduce SIRT1 expression [52, 25]. In contrast, short OGD and moderate ischemic insults do not modify SIRT1 protein levels [53]. However, all these lethal stimuli (mild or severe) lead to an early reduction in intracellular NAD+ levels and this will impair SIRT1 and PARP-1 activities.

Regulation of SIRT1 activity/levels

Perez-Pinzon's group was the first one to describe the positive effect of SIRT1 in cerebral ischemia/IPC models. As indicated earlier, IPC is highly protective. Among the different neuroprotective mediators, SIRT1 was shown to contribute to the beneficial effect of different PC treatments [13]. For instance, ischemic or hyperbaric oxygen (HBO) preconditioning reduced cell death induced by tMCAO and OGD in cortical neurons [25, 53] and the protective effect was abolished by pre-treatment with the SIRT1 inhibitors sirtinol or EX527. Similarly, the pro-survival effects of ischemic preconditioning in organotypic hippocampal cultures exposed to OGD or IPC before cardiac arrest were impaired by sirtinol [54, 55].

The administration of RSV by different ways (i.p., i.c.v., or p.o.) daily for 3 days, 15 days before tMCAO [25, 53], or at the beginning of the reperfusion [24] in a tMCAO model reduced the infarct volume and improved the neurological score. In a model of global ischemia, RSV preconditioning for 48h before cardiac arrest prevented the neurodegeneration of the CA1 hippocampal neurons [55]. RSV applied 3h before OGD in cortical neurons [53] or 48h prior to OGD in organotypic hippocampal cultures mimicked the protective effects of preconditioning [54]. Moreover, sirtinol or EX-527 abrogated the protective effect of RSV [54, 25, 53].

The piece of evidence confirming the prominent role of SIRT1 in post-ischemic survival was provided recently by targeting SIRT1 expression. Indeed, overexpression of SIRT1 with viral vectors and SIRT1 silencing with siRNA

demonstrated that changes in the SIRT1 protein levels significantly influenced post-ischemic recovery [25, 53]. SIRT1 silencing blocked the neuroprotective effect of HBO preconditioning and of RSV [25, 53].

However, two studies are in disagreement with a benefit of SIRT1 stimulation in stroke models. Liu et al. [52] reported that nicotinamide and sirtinol prevented cell death induced by tMCAO and excitotoxicity in cortical neurons. The authors of the study argued that enhanced SIRT1 activity may exacerbate the NAD+ depletion induced by the injuries. However, nicotinamide is an inhibitor of all the sirtuins and also a NAD+ precursor. Therefore this compound is not the most appropriate to discriminate between the effects of SIRT inhibition and those of NAD+ production. NAD supply was shown to prevent excitotoxic and ischemic damage [56, 48].

Furthermore, transgenic mice overexpressing human SIRT1 under the control of a neuron specific promoter were not protected against ischemic injury induced by tMCAO and presented memory deficit [44].

Mechanism of action

SIRT1 beneficial to stroke

Though most of the studies concluded that SIRT1 is advantageous in stroke, the underlying molecular mechanisms are still poorly understood. The demonstrated effects of SIRT1 are recapitulated below.

RSV preconditioning reduced the levels of the mitochondrial uncoupling protein 2 (UCP-2) and increased mitochondrial oxidative phosphorylation [55]. SIRT1 binds to the UCP-2 promoter and regulates UCP-2 transcription [57]. UCP-2 decreases the mitochondrial membrane potential and interferes with ATP production. SIRT1 is involved in the RSV effect since sirtinol restores UCP-2 expression and abolishes the protection afforded by RSV.

