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The hypoxia inducible factor HIF-1 functions as both a positive and negative modulator of aging

Scott F. Leiser¹ and Matt Kaeberlein^{1,2}

¹Department of Pathology, University of Washington, Seattle, WA 98195, USA

²Institute of Aging Research, Guangdong Medical College, Dongguan 523808, China

Abstract

In the past year and a half, five studies have independently established a direct connection between the hypoxic response transcription factor, HIF-1, and aging in *Caenorhabditis elegans*. These studies demonstrated that HIF-1 can both promote and limit longevity via pathways that are mechanistically distinct. Here we review the current state of knowledge regarding modulation of aging by HIF-1 and speculate on potential aspects of HIF-1 function that may be relevant for mammalian longevity and healthspan.

Keywords

Caenorhabditis elegans; dietary restriction; hypoxic response; longevity; stress response

Introduction

Aging is a complex process that is influenced by environmental and genetic factors. Studies from yeast and invertebrate organisms have identified hundreds of genes that modulate longevity in these simple eukaryotes, and several single gene mutations have also been shown to increase life span in rodents (Partridge 2009; Fontana et al. 2010; Kenyon 2010). Environmental components that modulate aging include temperature and diet. Indeed, the best characterized intervention for slowing aging involves reducing nutrient availability, a process referred to as dietary restriction (DR). DR has long been known to increase life span in organisms from yeast to rats (Masoro 2005; Kennedy et al. 2007), and was recently shown to improve healthspan and reduce deaths due to age-related causes in the rhesus monkey (Colman et al. 2009).

The nematode *Caenorhabditis elegans* has been a particularly fruitful model organism for identifying and characterizing factors that modulate aging. Several large-scale RNAi screens for enhanced life span in *C. elegans* have led to the identification of approximately 300 longevity-related genes, including many genes involved in nutrient response, mitochondrial function, chemosensation, and mRNA translation (Dillin et al. 2002; Lee et al. 2003; Hamilton et al. 2005; Hansen et al. 2005; Chen et al. 2007; Curran & Ruvkun 2007). Studies

in *C. elegans* have been instrumental in defining the role of insulin-like signaling in aging, with several mutations that reduce insulin-like signaling having been shown to increase life span. These include loss of function alleles of insulin-like receptor *daf-2* (Kenyon et al. 1993; Kimura et al. 1997; Gems et al. 1998), the PI-3 kinase *age-1* (Friedman & Johnson 1988; Morris et al. 1996), and Akt kinase homologs *akt-1*, *akt-2*, and *sgk-1* (Hertweck et al. 2004; Hamilton et al. 2005; Oh et al. 2005). A defining feature of these mutations and others in the insulin-like pathway is that they require the FOXO transcription factor DAF-16 in order to increase life span (Lin et al. 1997; Ogg et al. 1997).

As in other organisms, DR has been studied extensively in *C. elegans*. Several different methods for extending life span through DR have been described in nematodes (Greer & Brunet 2009; Mair et al. 2009). These include genetic manipulations that reduce food consumption due to a defect in pharyngeal pumping (referred to as *eat* mutants (Lakowski & Hekimi 1998)), as well as environmental methods of food restriction ranging from dilution of the bacterial food source (Klass 1977) to complete removal of the bacterial food during adulthood (Kaerberlein et al. 2006; Lee et al. 2006). With one exception (Greer et al. 2007), methods of DR in *C. elegans* extend life span by a mechanism that is genetically distinct from reduced insulin-like signaling, based on the observation that life span extension does not require DAF-16.

Recently, oxygen availability has emerged as another important environmental factor influencing the aging process in *C. elegans* (Kaerberlein & Kapahi 2009). In the natural environment, *C. elegans* are soil dwellers often found in compost. These animals likely experience oxygen concentrations ranging from atmospheric (around 20%) to only a few percent, or less. Appropriately altering cellular physiology and gene expression in response to changing oxygen availability is therefore essential for their growth and survival.

The hypoxia-inducible factor (HIF) is a highly conserved transcriptional regulator of genes necessary for the response to reduced oxygen availability, collectively referred to as the hypoxic response. HIF exists as a heterodimer of the regulated HIF- α subunit and the constitutively expressed HIF- β subunit (Wang et al. 1995). Under normoxic conditions, HIF- α is modified by oxygen-dependent proline hydroxylases (PHDs), leading to ubiquitination by the von Hippel Lindau protein (pVHL) and targeted proteasomal degradation. The hydroxylation and subsequent ubiquitination reaction is inhibited by hypoxia, causing stabilization of HIF- α and induction of the hypoxic response (Kim & Kaelin 2003). This hydroxylation and ubiquitin-mediated regulation of HIF- α stability is conserved from nematodes to humans.

