SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer’s disease and amyotrophic lateral sclerosis

Dohoon Kim1,2,7, Minh Dang Nguyen3,7,8, Matthew M Dobbin1, Andre Fischer1,9, Farahnaz Sananbenesi1,3,9, Joseph T Rodgers4,5, Ivana Delalle1, Joseph A Baur6, Guangchao Sui3, Sean M Armour6, Pere Puigserver4,5, David A Sinclair6,* and Li-Huei Tsai1,*

1Howard Hughes Medical Institute, Picower Institute for Learning and Memory, Riken-MIT Neuroscience Research Center, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Boston, MA, USA, 2Division of Medical Sciences, Harvard Medical School, Boston, MA, USA, 3Department of Pathology, Harvard Medical School, Boston, MA, USA, 4Dana Farber Cancer Institute and Department of Cell Biology, Harvard Medical School, Boston, MA, USA, 5Department of Cell Biology, Johns Hopkins University School of Medicine, Boston, MA, USA and 6Department of Pathology and Paul F Glenn Laboratories for the Biological Mechanisms of Aging, Harvard Medical School, Boston, MA, USA

A progressive loss of neurons with age underlies a variety of debilitating neurological disorders, including Alzheimer’s disease (AD) and amyotrophic lateral sclerosis (ALS), yet few effective treatments are currently available. The SIR2 gene promotes longevity in a variety of organisms and may underlie the health benefits of caloric restriction, a diet that delays aging and neurodegeneration in mammals. Here, we report that a human homologue of SIR2, SIRT1, is upregulated in mouse models for AD, ALS and in primary neurons challenged with neurotoxic insults. In cell-based models for AD/tauopathies and ALS, SIRT1 and resveratrol, a SIRT1-activating molecule, both promote neuronal survival. In the inducible p25 transgenic mouse, a model of AD and tauopathies, resveratrol prevented learning impairment, and decreased the acetylation of the known SIRT1 substrates PGC-1alpha and p53. Furthermore, injection of SIRT1 lentivirus in the hippocampus of p25 transgenic mice conferred significant protection against neurodegeneration. Thus, SIRT1 constitutes a unique molecular link between aging and human neurodegenerative disorders and provides a promising avenue for therapeutic intervention.

The EMBO Journal (2007) 26, 3169–3179. doi:10.1038/sj.emboj.7601758; Published online 21 June 2007

Introduction

Although neurodegenerative disorders are relatively cell type specific, many of the underlying pathogenic processes are similar, including protein misfolding, oxidative stress, cytoskeletal abnormalities, disruption of calcium homeostasis, and inflammation, all of which increase during aging (Bossy-Wetzel et al, 2004; Forman et al, 2004; Selkoe, 2004). The existence of related mechanisms underlying neurodegeneration raises the possibility of developing a class of therapeutic interventions that treat a variety of neurological disorders by activating the body’s own defenses against age-related deterioration and cell death (Bossy-Wetzel et al, 2004; Forman et al, 2004; Selkoe, 2004). Studies from yeast identified the evolutionarily conserved NAD+-dependent deacetylase Sir2 as a critical regulator of the aging process (Kaehberlein et al, 1999; Imai et al, 2000; Anderson et al, 2003a,b; Howitz et al, 2003; Cohen et al, 2004b). An additional copy of the Sir2 gene extends lifespan in yeast and metazoans by a process seemingly analogous to caloric restriction (Lin et al, 2000; Anderson et al, 2003a,b), a diet that delays diseases of aging in mammals including neurodegeneration (Luo et al, 2001; Vaziri et al, 2001; Langley et al, 2002; Howitz et al, 2003; Brunet et al, 2004; Cohen et al, 2004a,b; Motta et al, 2004; Qin et al, 2006). Mammals possess seven Sir2 homologues (SIRT1-7) whose biological functions remain poorly defined. The SIRT1 gene is believed to provide cell protection during times of cell stress (Brunet et al, 2004; Cohen et al, 2004a,b; Chen et al, 2005; Tang, 2006). Consistent with this, knockdown of the SIRT1 gene in cultured mouse dorsal root ganglion sensory neurons abrogates the protective effects of increased NAD+ synthesis on axonal degeneration following acute axotomy (Araki et al, 2004). On the other hand, a recent study suggests that SIRT1 is not required for NAD+-dependent protection, rendering the role of SIRT1 in peripheral axotomy unclear (Wang et al, 2005). Furthermore, Sir2 seems to block extreme lifespan in post-mitotic cells in yeast, raising the possibility that SIRT1 may play a dual role in the CNS (Fabrizio et al, 2005). Most importantly, the role of SIRT1 in vivo in age-dependent chronic neurodegenerative disorders remains undefined.
Results

