Experimental Gerontology 44 (2009) 70–74

Contents lists available at ScienceDirect

Experimental Gerontology

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

Linking sirtuins, IGF-I signaling, and starvation

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ARTICLE INFO

Article history:
Received 16 May 2008
Received in revised form 12 June 2008
Accepted 16 June 2008
Available online 24 June 2008

Keywords:
IGF-I
SirT1
Sirtuins
sch9
Ras
ERK

ABSTRACT

Our studies in yeast have shown that the down-regulation of major signal transduction mediators increases stress resistance and causes an up to 10 fold chronological life span extension. Whereas other laboratories have proposed that sirtuins (Sir2 and its homologs), a family of conserved proteins which are NAD^+-dependent histone deacetylases, can extend longevity in various model organisms, we propose that one sirtuin, i.e., Sir2, can also accelerate cellular aging and death. In *Saccharomyces cerevisiae* (yeast), the deletion of Sir2 increases DNA damage but in combination with longevity mutations in principal intracellular signal transduction mediators, or in combination with calorie restriction it causes a further increase in the chronological lifespan as well as an increase in the stress resistance and a major reduction in age-dependent genomic instability. Our recent results also provide evidence for a role of the mammalian Sir2 ortholog SirT1 in the activation of a highly conserved neuronal pathway and in the sensitization of neurons to oxidative damage. However, the mean lifespan of the $SirT1^{+/-}$ mice is not different from that of wild type animals, and the survival of $SirT1^{-/-}$ mice was reduced under both normal and calorie restricted conditions. Here, I review the studies linking SirT1, IGF-I signaling and starvation in various model organisms with a focus on the post-mitotic cells, which indicate that sirtuins can play both protective and pro-aging roles.

1. Introduction

Sirtuins, or Sir2 family proteins, are conserved NAD^+-dependent histone deacetylases (Frye, 2000) that have been shown to extend the lifespan of *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila* (Kaeberlein et al., 1999; Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). Though earlier studies proposed that Sir2 is required for the effect of calorie restriction (CR) on the lifespan of lower eukaryotes (Guarente and Picard, 2005), later studies found that CR can increase the yeast replicative lifespan (Kaeberlein et al., 2004) or the worm lifespan (Hansen et al., 2007; Kaeberlein et al., 2006; Lee et al., 2006) independently of Sir2. Our results with the chronological lifespan of non-dividing yeast cells indicated that Sir2 can also have the opposite effect on the longevity since the lack of Sir2 further extended the lifespan of calorie restricted cells (Fabrizio et al., 2005b). Sir2 deficiency also further extended the lifespan of long-lived mutants lacking SCH9, homologous to both mammalian S6 kinase and Akt (Geyskens et al., 2000; Urban et al., 2007), and of mutants with deficiencies in the Ras/cAMP pathway (Fabrizio et al., 2005b). Here, I review the connection between sirtuins, insulin like growth factor 1 (IGF) IGF-I-like signaling and calorie restriction with focus on non-dividing yeast and neurons.

2. Conserved regulation of lifespan

Genetic manipulations which reduce insulin/IGF-I-like signaling extend the lifespan of *C. elegans*, *Drosophila* and mammals (Kenyon, 2001; Longo and Finch, 2003). The reduction of insulin/IGF-I signaling also extends the lifespan of mice (Bluher et al., 2003; Holzenberger et al., 2003; Taguchi et al., 2007). Work in *C. elegans* and *Drosophila* points to one major longevity regulatory pathway which includes the IGF-I-like receptor, Akt and forkhead stress resistance transcription factors (Hwangbo et al., 2004; Kenyon et al., 1993). These studies and others have shown that reduced insulin/IGF-I-like signaling protects against oxidative damage and other forms of stress in simple model systems and mice (Holzenberger et al., 2003; Kenyon, 2001; Longo and Finch, 2003). Sir2/SirT1 (SirT1, the mammalian ortholog of yeast Sir2) has also been linked to the insulin/IGF-1 signaling pathway: in *C. elegans* Sir2.1 interacts with 14-3-3 proteins to activate DAF-16, which is a major stress resistance transcription factor in the IGF-like pathway (Berdichevsky et al., 2006; Brunet et al., 2004; Wang and Tissenbaum, 2006). Our studies of the chronological lifespan of yeast have revealed a similar longevity and stress resistance regulatory pathway in which glucose, instead of IGF-I, causes the activation of the serine threonine kinase Sch9 which results in the down-regulation of the downstream stress resistance kinase Rim15 (Cheng et al., 2007; Fabrizio et al., 2001; Wei et al., 2008). Others have shown a similar pro-aging effect of Sch9 in the regulation of the

