Experimental Gerontology 43 (2008) 1086–1093

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

The role of calorie restriction and SIRT1 in prion-mediated neurodegeneration

Danica Chen [a,1], Andrew D. Steele [a,b,*,1], Gregor Hutter [c], Joanne Bruno [a], Arvind Govindarajan [a], Erin Easlon [d], Su-Ju Lin [d], Adriano Aguzzi [c], Susan Lindquist [a,b], Leonard Guarente [a,*]

[a] Department of Biology, MIT, Cambridge, MA 02139, USA
[b] Whitehead Institute for Biomedical Research, Howard Hughes Medical Institute, MIT, Cambridge, MA 02142, USA
[c] Institute of Neuropathology, University Hospital Zurich, Zurich, Switzerland
[d] Section of Microbiology, University of California, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 4 July 2008
Received in revised form 20 August 2008
Accepted 21 August 2008
Available online 30 August 2008

Keywords:PrP:
Sirtuin
Aging
Dietary restriction
Amyloid
Transmissible spongiform encephalopathy

ABSTRACT

A central focus of aging research is to determine how calorie restriction (CR) extends lifespan and delays diseases of aging. SIRT1, the mammalian ortholog of Sir2 in yeast, is a longevity factor which mediates dietary restriction in diverse species. In addition, SIRT1 plays a protective role in several models of neurodegenerative disease. We tested the role of SIRT1 in mediating the effects of CR in a mouse model of prion disease. Prion diseases are protein misfolding disorders of the central nervous system with many similarities to other neurodegenerative diseases, including deposition of aggregated protein, gliosis, and loss of synapses and neurons. We report that the onset of prion disease is delayed by CR and in the SIRT1 KO mice fed *ad libitum*. CR exerts no further effect on the SIRT1 KO strain, suggesting the effects of CR and SIRT1 deletion are mechanistically coupled. In conjunction, SIRT1 is downregulated in certain brain regions of CR mice. The expression of PrP mRNA and protein is reduced in the brains of CR mice and in SIRT1 knockout mice, suggesting a possible mechanism for the delayed onset of disease, as PrP levels are a critical determinant of how quickly mice succumb to prion disease. Surprisingly, CR greatly shortens the duration of clinical symptoms of prion disease and ultimately shortens lifespan of prion-inoculated mice in a manner that is independent of SIRT1. Taken together, our results suggest a more complex interplay between CR, SIRT1, and neurodegenerative diseases than previously appreciated.

1. Introduction

Aging research has the potential to impact a broad array of aging-related diseases (Sinclair and Guarente, 2006; Chen and Guarente, 2007). Calorie restriction (CR) is a dietary regimen that extends lifespan in a wide spectrum of species ranging from yeast to mammals (Guarente and Picard, 2005). CR also delays many diseases with seemingly different causes, such as kidney disease, cancer, autoimmune disease, metabolic syndromes, and neurodegenerative diseases including Parkinson's and Alzheimer's disease (Koubova and Guarente, 2003; Chen et al., 2005b; Longo and Kennedy, 2006). Mediators of CR would serve as potential targets for a CR mimetic (Baur and Sinclair, 2006).

The Sir2 gene was first identified as a longevity factor in yeast, and this function is conserved in higher organisms (Guarente and Picard, 2005). The Sir2 protein is an NAD-dependent deacetylase (Imai et al., 2000). Thus, its activity is amenable for regulation by the metabolic status of the cell, and it may serve as a prime candidate in mediating the cellular responses to CR. There is evidence that Sir2 is required for CR-induced lifespan extension in yeast and flies, although in yeast the requirement of Sir2 might depend on the specific conditions of CR (Longo and Kennedy, 2006; Chen and Guarente, 2007). SIRT1, the mammalian ortholog of the yeast Sir2, is required for the increased physical activity exhibited in CR mice (Chen et al., 2005a). In addition, transgenic mice overexpressing SIRT1 in certain tissues show some metabolic phenotypes resembling those seen in mice on a CR regimen (Bordone et al., 2007). Thus, it is relevant and critical to address whether SIRT1 mediates both CR-induced lifespan extension and the delay of aging-related diseases in mammals.