Levels of SIRT1 in models of neurodegeneration

We hypothesized that SIRT1 levels may increase as a protective response to neurodegenerative conditions and examined levels of SIRT1 in various mouse models for human age-dependent neurodegeneration. Mice inductively overexpressing a toxic coactivator of cyclin-dependent kinase 5 (CDK5), p25, display massive degeneration of forebrain with features of AD (Cruz et al., 2003, 2006), whereas transgenic mice expressing a mutant form of superoxide dismutase 1 (SOD1G37R), which has been linked to human amyotrophic lateral sclerosis (ALS), exhibit severe motor neuron and axon degeneration in spinal cord (Gurney et al., 1994; Wong et al., 1995). Interestingly, in the forebrains of p25 transgenic mice (n = 9), SIRT1 protein levels increased as early as 2 weeks after p25 induction and persisted throughout the progression of the pathology to 12 weeks (Figure 1A and B). In accordance with increased protein levels of SIRT1, there was a decrease in the acetylation state of PGC-1alpha, a target for SIRT1 deacetylase activity (Nemoto et al., 2005; Rodgers et al., 2005; St-Pierre et al., 2006) (Figure 1C). In the spinal cords of mutant SOD1G37R mice, SIRT1 was only slightly upregulated at 4 months (n = 4), a stage with limited degeneration; however, levels of SIRT1 were significantly upregulated when severe neurodegeneration was evident at 10–12 months (n = 8) (Nguyen et al., 2001) (Figure 1D and E). Mice expressing a mutant form of amyloid precursor protein (APP) linked to Familial AD (PDAPP-V717F, n = 7; 2–12 months) (Games et al., 1995) do not exhibit significant neuronal loss, although they display, in an age-dependent manner, substantial β-amyloid plaques, a hallmark of AD (Games et al., 1995). These mice showed no significant increase in SIRT1 in the forebrain (Supplementary Figure 1). Together, these results indicate that SIRT1 levels correlate with neurodegeneration accompanied by progressive and severe loss of neurons, but not with β-amyloid plaque pathology in the absence of neuronal loss. In all the mouse models analyzed, as well as in human brains, SIRT1 is not only enriched in the nucleus but also localized in the cytoplasm (Supplementary Figure 2; unpublished data).

Since p25 and mutant SOD1 trigger disruption of calcium homeostasis and generate oxidative stress (Bruinj et al., 2004; Cruz and Tsai, 2004), we tested whether SIRT1 is induced in neurons in response to ionomycin (1 μM), a calcium ionophore, or hydrogen peroxide (H2O2) (25 μM), a free radical generator. These specific stresses have previously been shown to trigger the deterioration of neuronal morphology and the formation of p25 in cultured neurons (Kusakawa et al., 2000; Lee et al., 2000; Nath et al., 2000). Treatment of primary cortical neurons with either ionomycin or H2O2 rapidly induced SIRT1 protein expression, and did so in a dose-dependent manner (Figure 1F and G). Thus, SIRT1 is not only induced in mouse models of neurodegeneration but also in primary cultured neurons under neurototoxic stresses.