The *C. elegans* hypoxic response has been characterized largely through genetic and microarray studies. HIF- α is encoded by the *hif-1* gene, and oxygen-dependent proteasomal degradation of the HIF-1 protein is mediated by the EGL-9 prolyl hydroxylase and the VHL-1 cullin E3 ligase subunit. While EGL-9 and VHL-1 are both necessary for HIF-1 targeted degradation, EGL-9 and perhaps VHL-1 also independently regulate HIF-1 activity (Shen et al. 2006). Microarray and qPCR analyses show that EGL-9 down-regulates HIF-1 activity in the absence of VHL-1, and *vhl-1* and *egl-9* transcriptional profiles differ significantly. Deletion of *hif-1* causes sensitivity to hypoxia, and many mRNAs are induced

by hypoxia or by deletion of either *vhl-1* or *egl-9* (Bishop et al. 2004; Shen et al. 2005; Mabon et al. 2009b). Interestingly, a relationship between longevity and resistance to hypoxic stress has been noted from the fact that several mutant alleles of *daf-2* confer resistance to hypoxia (Scott et al. 2002; Mabon et al. 2009a), and a recent genome-wide RNAi screen identified 11 previously known longevity genes as regulators of hypoxia resistance (Mabon et al. 2009a).

Stabilization of HIF-1 increases life span in *C. elegans*

Mehta et al. (Mehta et al. 2009) were the first to establish HIF-1 as a longevity factor by showing that stabilization of HIF-1 is associated with a significant increase in life span of approximately 30–50% (Mehta et al. 2009). Stabilization of HIF-1 in this study was accomplished by RNAi knockdown or deletion of *vhl-1*. These findings were replicated in a second study of *vhl-1* mutant animals (Muller et al. 2009). Importantly, loss of *vhl-1* also appeared improve age-associated measures of healthspan in *C. elegans*, including resistance to amyloid beta and polyglutamine toxicity, as well as accumulation of auto-fluorescent age pigment (Mehta et al. 2009). Both studies of *vhl-1* mutant animals established that the life span extension was largely dependent on HIF-1, consistent with the model that loss of *vhl-1* slows aging by stabilization of HIF-1 (Figure 1). This model was further solidified by a third study showing that stabilization of HIF-1 by transgenic expression of a non-degradable allele is sufficient to increase life span (Zhang et al. 2009)

Although the mechanism by which stabilization of HIF-1 slows aging remains to be determined, evidence suggests that HIF-1 acts in a genetic pathway that is distinct from both insulin-like signaling and DR (Kaeberlein & Kapahi 2009). Life span extension from deletion of *vhl-1* or expression of non-degradable HIF-1 occurs even in animals carrying a homozygous null allele of *daf-16*, and knockdown of *daf-2* is sufficient to increase life span in *hif-1* deletion mutants (Mehta et al. 2009; Muller et al. 2009; Zhang et al. 2009). Likewise, two different methods of DR, mutation of *eat-2* and bacterial deprivation, have been shown to increase life span in *hif-1* knockout animals (Mehta et al. 2009). Thus, HIF-1 and the hypoxic response appear to slow aging in a new longevity pathway that is distinct from both insulin-like signaling and DR.

In addition to knockdown of *vhl-1*, Mehta et al. (Mehta et al. 2009) also reported that maintaining animals under hypoxia at 0.5% oxygen is sufficient to increase adult life span. HIF-1 is known to be stabilized under these conditions (Jiang et al. 2001), suggesting the hypothesis that life span extension from hypoxic growth occurs by a mechanism similar to life span extension from stabilization of HIF-1 under normoxic conditions. Although it seems likely to be at least partially correct, this hypothesis has yet to be experimentally validated.

Deletion of *hif-1* increases life span in *C. elegans*

In the same study where they reported that transgenic expression of non-degradable HIF-1 increases life span, Zhang et al. (Zhang et al. 2009) made the surprising observation that deletion of *hif-1* can also increase life span. This differed from Mehta et al. (Mehta et al. 2009), who observed no change in life span upon deletion of *hif-1*. Zhang et al. (Zhang et al.

2009) further examined the long life span of *hif-1* deletion mutants, and found that the extension was fully suppressed by mutation of *daf-16*. This led them to conclude that loss of *hif-1* increases life span by a mechanism similar to reduced insulin-like signaling (Figure 1).