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doi:10.1016/j.exger.2008.06.005

replicative lifespan (Kaeberlein et al., 2005). Our work has also pointed to a second pro-aging pathway which includes Ras, adenylate cyclase, PKA and the stress resistance transcription factors Msn2/Msn4 (Fabrizio et al., 2003, 2004), which are also implicated in the regulation of the yeast replicative lifespan (Kaeberlein et al., 2005; Medvedik et al., 2007). Recently, the down-regulation of the adenylate cyclase/PKA pathway by the omission of five adenylyl cyclase was shown to extend the lifespan of mice and to protect them from reduced bone density and aging-induced cardiomyopathy (Yan et al., 2007). Analogously to our findings in yeast (Fabrizio et al., 2001, 2003), the five adenylyl cyclase deficient mice displayed increased levels of MnSOD and stress resistance (Yan et al., 2007). These data support the hypothesis that the mechanisms of lifespan regulation are conserved and that the studies in *S. cerevisiae* can point to additional pathways and mechanisms important for mammalian aging and diseases.

3. Regulation of oxidative stress by mammalian signal transduction pathways

Ras, Akt, and S6K are among the principal intracellular signal transduction mediators of the many growth, survival and metabolic effects of IGF-I (Fig. 1). These proteins are also homologs or orthologues of yeast Ras2 and Sch9. Before introducing the potential link between SirT1, IGF-I signaling and oxidative stress in mammals, I will briefly review some of the studies on the role of proteins in the Ras and Akt pathways in the production of oxidants and in the regulation of stress resistance. Oxidants have recently gained attention as mediators of growth factor signaling. In PC12 cells, the generation of nitric oxide (NO) is required for NGF-dependent differentiation, whereas EGF stimulates the generation of superoxide by a Ras-dependent mechanism (Mills et al., 1998; Peunova and Enikolopov, 1995). The small G protein p21Ras plays a critical role in transmitting growth factor signals in many cell types through the activation of Raf, MEK, and ERK. In PC12 cells, EGF induces the generation of high levels of superoxide by a Ras- and MEK-dependent mechanism (Mills et al., 1998). Superoxide generation in EGF-treated PC12 cells is blocked by the inhibitors of the superoxide-generating enzyme lipoxygenase (Mills et al., 1998). A constitutive active form of p21Ras stimulates the generation of high levels of superoxide and mitogenesis by a Rac1-dependent mechanism in 3T3 fibroblast cells (Irani and Goldschmidt-Clermont, 1998; Irani et al., 1997). This mitogenic activity of Ras is blocked by the antioxidant enzymes (Irani et al., 1997). Down-regulation of mox1, a homologue of the catalytic subunit of a superoxide-generating NADPH oxidase, decreases superoxide generation and growth in smooth muscle cells and 3T3 cells (Suh et al., 1999). As shown in other cell types, in 3T3 cells NADPH oxidase is activated in cells with a constitutively active Ras (Benhar et al., 2001). Ras can induce the generation of NO in neuronal cell lines and astrocytes. The Ras–ERK pathway is required for the activation of neuronal NO synthase in PC12 cells and for the microglial-dependent stimulation of cholinergic neurons, which is attenuated by antioxidants (Jonakait et al., 2000; Schonhoff et al., 2001). A dominant negative form of p21Ras also inhibits the induction of NO synthase in LPS-stimulated primary astrocytes (Pahan et al., 2000) and 3T3 cells expressing a constitutively active Ras increase the levels of hydrogen peroxide, which induce apoptosis (Liou et al., 2000). In embryonic cortical neurons brain-derived neurotrophic factor (BDNF) accelerates NO induced cell death by a mechanism that requires p38 or MAPK/ERK kinase 1 activation, suggesting that these proteins, which function downstream of Ras, can increase the sensitivity of neurons to oxidative damage (Ishikawa et al., 2000). The free radical nitric oxide (NO) plays a role in synaptogenesis and IGF-I signaling (Contestabile, 2000; Schini-Kerth, 1999). The protein kinase Akt is also involved in the generation of oxidants (Erlich et al., 2001). However, Akt is better known for its induction of anti-apoptotic genes: it blocks apoptosis induced by oxidants and serum withdrawal in many cell types, including B cells and 3T3 cells (Ding et al., 2000; Wang et al.,