Resveratrol-mediated SIRT1 activation protects against p25 and mutant SOD1

To understand the physiological significance of SIRT1 activation in context of p25 and mutant SOD1 toxicity, we first tested the effects of resveratrol, a polyphenolic SIRT1-activating compound (STAC) (Howitz et al., 2003), on the viability of primary mouse neurons overexpressing p25 or a mutant form of SOD1 linked to ALS (SOD1G93A). The SOD1G93A mutation, which has been linked to ALS, has been widely used in primary neurons to examine neurotoxicity related to ALS. Doses of up to 500 nM resveratrol showed no evidence of toxicity to primary neurons transfected with GFP (Figure 2A). As previously reported (Patrick et al., 1999; Lee et al., 2000; Zhang et al., 2002; Hamdane et al., 2003), transfection with p25-GFP resulted in a high degree of cell death (54% after 24 h), which were scored on the basis of neuritic integrity and nuclear morphology, as described in Materials and methods (Figure 2B and C). Remarkably, resveratrol treatment significantly reduced the extent of cell death caused by p25 (Figure 2B and C) (54 versus 27%; T < 0.003). The protective effect of resveratrol against p25-mediated toxicity was further confirmed by examination of propidium iodide uptake as a marker for loss of membrane integrity and viability (Supplementary Figure 3A and B). In this experiment, resveratrol treatment reduced p25-induced propidium uptake (37 versus 18.2%; T < 0.007). We also examined whether resveratrol protected against neurotoxicity elicited by expression of SOD1G93A. Approximately 52% of primary neurons transfected with mutant SOD1G93A for 48 h exhibited cytoskeletal disruption and SOD1 aggregates, two hallmarks of ALS-associated SOD1 toxicity (Figure 2D and E). Resveratrol treatment significantly attenuated SOD1G93A-mediated neurotoxicity (52 versus 28%; T < 0.001) (Figure 2E). These results are in line with a recent report showing that in cultured neurons derived from transgenic mice overexpressing a mutant (109Q) huntingtin, resveratrol suppressed the neurotoxic effects of the mutant protein (Parker et al., 2005). In addition, we examined whether the acetylation of PGC-1alpha was decreased following resveratrol treatment. Indeed, we observed a decrease in PGC-1alpha (Supplementary Figure 4), suggesting that SIRT1 activity is increased following resveratrol treatment of primary neurons.

Deacetylase activity of SIRT1 confers neuroprotection against p25 and mutant SOD1

To directly verify the protective role of SIRT1 in neurodegeneration, we transfected primary neurons with p25-GFP or SOD1G93A, together with either SIRT1 or SIRT1 lacking catalytic activity (H363Y). Overexpression of SIRT1, but not H363Y, significantly rescued the rate of p25-GFP-mediated cell death (54 versus 28.7%; P < 0.01; 54 versus 56.5%; P > 0.05; one-way ANOVA with post hoc Newman–Keuls Multiple Comparison Test) (Figure 3A and B). The morphology of the p25-GFP/SIRT1-overexpressing neurons appeared normal and indistinguishable from control GFP-transfected neurons. This protective effect was unlikely to be an effect of SIRT1 on the stability of p25-GFP, because similar levels of p25-GFP were detected in the presence or absence of SIRT1 overexpression in CAD cells (Figure 3C). Similarly, a neuroprotective effect of SIRT1, but not the H363Y mutant, was observed when examining p25-induced propidium iodide uptake (37 versus 17.7%; P < 0.05; 37 versus 41.6%; P > 0.05; one-way ANOVA with post hoc Newman–Keuls Multiple Comparison Test) (Supplementary Figure 3A and C).

We also sought to determine whether SIRT1 overexpression protected against mutant SOD1-induced neurotoxicity. The overexpression of SIRT1, but not H363Y, protected...
against SOD1G93A toxicity (52 versus 32.3%; \(P<0.001\); 52 versus 48%; \(P>0.05\); one-way ANOVA with post hoc Newman–Keuls Multiple Comparison Test) (Figure 3D and E). Together, these results indicate that increased levels of SIRT1 in primary neurons confer protection against neurotoxicity induced by p25 or mutant SOD1. The observation that the H363Y mutant did not confer protection demonstrates that the deacetylase activity of SIRT1 is required for neuroprotection. Interestingly, siRNA-mediated knockdown of SIRT1 in primary neurons was not neurotoxic per se and did not result in increased sensitivity of neurons to neurotoxic stimuli (Supplementary Figure 5). This suggests that in contrast to the survival benefit of increased SIRT1 activation, SIRT1 knockdown per se does not sensitize neurons to acute neurotoxic stimuli; alternatively, other SIRT family members may compensate following loss of SIRT1 function.