A prior study from the Kapahi group had also concluded that deletion of *hif-1* is sufficient to increase life span in *C. elegans* (Chen et al. 2009). Unlike Zhang et al. (Zhang et al. 2009), they reported that life span extension from deletion of *hif-1* occurred independently of *daf-16* (Chen et al. 2009). They also found that *hif-1* deletion failed to further extend the life spans of animals subjected to DR or animals deleted for the S6 kinase homolog *rsks-1*. S6 kinase is a substrate of the target of rapamycin kinase, which is thought to act downstream of DR to promote longevity in *C. elegans* and a variety of other species (Hansen et al. 2008; Stanfel et al. 2009). Interestingly, they also found that mutation of *ire-1* or knock-down of *xbp-1*, two factors required for the ER stress response, blocked life span extension from loss of *hif-1* (Chen et al. 2009). Based on these observations, they proposed that HIF-1 acts as a negative regulator of longevity in a pathway downstream of DR and TOR signaling and upstream of the ER stress response (Figure 1).

A more recent study by Bellier *et al.* (Bellier et al. 2009) has added additional confirmation that *hif-1* can act as a negative regulator of longevity. In studying the role of HIF-1 in defense against bacterial toxins they found that deletion of *hif-1* led to increased life span, despite its being required for toxin defense (Bellier et al. 2009). Interestingly, they also found that animals with activated HIF-1, such as *vhl-1* or *egl-9* mutants, are resistant to multiple pore-forming toxins and toxic bacterial strains. It is unknown whether the resistance to bacterial toxins of *vhl-1* mutant animals is related to their increased life span; however, at least some *egl-9* loss of function mutants are not long-lived (Bellier et al. 2009; Chen et al. 2009), suggesting that enhanced longevity does not always accompany toxin resistance.

HIF-1 and aging: consensus amid confusion

The picture that has emerged for the role of HIF-1 in aging is complex, and a bit confusing. Three independent studies have shown that stabilization of HIF-1 can increase life span, while three studies have found that deletion of *hif-1* can increase life span. There seems to be consensus that life span extension from stabilization of HIF-1 occurs by a mechanism that is genetically distinct from both insulin-like signaling and DR. There is no indication to date, however, as to what that mechanism might entail. One possible explanation for HIF-1 mediated lifespan extension is that HIF-1 down-regulates mitochondrial activity (Tormos & Chandel 2010). RNAi knockdown of several dozen mitochondrial factors has been shown to increase life span in *C. elegans* (Dillin et al. 2002; Lee et al. 2003; Rea et al. 2007), and it may be that HIF-1 activation promotes longevity via a similar mechanism. Alternatively, HIF-1 could act as a stress response factor to up-regulate protection against multiple stresses, similarly to other known aging modifiers such as DAF-16 and SKN-1. HIF-1 and DAF-16 are known to share a subset of target genes (McElwee et al. 2004; Hoogewijs et al. 2007), suggesting this is a reasonable explanation. A more complete understanding of HIF-1-mediated life span extension will likely be accomplished through experiments

characterizing factors involved in longevity-control both upstream and downstream of HIF-1.

One question that remains to be resolved is why loss of function mutations in *egl-9* do not always increase life span. *Egl-9* mutants show stabilization of HIF-1 and robust expression of HIF-1 target genes, usually exceeding that of *vhl-1* mutants (Chuan Shen 2006; Shao et al. 2009). Why then are these animals not long-lived? One possible explanation is that HIF-1 expression in *egl-9* mutants exceeds a threshold that is beneficial, and actually becomes detrimental – “too much of a good thing”, so to speak. Consistent with this possibility, RNAi knockdown of *egl-9*, which only partially impairs *egl-9* expression, can increase life span and enhance resistance to polyglutamine and amyloid beta toxicity (Mehta et al. 2009). It is also possible that pleiotropic effects associated with mutation of *egl-9*, but not *vhl-1*, limit longevity in a manner that prevents life span extension from HIF-1 stabilization in these animals. For example, *egl-9* mutants have a defect in egg laying that is not apparent in *vhl-1* deletion animals (Trent et al. 1983; Epstein et al. 2001).

Compelling evidence has been provided for two different mechanisms for how deletion of *hif-1* might be increasing life span. The first model, that loss of HIF-1 leads to activation of DAF-16, is satisfying given the abundant data that DAF-16 acts as a master regulator coordinating growth, stress resistance, and longevity (Landis & Murphy 2010). This model is also consistent with the noted links between mutations in the insulin-like pathways and resistance to hypoxic stress (Scott et al. 2002; Mabon et al. 2009b). DAF-16 and HIF-1 are both stress regulated transcription factors, and there is some evidence that they have a subset of shared targets (McElwee et al. 2004; Hoogewijs et al. 2007). One possibility is that loss of HIF-1 provides sufficient stress, even under normoxia, to induce DAF-16 and extend life span. If correct, future studies may shed light on the mechanism by which DAF-16 is activated and which DAF-16 target genes are involved in the enhanced longevity of *hif-1* mutant animals.