The expression of SIRT1 promotes longevity by an as yet undetermined mechanism that may involve its upregulation in many tissues of mice on a CR diet (Cohen et al., 2004). It is also thought to have a protective role in the progression of many neurodegenerative diseases. SIRT1 may serve as a downstream effector of increased NAD biosynthesis and delay axonal degeneration in a mouse model of Wallerian degeneration (Araki et al., 2004). Importantly, overexpression of SIRT1 also protects against Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis in

* Corresponding authors. Address: California Institute of Technology Pasadena, 1200 E. California Boulevard, CA 91125, USA.
E-mail addresses: steelea@caltech.edu (A.D. Steele), leng@mit.edu (L. Guarente).
[1] These authors contributed equally to this work.

0531-5565/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2008.08.050

various model systems (Parker et al., 2005; Qin et al., 2006; Kim et al., 2007), consistent with its proposed neuroprotective function. However, the effect of deleting SIRT1 in a neurodegenerative disease model in a mammalian system is not known.

Prion diseases are unique among neurodegenerative diseases in that they are transmissible while still sharing commonalities with other neurodegenerative diseases such as the accumulation of aggregates of misfolded protein, a prominent astrocytic and microglial response, and loss of neurons in the central nervous system (Prusiner, 1998; Aguzzi et al., 2007). The prion protein (PrP) is an N-linked glycoprotein tethered to the cell surface via a GPI anchor. Although the normal function of PrP is poorly defined (Steele et al., 2007a), numerous lines of evidence point toward a pivotal role for PrP in the pathogenic mechanism of prion diseases (Prusiner, 1998; Aguzzi et al., 2007). In prion diseases, the normal isoform of PrP (termed PrP^{C}) is structurally converted into PrP^{Sc}, a self-perpetuating and aggregation-prone conformation of the protein (Prusiner, 1998). The ongoing conversion of PrP^{C} to PrP^{Sc} in neurons is required for prion toxicity (Brandner et al., 1996; Mallucci et al., 2003; Aguzzi and Heikenwalder, 2006). Yet beyond this basic observation, the pathways leading to neurotoxicity are almost completely unknown (Aguzzi et al., 2007; Steele et al., 2007b).

Here we investigate the effects of CR and SIRT1 deletion in a mouse model of infectious prion disease. In contrast to both what has been observed with Sir2 deletion in a worm model of polyglutamine disease (Parker et al., 2005) and what would be predicted based on studies where SIRT1 is overexpressed in mouse models of neurodegeneration (Qin et al., 2006; Kim et al., 2007), SIRT1 deletion delays the onset of disease. As expected, CR delays the onset of disease, consistent with what has been observed with other models of neurodegeneration (Duan et al., 2003; Patel et al., 2005). Both CR and SIRT1 deletion delay the onset of prion disease by mechanisms that may involve a reduction in PrP expression, a well characterized determinant of the onset and duration of prion disease (Weissmann et al., 1998). Unexpectedly, CR also greatly shortens the duration of the clinical period of the disease in a manner that is independent of SIRT1. Despite the delay in disease onset, mice on a CR diet ultimately succumb to disease slightly faster than controls on a normal diet, in contrast to what has been observed with CR and other neurodegenerative disease models (Duan et al., 2003; Patel et al., 2005). Regulation of SIRT1 expression by CR differs in various brain regions; expression of SIRT1 was upregulated by CR in the cortex and hippocampus, and downregulated in the cerebellum and midbrain. Thus, SIRT1 regulation in the brain during CR is complex, a finding which is consistent with the unexpected effects of CR and SIRT1 deletion on prion-mediated neurodegeneration.

2. Results

2.1. The onset of prion disease is delayed by CR and in SIRT1 knockout mice

To assess the effects of CR and SIRT1 on prion disease, we compared the response to prion infection of SIRT1 knockout mice (KO) (McBurney et al., 2003) and their wild type (WT) littermates either fed *ad libitum* (AL) or on a CR diet. One month after mice were adjusted to CR, we challenged them (WT/AL, WT/CR, SIRT1 KO/AL, and SIRT1 KO/CR) with 3.5 $\log LD_{50}$ Rocky Mountain Laboratory (RML) strain of murine prions inoculated directly into the brain.