**Resveratrol prevents neurodegeneration and cognitive decline in p25 transgenic mice**

To test the neuroprotective effects of resveratrol in vivo, resveratrol (Resv) or vehicle (Veh) was introduced by intracerebroventricular (ICV) injection in 2 week induced p25 mice for 3 weeks at a dose of 5 μg/μl, 0.5 μl bilateral injections injected every 2–3 days (Veh-treated animals, \(n=5\); Resv-treated animals, \(n=9\)) (Figure 4A). We determined that levels of acetylation of the SIRT1 substrate PGC-1alpha were
decreased in these resveratrol-treated animals compared to the vehicle-treated controls, suggesting that ICV injection of resveratrol resulted in the activation of SIRT1 (Figure 4B). After 5 weeks of p25 induction, cell death and neurodegeneration were evident in the hippocampus of the vehicle-treated animals, consistent with previous observations (Cruz et al., 2003). In contrast, administration of resveratrol reduced neurodegeneration in CA1 and CA3 regions of the hippocampus, as revealed by lower levels of the apoptotic marker activated caspase 3, and a marker of astrogliosis, GFAP (Figure 4C–E). Of note, GFP immunostaining, which labels p25-GFP expressing neurons, was more robust in the hippocampus of resveratrol-treated animals, suggesting the neurons were better able to tolerate and survive p25 expression (Figure 4E). We previously reported that by 4–6 weeks of induction, p25 mice have dramatically decreased associative learning capability, as revealed by contextual fear conditioning paradigm (Fischer et al., 2005). Associative learning was significantly rescued in p25 mice treated with resveratrol for 3 weeks, in comparison to the vehicle group or untreated p25 mice (Figure 4F). Together, these results show that resveratrol provides neuroprotection and prevents cognitive decline in an animal model of CNS degeneration that features massive neuronal loss and tau pathologies.

**Deacetylation of p53, a SIRT1 target, by resveratrol protects against p25 toxicity**

Next, we sought to gain insights into the mechanism by which resveratrol confers neuroprotection in vivo. We hypothesized that the tumor suppressor p53, an important mediator of cell death, may play an important role in mediating neuroprotection for the following reasons. First, p25/Cdk5 is known to phosphorylate p53 and upregulate its transcriptional activity (Zhang et al., 2002). Second, we found that p53 protein levels are significantly increased in p25 transgenic mice (Figure 5A). Third, this increase is accompanied by an increase in the acetylation status of lysine 382 of p53 (Figure 5B), a modification known to stabilize p53 and potentiate its apoptotic function (Luo et al., 2001; Vaziri et al., 2001; Langley et al., 2002). Fourth, lysine 382 of p53 (K382-p53) is a well-characterized target for SIRT1 deacetylase activity, and activation of SIRT1 by resveratrol would be consistent with the neuroprotective effects we observed in vivo.
We first tested whether p53 contributes to p25-induced cell death by cotransfecting cortical neurons with p25-GFP, together with either a control siRNA vector or p53 siRNA vector. Knockdown of p53 provided a 25% increase in cell survival (Figure 5C), which is similar to what we observed for resveratrol-treated neurons (Figure 2). Next, we asked whether resveratrol had a similar effect in vivo. As shown in Figure 5E, the acetylation status of K382-p53 was lower in...
resveratrol-treated hippocampal tissue of p25 transgenic mice, relative to vehicle. Overall p53 levels were also decreased by resveratrol treatment, which is consistent with the deacetylated form being less stable (Luo et al., 2001).

**Injection of SIRT1 lentivirus into the CA1 of p25 transgenic mice protects against neurodegeneration**

Finally, to directly ascertain a role for SIRT1 in neuroprotection in vivo, we introduced lentivirus carrying HA-tagged SIRT1, or control virus, into the hippocampus of p25 mice (n = 4; SIRT1-injected and control-injected hemispheres compared) by stereotaxic injection, as described in Materials and methods. Briefly, 2 week induced p25 transgenic mice were subjected to a single injection of control or SIRT1 expressing virus in each side of the brain. Mice were killed 3 weeks after the viral injections. GFP immunofluorescence staining showed that GFP-positive p25-expressing neurons are more prominent in the CA1 regions receiving the SIRT1 virus, compared to those receiving the control virus (Figure 6A–E), indicating that they can tolerate higher levels of p25, as seen in resveratrol-treated animals (Figure 4). In general, the SIRT1 virus-treated side exhibited 38 ± 13%
higher number of neurons than the control virus-treated side (Figure 6A; Supplementary Table 1), and the neurons were morphologically healthier. At higher magnification, it was clear that the surviving neurons expressed SIRT1, as detected by co-immunostaining with HA and GFP antibodies (Abs) (Figure 6F–H). These results provide further evidence that that resveratrol’s protective effects are due to SIRT1 activation and demonstrate a neuroprotective role of SIRT1 in vivo.