Lanzillotta et al. [51] discovered that ischemia leads to disturbances in NFκB acetylation. Particularly, the lysine residue K310 of p65, which is a SIRT1 substrate, was acetylated after tMCAO, in spite that this condition induced general deacetylation at other residues of p65. Under this specific lysine acetylated form, p50/p65 dimers induced the expression of the pro-apoptotic protein Bim. The combined use of MS-275, an inhibitor of HDAC1-3 and RSV promoted the acetylation of the H3 histone and the deacetylation of p65 K310. This status switches the NFκB binding to the Bim promoter for the Bcl-$_{XL}$ promoter, thereby regulating the transcription of two proteins inversely involved in apoptosis in favor of a pro-survival outcome [24]. In line with these data, the overexpression of SIRT1 prevented the loss of Bcl-2 expression and caspase-3 cleavage induced by OGD in cortical neurons [25].

Ischemia leads to NAD+ depletion, an effect reversed by IPC and RSV [53]. Increased levels of NAD+ activate SIRT1 that, among other actions, preserves the axons from injury in the Wallerian degeneration slow (wlds) mice [58] and promotes axogenesis through Akt deacetylation and activation [59]. Though NAD+ is indispensable for SIRT1 catalytic activity, it is also a substrate for the six additional sirtuins and other enzymes: mono-ADP-ribose transferases, poly ADP-ribose polymerases, cyclic-ADP-ribose synthases [60]. Therefore, only silencing or pharmacological blockade of SIRT1 can discriminate between SIRT1-specific effects and the broad effects of NAD supply.

It is certainly worth pursuing the exploration of SIRT1 mechanism of action in the ischemic brain.

SIRT1 was shown to mediate the neuroprotective effect of Nampt. As indicated earlier, Nampt is the rate-limiting enzyme in the salvage pathway to synthesize NAD+ in brain [60]. However, Nampt was found in neurons and astrocytes in culture and tMCAO in mice upregulated it at 24h reperfusion and 12h after OGD [61]. Nampt

overexpression dramatically reduced infarct volume and OGD toxicity in a SIRT1-dependent manner. In this case, SIRT1 activates AMPK and the protective effect of Nampt is abolished by SIRT1 and AMPKα-2 silencing [61]. SIRT1 was involved in the activation of autophagy promoted by Nampt overexpression [62]. Furthermore, overexpression of SIRT1 activated autophagy and prevented prion toxicity in cultured neurons [63].

Autophagy can also be triggered by the blockade of the mammalian target of rapamycin C1 (mTORC1), a target of SIRT1 and another important master regulator of cell metabolism. SIRT1 overexpression in cultured cortical and hippocampal neurons abrogated the phosphorylation and subsequent activation of mTORC1, promoted neurite outgrowth and prevented neuronal death induced by nutrient deprivation [64]. Rapamycin mimicked the effect of SIRT1 and reverted the impairment of neurite outgrowth in neurons transfected with SIRT1 siRNA. Therefore, SIRT1 seems to be located at the crossroad of multiple metabolic pathways with mutual interactions and it also promotes organelle recycling.

SIRT1 deacetylates the DNA repair protein Ku70 that sequesters Bax, thereby blocking its pro-apoptotic promoting effect. This effect was demonstrated in the mechanism underlying polyglutamine toxicity in neuroblastoma [65]. Ku70 is reduced after tMCAO [66] but whether reduced levels of SIRT1 after cerebral ischemia impair DNA repair by maintaining Ku70 acetylation has not been explored. Ischemia triggers apoptosis by the intrinsic and extrinsic pathways [67]. The tumor suppressor transcription factor p53 is upregulated after cerebral ischemia and its inhibition reduces apoptosis [68, 69]. Changes in acetylated p53 after treatment RSV or SIRT1 overexpression have not been reported so far.