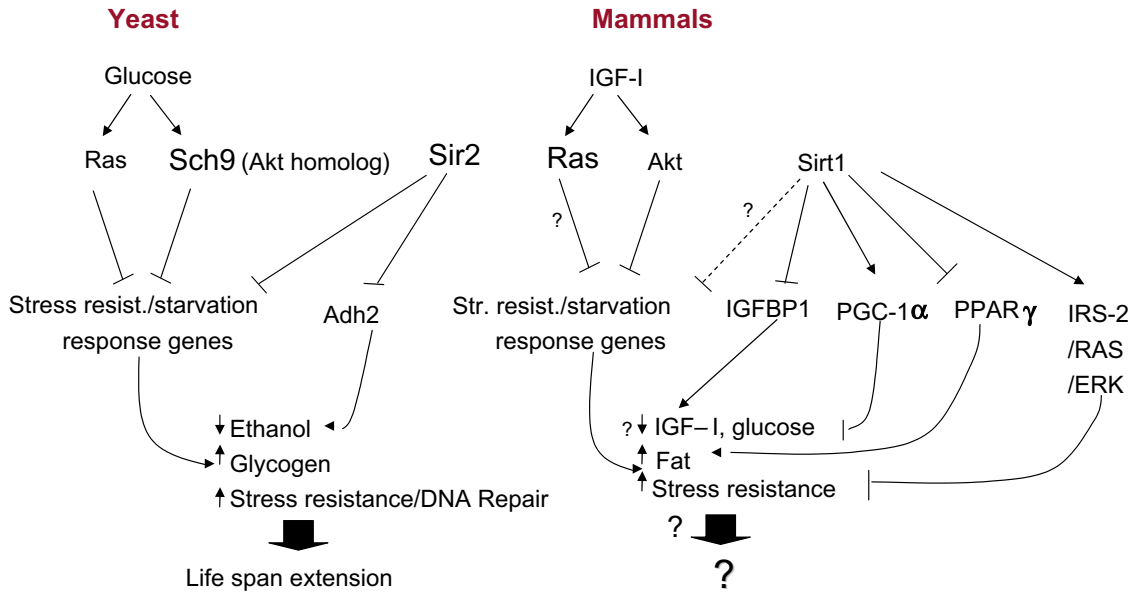

Fig. 1. Models for the role of sirtuins on stress resistance and metabolic pathways in yeast and mammals. Deletion of *SIR2* has been shown to decrease the replicative lifespan and to cause some genomic instability during growth. However, deletion of *SIR2* combined with calorie restriction/starvation or with longevity mutations in nutrient responsive pathways such as the Ras or Tor/Sch9 (Akt, S6K) pathways further enhances the chronological survival of non-dividing cells. *S. cerevisiae sir2Δ* strains show elevated stress resistance and enhanced alcohol dehydrogenase activity, which results in the depletion of extracellular ethanol during the early phases of starvation. In contrast, mice heterozygote for SirT1 display a normal lifespan whereas homozygote SirT1$^{-/-}$ null mutants are short-lived under both normal and calorie restricted conditions. The reduced systemic glucose levels in SirT1 knockdown mice is reminiscent of the reduced ethanol level in *sir2* deficient yeast. At least in rat neurons, inhibition of sirtuins activity increases resistance to oxidative stress but not to other stresses. Note that the many functions of SirT1 in mammals, are occurring in different organs/cell types.