**Discussion**

Collectively, our results demonstrate that it is possible to slow in vitro cell death as well as in vivo neurodegeneration and cognitive decline with resveratrol, a SIRT1-activating molecule, and by expression of SIRT1. We also provide evidence that the neuroprotective effect is due, at least in part, to deacetylation of K382-p53 (Figure 5). We do not rule out the possibility that other known substrates of SIRT1 are involved, such as Ku70, a protein that sequesters the apoptotic protein Bax from mitochondria (Brunet et al., 2004; Cohen et al., 2004a). Resveratrol may also stimulate the deacetylation of FOXO3/4 transcription factors, thereby enhancing gene expression of antioxidative molecules and upregulating DNA repair (Nguyen et al., 2002; Brunet et al., 2004; Smith et al., 2004). Another interesting candidate target for SIRT1 deacetylation activity is PGC-1alpha, which we have shown for the first time to be deacetylated in a model for neurodegeneration (p25 Tg) and in the brain, in response to resveratrol treatment. PGC-alpha activity was recently shown to play an important role in neuronal metabolism and detoxification of reactive oxygen species (St-Pierre et al., 2006), and our finding suggests that deacetylation and activation PGC-1-alpha by enhanced SIRT1 activity may also be involved in...
the neuroprotection observed in our experiments. On the other hand, while our study and previous reports suggest that the activation of SIRT1 constitutes an important aspect of resveratrol action, we cannot rule out that resveratrol may interact with other biomolecules, besides SIRT1, to exert its neuroprotective effects.

SIRT1 is thought to be a key regulator of an evolutionarily conserved pathway that allows organisms to cope with adversity. Consistent with this notion, yeast Sir2 and mammalian SIRT1 are upregulated by various biological stresses, including caloric restriction, which has been shown to prevent numerous diseases of aging in mammals such as Alzheimer’s disease (AD) (Lamming et al., 2004; Bordone and Guarente, 2005; Lombard et al., 2005). Of note, a reduction of β-amyloid peptide, a hallmark of AD, occurs in brain of calorie-restricted animals and can be reproduced in mouse neurons in vitro by manipulating cellular SIRT1 expression/activity (Marambaud et al., 2005; Tang, 2005; Qin et al., 2006).

In this study, we show for the first time the ability of SIRT1 and SIRT1-activating molecules to prevent an age-dependent neurodegenerative disease caused by the toxic Cdk5 coactivator, p25, which has been implicated in various neurotoxic conditions such as AD, ALS and stroke. The induction of SIRT1 expression levels in various neurotoxic conditions may be interpreted as a neuroprotective adaptation response, implying role for SIRT1 as an important stress sensor molecule that links aging to neurodegeneration. Future research efforts will investigate the underlying mechanism for SIRT1 induction by neurotoxic stresses. Microarray analyses of p25 transgenic mice indicated elevated mRNA levels of SIRT1 (not shown), suggesting a transcriptional component for SIRT1 induction. On the other hand, rapid induction of SIRT1 in cultured neurons (Figure 1F) implies that post-translational mechanisms may also be involved.
Our results predict that positive intervention into SIRT1 activity, such as through intake of SIRT1-activating molecules, may have profound therapeutic benefits against various age-dependent neurodegenerative diseases. Conversely, it may be worthwhile to explore whether mechanisms that decrease SIRT1 activity or levels result in enhanced susceptibility to age-dependent neurodegeneration. While knock-down of SIRT1 did not appear to result in increased susceptibility to acute neurotoxic stimuli in cultured neurons (Supplementary Figure 5), the long-term effects of decreased SIRT1 levels per se or in chronic neurodegenerative conditions is an important question for future studies. Interestingly, the SIRT1 gene resides in a locus on chromosome 10 that is associated with familial AD (WIPO, international publication WO 2005/004815 A2) and future studies are planned to determine whether mutations or polymorphisms in SIRT1 affect the susceptibility of individuals to AD pathology.