Hypoxia and cerebral ischemia induce the stabilization of the transcription factor HIF-1α and the subsequent expression of genes involved in neuroprotection and angiogenesis [70]. Changes in acetylation and transcriptional activity of HIF-1 have not been explored in cerebral ischemia and the relationship between SIRT1 and HIF1 certainly deserves interest, since target genes of HIF1, such as the vascular endothelial growth factor (VEGF) play a role in neurovascular plasticity after ischemic stroke [71]. SIRT1 interacts with and deacetylates HIF-1α in cancer cells subjected to hypoxia, however there is a discrepancy about the transcriptional efficiency of the acetylated HIF-1. In cultured human fibrosarcoma cells hypoxia caused the loss of NAD+, the slow decline in SIRT1 and the induction and acetylation of HIF-1α with the enhanced expression of VEGF [72]. Conversely, in a hepatocellular carcinoma cell line, hypoxia led to the stabilization of HIF-1α but did not alter SIRT1 expression, and the knockdown or inhibition of SIRT1 led to HIF-1α acetylation but reduced the accumulation and transcriptional activity of HIF-1α induced by hypoxia [73]. Therefore the regulation of the transcriptional activity of HIF-1α by SIRT1 under ischemic conditions is complex and seems to depend on the cell type and on the metabolic and redox state of the cell.

Potential detrimental effects of SIRT1 in stroke

Detrimental effects of SIRT1 in the brain have also been reported.

Among the undesirable effects of exacerbated SIRT1 functioning in the post-ischemic brain, SIRT1 may impair the transcription of protective genes such as *grp78* and *gadd34* through deacetylation of XBP-1, a transcription factor activated during the unfolded protein response (UPR) following endoplasmic reticulum stress [74]. In this paper, it was shown that SIRT1-/- MEF were more resistant to ER stress than SIRT1+/+ MEF.

As indicated earlier, SIRT1 binds TSC2 that inhibits mTOR, which in turn phosphorylates several proteins involved in the control of global protein synthesis, such as p70S6 kinase and the eukaryotic translation factor 4E-BP. The

phosphorylation of 4E-BP1 impedes the sequestration of eIF4E and enables global protein synthesis. Sustained SIRT1 inhibition or SIRT1 deletion increased the protein synthesis rate [75], an action extremely significant for neuronal survival. Indeed, ischemia causes the transient inhibition of translation, partly due to changes in the phosphorylation of several translation factors (eIF2α and 4E-BP). The recovery of protein synthesis during the reperfusion is essential to neuron survival whereas the persistent blockage of translation is lethal [76-78].

SIRT1 inhibition with NAM or sirtinol, as well as SIRT1 silencing, prevented cell death in cultured neurons subjected to oxidative stress [79] through reducing the IGF-1/Erk1/2 signaling pathway. Surprisingly, the same treatments increased the resistance of differentiated neurons challenged with compounds that induce ER stress or DNA damage, by promoting IGF-1 secretion and subsequent activation of the Akt and Erk1/2 pro-survival pathways [80].

Points to discuss

Enzymatic versus non-enzymatic activity of SIRT1

Many of the approaches in the studies that tackled the neuroprotective effect of SIRT1 are based on the use of pharmacological compounds that activate or inhibit the deacetylase activity. The non-catalytic effects of SIRT1 have hardly been explored but they certainly warrant further investigation. A clear example of it was provided by a study in which overexpression of SIRT1 blocked apoptosis induced by low potassium in cerebellar granule cells [26]. The overexpression of two mutants without catalytic activity reproduced the protective effects of full-length SIRT1 whereas pharmacological activators and inhibitors of SIRT1 were unable to modify SIRT1 effect [26]. SIRT1 interacts with TSC2 and reduces mTOR phosphorylation. However, acetylated TSC2 has not been detected [75]. Therefore, some deacetylase-independent SIRT1 functions remain to be unveiled to explain some unexpected observations or possible discrepancies between the effects of the regulation of SIRT1 enzymatic activity and changes in SIRT1 protein levels.