2000). In summary, the Ras and Akt pathways, which function in mammalian IGF-I signaling, are implicated in the generation of oxidants and in the response to oxidative stress. However, both pathways can play both protective and pro-apoptosis roles depending on the cell type and conditions. The role of each pathway in the generation of specific oxidants and in the regulation of antioxidant systems and oxidative damage remains poorly understood, particularly in neurons.

4. Linking IGF-I signaling and sirtuins

SirT1 affects many metabolic and stress resistance pathways including those involved in DNA repair, apoptosis, glucose and fat metabolism (Bordone et al., 2006; Cohen et al., 2004; Luo et al., 2001; Moynihan et al., 2005; Rodgers et al., 2005; Sun et al., 2007a). In mammalian cells, SirT1 directly regulates stress-response transcription factors, such as the p53 tumor suppressor factor (Langley et al., 2002; Vaziri et al., 2001), forkhead transcription factors (Brunet et al., 2004; Motta et al., 2004), and NF-κB (Yeung et al., 2004). It also induces gluconeogenic genes and hepatic glucose output through PGC-1 (Rodgers et al., 2005). Evidence is also accumulating in support of a synergic relationship between Sir2 and insulin/IGF-1. SirT1 can increase the release of insulin and affect insulin sensitivity (Bordone et al., 2006; Moynihan et al., 2005; Sun et al., 2007a) and it reduces the expression of IGF-binding proteins (IGFBPs), which can inhibit IGF-I function (Yang et al., 2005). In fact, SirT1 knockout mice have increased expression of IGFBP1 (Lemieux et al., 2005). In agreement with a gluconeogenic/hepatic output role for SirT1, knockdown of liver SirT1 in mice displays reduced blood glucose concentration (Rodgers and Puigserver, 2007). Furthermore, the sirtuin activator resveratrol was shown to increase insulin sensitivity and extend the survival of mice on a high calorie diet (Baur et al., 2006) although Bordone et al. showed that mice lacking SirT1 are also insulin sensitive (Bordone et al., 2006). In non-dividing *S. cerevisiae*, the omission of SIR2 Sir2 caused a depletion of the ethanol which was generated during fermentation and was released into the medium (Fabrizio et al., 2005a), reminiscent of the reduction in glucose in mice (Fig. 1). In *S. cerevisiae* the deletion of SIR2 increases further the resistance to oxidative stress and heat shock in mutants lacking sch9 but not in mutants with defects in the Ras/cAMP pathway raising the possibility that Sir2 and Ras function in overlapping pathways. Although Ras has not been linked to aging in mammals in our recent study we describe a mechanism linking SirT1 activity, the IGF-I/IRS-2/Ras/ERK pathway and stress resistance in neurons (Fig. 1). Our experiments indicate that the inhibition of SirT1 in neurons decreases the insulin/IGF-I-dependent activation of ERK1/2 in part through increased IRS-2 acetylation decreased IRS-2 phosphorylation and decreased Ras activation (Li, Y., Longo, V.D., unpublished results). These results are consistent with our work revealing a potential link between Sir2 and Ras in yeast but are also consistent with mammalian studies showing that Ras induces premature replicative senescence in mammalian cells (Serrano et al., 1997) and that SirT1-deficient mouse embryonic fibroblasts (MEF) display a major extension in replicative lifespan (Chua et al., 2005). As it would be expected if SirT1 functions upstream of Ras, SirT1 was not required for the Ras-dependent effect on replicative senescence (Chua et al., 2005). Notably, this protective effect of SirT1 inhibitors was limited to oxidative stress, which may explain why others have proposed that SirT1 protects cell-based models for neurodegenerative diseases (Kim et al., 2007a). Thus, the IRS-2 regulation upstream of Ras may be responsible for this effect of SirT1 deficiency on stress resistance and replicative senescence. Interestingly, the PI3K/Akt pathway, another major effector downstream of IRS-2, was unaffected by SirT1 inhibition (Li et al., 2008). Others have reported that the overexpression of SirT1 causes the activation of Akt under insulin-resistant conditions, but not under normal conditions (Sun et al., 2007b). The effect of SirT1 on IRS-2 may have important implications for aging considering that brain irs2$^{-/-}$ mice are long-lived (Taguchi et al., 2007).