Materials and methods

**Protein preparation and Western blots**

Total protein extracts of mouse spinal cord, mouse forebrain, mouse hippocampus or human prefrontal cortex were obtained by homogenization in SDS–urea β-mercaptoethanol (0.5% SDS, 8 M urea in 7.4 phosphate buffer) or Triton X-100 (10 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA (pH 8.0) and 1% Triton). The protein concentration was estimated by the Bradford procedure (Bio-Rad Laboratories, Hercules, CA). Proteins were fractionated on 7.5% SDS–PAGE and blotted on a nitrocellulose or PVDF membrane for Western blot analysis. Membranes were incubated with Abs against SIRT1 (07–131, Upstate), against tubulin (B-5, Sigma), actin (MAB 1501, Chemicon), FAK (C-20, Santa Cruz Biotechnology), Bax (N-20, Santa Cruz) and GFP (B-2, Santa Cruz). The Western blots were examined using RENAISSANCE, a Western blot chemiluminescence kit from PerkinElmer (Boston, MA). Quantitations were corrected with levels of actin, α-tubulin and FAK and performed with the Labscan program (Image Master, 2D software v 3.10, Amersharm Pharmacia Biotech).

**Culture, transfection and treatment of primary neurons**

Rat cortical primary neurons were isolated, cultured and transfected at DIV 5–7 with Lipofectamine 2000, according to Nguyen et al. (2004), in a ratio of 3:1 ((SIRT1 or SIRT1 H363Y or p53 RNAi): (p25-GFP, WT SOD1 or SOD1G93A or GFP)). Treatment of these neurons was performed on a 60 s period, so that a volume of 0.5 μl was injected into each side. Resveratrol (25% DMSO/artificial cerebrospinal fluid) was prepared fresh immediately before each injection. For SIRT1 lentivirus infection, canine were placed in the dorsal hippocampus, AP—0.5 mm, lateral 1 mm, depth 2 mm. Resveratrol (5 μg/μl) or vehicle was injected bilaterally 2–3 x /week using a microinjector (CMA/ microdialysis) over a 60 s period, so that a volume of 0.5 μl was injected into each side. Resveratrol (25% DMSO/artificial cerebrospinal fluid) was prepared fresh immediately before each injection. For SIRT1 lentivirus infection, canine were placed in the dorsal hippocampus, AP—1.5 mm, lateral 1 mm, depth 2 mm. SIRT1-HA lentivirus (1.5 μl) was injected as described above into the left hippocampus, whereas SIRT1-HA lentivirus (1.5 μl) was injected into the right hippocampus of 1 week induced CK-p25 mice. Number of GFP neurons was counted 1–2 mm caudal to the injection site. A ratio neurons control side/neurons SIRT1 side was calculated to quantify variations in percentage of neurons between both sides.

**Fear conditioning**

The fear conditioning apparatus (TSE Systems) consisted of two test boxes with defined light and background noise that were connected to a control unit and a PC computer. The experimental protocols were designed and performed using TSE fear conditioning software. A random sequence without homology to any known mRNA open reading frames (2500 nucleotides) and have been maintained on a pure C57BL6 background (Nguyen et al, 2001; Cruz et al, 2003). Double cannulae (Plastic1) were implanted 7 days before the experiments, under 1.2% avertin anesthesia (0.4 ml/mouse), as described previously (Fischer et al, 2004). For resveratrol injection, the cannulae were placed in both lateral brain ventricles, AP—0.5 mm, lateral 1 mm, depth 2 mm. Resveratrol (5 μg/μl) or vehicle was injected bilaterally 2–3 x /week using a microinjector (CMA/ microdialysis) over a 60 s period, so that a volume of 0.5 μl was injected into each side. Resveratrol (25% DMSO/artificial cerebrospinal fluid) was prepared fresh immediately before each injection. For SIRT1 lentivirus injection, cannulae were placed in the dorsal hippocampus, AP—1.5 mm, lateral 1 mm, depth 2 mm. SIRT1-HA lentivirus (1.5 μl) was injected as described above into the left hippocampus, whereas SIRT1-HA lentivirus (1.5 μl) was injected into the right hippocampus of 1 week induced CK-p25 mice. Number of GFP neurons was counted 1–2 mm caudal to the injection site. A ratio neurons control side/neurons SIRT1 side was calculated to quantify variations in percentage of neurons between both sides.