SIRT1 regulation

Milner [81] recently reviewed the mechanisms involved in the modifications of SIRT1 expression and activity. So additional questions about the regulation of SIRT1 have to be posted in the context of stroke:

- AROS and DBC-1 are the physiological activator and inhibitor of SIRT1 respectively. Whether these SIRT1 regulators are involved in changes in SIRT1 levels/activity after stroke is currently unknown.
- The SUMOylation of human SIRT1 regulates its enzymatic activity [81]. However, it has not been reported whether SIRT1 undergoes post-translational modifications (phosphorylation, SUMOylation) or proteolytic cleavage that affect its activity after stroke.
- It is not known either whether glial SIRT1 plays a role in ischemic damage.
- SIRT1 levels decrease after an ischemic insult. Therefore it is quite surprising that the indirect stimulation of residual levels of the enzyme with RSV have such a potent effect on survival, unless the enzyme is modified by the stress, a possibility that deserves further studies. Another explanation would be that RSV supports many SIRT1-independent benefits. In the same line of reasoning, whether SIRT1 can function properly when NAD+ levels are depleted needs to be unraveled.

SIRT1 polymorphism in humans

Two binding sequences for p53 are present in the SIRT1 promoter. The sequence most proximal to the initiation codon represses SIRT1 transcription while the second consensus sequence stimulates SIRT1 expression under nutrient deficiency. Interestingly, the latter has a single nucleotide polymorphism (SNP) C/T. The C allele significantly diminishes the promoter activity in nutrient depletion conditions [82]. In humans, 5 different SIRT1 SNP have been reported so far but no association between a specific allele and exceptional longevity was observed [83, 84]. Whether these polymorphisms influence stroke risk or stroke outcome has not been investigated.

Concluding remarks

Despite the fact that most of the reports point to a benefit of targeting SIRT1 to promote neuronal survival, there is a debate on whether it is important to increase SIRT1 level, to stimulate its activity, or whether SIRT1 activity should be blocked since it exacerbates energy depletion. Additional studies overexpressing/suppressing SIRT1 expression/activity in the different brain cell types are necessary to draw more convincing conclusions.

Since the energy status of the brain is critical after stroke, combined therapies stimulating SIRT1 activity and simultaneously either supporting energy supply or lowering energy demand deserve exploration as strategies to enhance the putative beneficial effects of SIRT1 in stroke while preventing its possible deleterious actions.

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Compliance with Ethics Requirements

Valérie Petegnief declares that she has no conflict of interest. Anna M. Planas declares that she has no conflict of interest.

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Fig 1. SIRT1 enzymatic activity