Prior to the onset of overt symptoms we sacrificed a cohort of mice at 4 months post-inoculation (MPI) to conduct neuropathological analysis. RML prion strain pathology is characterized by dramatic vacuolation in white and gray matter, neuronal loss, and severe gliosis. Histological analysis for spongiform changes with hemotoxylin and eosin (HE) staining of brains taken at 4 MPI revealed vacuolation throughout the WT/AL group. However, the WT/CR mice or SIRT1 KO mice on either diet showed much less spongiform pathology (Fig. 1A). In addition, immunohistochemical staining with an antibody against glial fibrillary acidic protein (GFAP) and a microglial marker ionized calcium-binding adaptor molecule 1 (IBA1) indicated that gliosis was much more advanced in WT mice fed AL than in other groups (Fig. 1A). While both CR and SIRT1 deletion alleviated spongiosis and gliosis, there was no further improvement in SIRT1 KO mice on CR, suggesting that the effects of CR and SIRT1 depletion may be mechanistically coupled.

The formation of proteinase K (PK)-resistant PrP is a classic surrogate marker for prion disease and typically correlates well with disease state in RML inoculated mice (Steele et al., 2007b). We assayed the amount of PK-resistant PrP accumulation in these mouse brains at 4 MPI by subjecting brain homogenates to PK digestion, SDS–PAGE, and immunodetection of the three glycoforms (di-, mono-, and un-glycosylated) of PrP by immunoblotting (Fig. 1B). The amount of PK-resistant PrP that had accumulated in the brains of WT/CR, SIRT1 KO/AL, and SIRT1 KO/CR mice was reduced in comparison to WT/AL (Fig. 1B).

During prion disease, PrP forms dense protein deposits in the brain that can be visualized by immunostaining for PrP after treatment of tissue sections with formic acid, which removes PrP^{C} but spares PrP aggregates. Prion aggregate staining was clearly detected in WT/AL brains at 4 MPI, but was not apparent in WT/CR, SIRT1 KO/AL, or SIRT1 KO/CR brain sections at 4 MPI (Fig. 1A, "SAF"). The higher amount of PK-resistant PrP in WT/AL mice is consistent with the more severe pathology observed in these mice. We also harvested the brains of all four groups of mice when death was imminent. Similar levels of PK-resistant PrP were detected in terminal samples taken from WT/AL, WT/CR, SIRT1 KO/AL, and SIRT1 KO/CR (Supplemental Fig. 1B). Thus, while CR and SIRT1 deletion suppress prion aggregation levels in pre-symptomatic mice, all groups of mice arrive at a comparable level of prion deposition at death.

2.2. The effects of calorie restriction and SIRT1 deletion on the lifespan of mice with prion disease

Prion diseases are characterized by a long asymptomatic period followed by a rapid deterioration. At a gross level, mice inoculated with RML strain of prions develop ataxia and imbalance, lose weight, and show hunched posture (Kingsbury et al., 1983). Mice were monitored for behavioral changes daily. Prion inoculated WT/AL mice first showed symptoms of disease at 155 days post-inoculation and survived for an average of 20 days before reaching the terminal stage of the disease (Fig. 2). Both the onset of clinical symptoms and the death of SIRT1 KO mice fed AL were delayed for about 10 days when compared to WT controls (Fig. 2). WT/CR mice also showed a similar delay in the onset of clinical symptoms when compared to WT mice fed AL. The onset of symptoms in SIRT1 KO mice on the CR diet was not further delayed. These results are consistent with the idea that CR delays the onset of prion disease via SIRT1, perhaps by reducing levels of this sirtuin in some critical brain region(s).

Surprisingly, even though CR delayed the onset of prion disease, the CR mice had a shorter lifespan (median survival of 164.5 days for WT/CR and 167 for SIRT1 KO/CR), compared to mice fed AL (median survival of 172 days for WT/AL and 182 for SIRT1 KO/AL) (Fig. 2A–C). This was because CR mice had a strikingly short duration of clinical symptoms (on average 2 days) (Fig. 2D). Moreover, many CR mice remained apparently healthy and active until they experienced sudden death. The shortened duration of clinical