The role of ERK in the stress resistance of mammalian cells has been studied extensively. ERK signaling is known to exhibit dual effects on cell death depending on the type of cell and duration, and the intensity of treatment (Chu et al., 2004). It has been shown to increase survival under some conditions (Cheung and Slack, 2004), but to sensitize cells to oxidative stress under other conditions. The inhibition of ERK abrogates H_2O_2-dependent cell death in pancreatic cancer cells (Osada et al., 2008) but protects against glutamate-induced neuronal death (Satoh et al., 2000). Furthermore, certain drugs can protect neurons partly by inhibiting ERK activation (Xu et al., 2007). Our results suggest that SirT1 inhibition protects neurons by decreasing Ras/ERK signaling and provides evidence for a pro-aging role of the Ras/ERK pathway downstream of IGF-I (Fig. 1). In agreement with this hypothesis, a reduction in brain IRS-2 increases the lifespan of mice and MnSOD activity after fasting (Taguchi et al., 2007). We had also shown that *S. cerevisiae* MnSOD is required for lifespan extension in both mutants with defects in SCH9 or in RAS/cAMP signaling (Fabrizio et al., 2003).

5. Calorie restricted SirT1 knockout mice are short-lived

The ubiquitous and complex role of SirT1 in many different cells and pathways was confirmed by our recent study of SirT1$^{-/-}$ mice. We show that the level of markers of oxidative damage in the brain was reduced compared to the controls (Li et al., 2008) but these apparently protective effects were not sufficient to counterbalance the defects caused by the lack of SirT1. Whereas heterozygote mice had a normal mean life span, homozygote SirT1 knock out mice died early under both ad lib or calorie restricted diets (Li et al., 2008). Because of such complexity, it is important to investigate the various functions of SirT1 in different organs and cell types, but to also begin to define the "general purpose" of all these poorly understood functions of SirT1. For example, is SirT1 mediating some of the anti-aging effects or signals induced by calorie restriction or is it instead coordinating gluconeogenesis and hepatic glucose output (ethanol in yeast) required for the adaptation to temporary starvation conditions?

Our results suggesting that sirtuins sensitize neurons to stress may reveal a conserved pro-aging role for sirtuins but could also simply be reflecting a pro-signaling role in neurons, possibly aimed at altering the behavioral functions. For non-neuronal cells, this effect of SirT1 may result in cell growth. In fact others have shown that the SirT1 inhibitor sirtinol induces a cell cycle arrest (Ota et al., 2006). Consistent with the discovery of a cytosolic function of SirT1 in addition to its nuclear role, Tanno et al. examined different tissues and found SirT1 predominantly localized to the cytoplasm in mouse brain tissue (Jin et al., 2007; Kim et al., 2007b; Tanno et al., 2007; Zhang, 2007). Our results also indicate that SirT1 can be localized both in the nucleus and in the cytoplasm of neurons (Li et al., 2008). It will be important to further investigate the role of sirtuins in the cytosol, to determine whether they may play a role in the deacetylation of multiple nuclear and cytoplasmic targets to coordinate the response to short-term fasting or long-term calorie restriction.

6. Conclusion

In summary, our results and those of others are consistent with a role for SirT1 in increasing signaling downstream of IGF-I in neu-

rons and other cell types. This effect and other effects of sirtuins appear to sensitize some cell types and protect others against oxidative stress. However, SirT1 activities, such as its effect on gluconeogenesis and glucose output in response to starvation, appear to be so important for survival that its absence causes many defects and reduces lifespan, particularly under calorie restricted conditions (McBurney et al., 2003; Moynihan et al., 2005; Picard et al., 2004). A brain specific SirT1 knockout mouse model will be important to determine whether reduced brain SirT1 expression may result in increased lifespan extension but also to understand whether it may promote important functions such as learning and memory. A major conclusion that can be made from the studies of sirtuins is that they are involved in many cellular processes some of which increase the cellular sensitivity to stress and organismal aging and some which are protective.

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