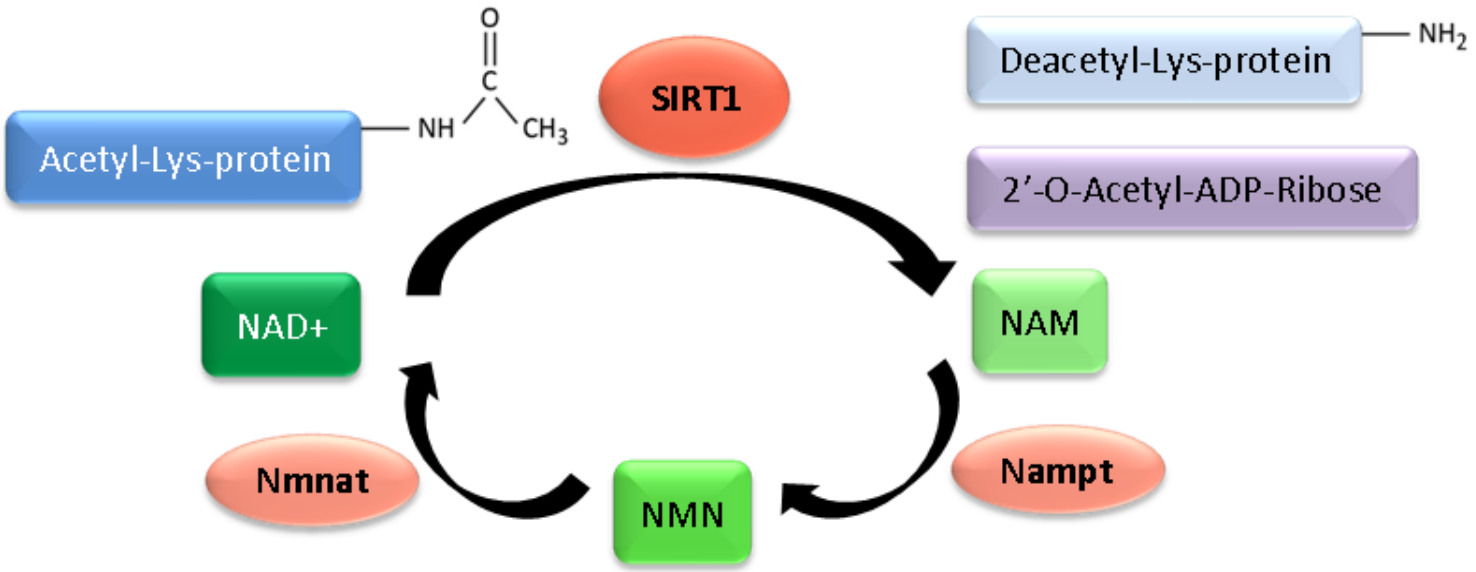

Figure 1. Enzymatic reaction catalyzed by SIRT1. SIRT1 hydrolyzes NAD+ to remove the acetyl group from acetylated lysine substrates and generates nicotinamide (NAM) and 2'-O-Acetyl-ADP Ribose. NAM is converted back to NAD+ in a two-step reaction catalyzed by Nampt and Nmnat.

Fig 2. SIRT1 main functions

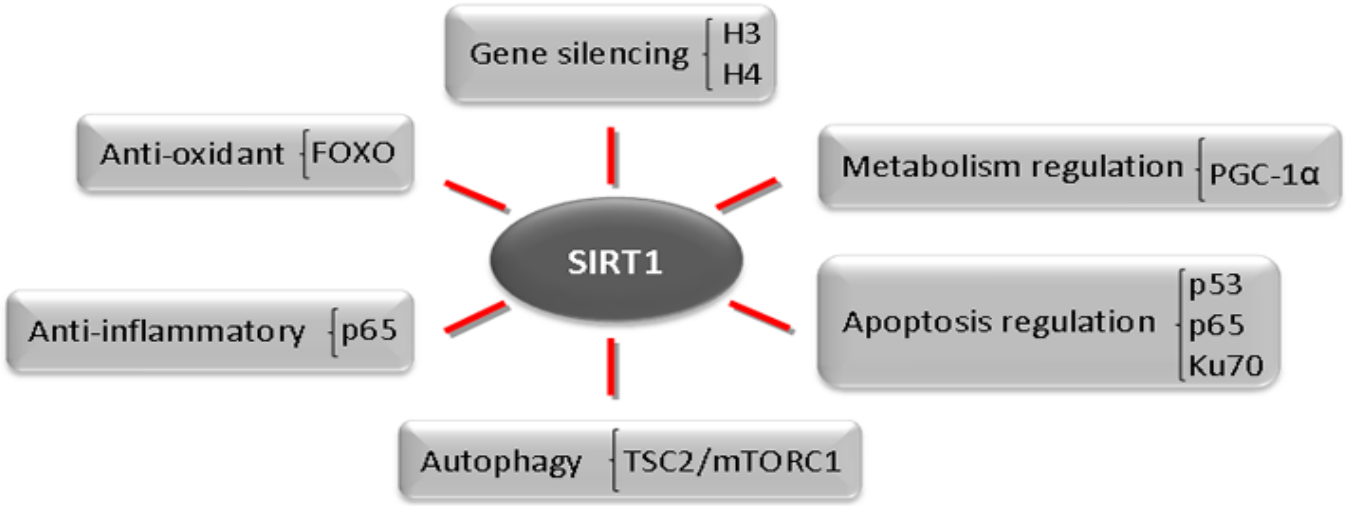

Figure 2. SIRT1 through its interaction with multiple targets (histones, transcription factors and cytoplasmic proteins) is involved in the regulation of gene expression, metabolism, apoptosis, autophagy, inflammation and oxidative stress.