**Generation of RNAi**

P53 RNAi sequence were selected based on the criteria proposed by Sui et al (2002). Complementary hairpin sequences were commercially synthesized and cloned into pSilencer 2.0 under promoter U6 (Ambion). Sequence for p53 are basepairs: gga gtc ttc cag tgt gat for p53-GFP, WT SOD1 or SOD1G93A or GFP). Treatment of these neurons was performed on a 60 s period, so that a volume of 0.5 μl was injected into each side. Resveratrol (25% DMSO/artificial cerebrospinal fluid) was prepared fresh immediately before each injection. For SIRT1 lentivirus infection, canine were placed in the dorsal hippocampus, AP—1.5 mm, lateral 1 mm, depth 2 mm. SIRT1-HA lentivirus (1.5 μl) was injected as described above into the left hippocampus, whereas SIRT1-HA lentivirus (1.5 μl) was injected into the right hippocampus of 1 week induced CK-p25 mice. Number of GFP neurons was counted 1–2 mm caudal to the injection site. A ratio neurons control side/neurons SIRT1 side was calculated to quantify variations in percentage of neurons between both sides.

**Immunofluorescence of primary neurons and human prefrontal cortex tissues**

Cells were stained according to Nguyen et al (2004) with Abs against tubulin (α-tubulin, Sigma Aldrich), GFP (Molecular Probes), SOD1 (Biodesign), FLAG (M2, Sigma), SIRT1 (Upstate). Staining of spinal cord tissues was performed according to Cruz et al (2003) with Abs against SIRT1 (Upstate).

**Generation of SOD1G93R transgenic mice and p25 inducible transgenic mice**

Transgenic mice overexpressing SOD1G93R (line 29) (G37R) and p25-CK transgenic have been generated as described previously, and have been maintained on a pure C57BL6 background (Nguyen et al, 2001; Cruz et al, 2003).
(WT) mice, with a monoclonal Ab against p53 (Ab-3, Calbiochem/ Oncogene). Membranes were probed with a homemade Ac-p53 Ab and a mouse monoclonal p53 Ab (Ab-240, Abcam).

For examination of PGC-1alpha acetylation, brain samples or cultured primary neurons were lysed in RIPA buffer then diluted three-fold with PBS and protease inhibitors. Samples were immunoprecipitated using an anti-PGC-1alpha Ab (H-300, Santa Cruz), washed extensively with a 1:2 solution (RIPA-PBS + protease inhibitors, noncitrination, and TSA) and membranes were probed using acetylated lysine Abs (Cell Signaling) and PGC-1alpha Abs (H-300, Santa Cruz).

**Supplementary data**

Supplementary data are available at The EMBO Journal Online (http://www.embojournal.org).

**Acknowledgements**

We thank Dr B Samuels for critical reading of the manuscript, Dr L Moy for helpful discussions, and Drs M Urushitani and J-P Julien for SOD1 constructs and mice. This work was supported by the National Institutes of Health (NIH) (DAS and L-HT), POI Grant (PGC-1alpha27916) the National Institute of Aging (DAS), the Canadian Institutes of Health Research (MDN) and the Paul F Glenn Foundation for Medical Research (DAS). L-HT is an investigator at the Howard Hughes Medical Institute. DAS is an Ellison Medical Research Foundation fellow. MDN is the Investigator at the Brenda Strafford Foundation Chair in Alzheimer research and a recipient of a Career Development Award from the Human Frontier Science Program Organization. AF held a Humboldt post-doctoral fellowship. FS was a fellow of the DFG (German Research Organization). JB holds an American Heart Association postdoctoral fellowship.

**References**


**Author Information**

D Kim et al.