The alternative model proposed by Chen et al. (Chen et al. 2009) in which HIF-1 negatively regulates longevity downstream of TOR by repressing ER stress resistance is intriguing, as well. There is accumulating evidence in mammals that HIF, mTOR, and the ER unfolded protein response (UPR) interact with each other (reviewed in (Wouters & Koritzinsky 2008)). The data on TOR and HIF are somewhat mixed, but suggest that while hypoxia inhibits signaling from mTOR and lowers translation rates (Arsham et al. 2003; Sofer et al. 2005; Koritzinsky et al. 2006), HIF-1 is preferentially translated in a TOR dependent manner (Stein et al. 1998; Lu et al. 2004). Both the TOR pathway and hypoxia are known to activate the UPR, and the UPR is known to protect cells from hypoxia (Romero-Ramirez et al. 2004; Bi et al. 2005). Thus, while the mechanisms for signaling between TOR, HIF and the UPR are not yet clear, these pathways clearly overlap and future work is likely to uncover additional links between them.

Regardless of the mechanism of life span extension from deletion of *hif-1*, the fact that loss of *hif-1* is sufficient to increase life span under normoxic conditions is striking. Since *hif-1* is not stabilized in these animals, the longevity phenotype must result from a loss of basal

activity of HIF-1. Basal activity of HIF-1 in normoxia is an area that has not been adequately explored, but clearly warrants further examination.

A role for HIF in mammalian aging?

Perhaps not surprisingly, HIF function seems to be relatively simple in *C. elegans* compared with mammals. Current evidence suggests that worms have only one HIF transcription factor (HIF-1), one proline hydroxylase specific for HIF-1 (EGL-9), a primary HIF-1 E3 ubiquitin ligase (VHL-1) and one negative effector protein (RHY-1) (Bishop et al. 2004; Shen et al. 2005; Shen et al. 2006). Loss of function of any of the negative regulators of HIF-1 leads to variable increases in HIF-1 activity. In addition, all three factors are likely to have other protein targets, resulting in pleiotropic effects that, as mentioned previously with respect to EGL-9, can complicate observations (Shen et al. 2006; Shao et al. 2009). Despite this, it is clear that increased HIF-1 activity can lead to increased lifespan and resistance to multiple stresses including proteotoxicity (Bellier et al. 2009; Mehta et al. 2009; Budde & Roth 2010). It is also clear that, in at least certain conditions, loss of HIF-1 function under normoxic conditions leads to increased lifespan and resistance to certain stresses (Bellier et al. 2009; Zhang et al. 2009). The seemingly paradoxical idea that *C. elegans* maintain HIF-1 levels at suboptimal levels for lifespan and stress resistance may arise from differences between the laboratory conditions used to study *C. elegans* and their natural environment or may be due to currently unappreciated selective advantages associated with this mode of HIF-1 regulation.

Whereas the hypoxia response system is relatively simple in worms, the mammalian system is much more complex. There are three HIF α proteins in mammals (HIF-1 α , HIF-2 α , and HIF-3 α), the first two of which are known to activate an overlapping but distinct set of genes (Schofield & Ratcliffe 2004; Koh et al. 2008). While there is only one known HIF ubiquitin ligase in mammals (pVHL), there are three proline hydroxylases (PHD-1, PHD-2, and PHD-3), and a myriad of other possible effectors of HIF function. Also, because mammals have much less surface area to volume than worms, cellular oxygen tension is much lower in mammals and is under homeostatic control. Thus, mammalian hypoxic response is important for both cellular and systemic response to changing oxygen conditions (Koh et al. 2008).

Hif-1 α in mammals was discovered by its ability to increase production of erythropoietin (Wang et al. 1995), but was quickly linked to the hereditary syndrome, von-Hippel Lindau (VHL) disease (Kaelin 2002). This heterozygous disease is characterized by development of angiomas in the retina and central nervous system and carcinomas in the kidneys (Kaelin 2002). Because HIF-1 α increases are involved in VHL disease and are a frequent negative predictor in metastatic tumors (Semenza 2009), there is little data on the role of HIF-1 α in mammalian aging. It is clear that globally disrupting pVHL function in mammals is unlikely to increase life span as it does in worms, and it is also clear that knocking-out HIF-1 α or HIF-2 α in mammals will not show enhance life span, because both proteins are necessary for development (Iyer et al. 1998; Ryan et al. 1998). Therefore, the relationship between HIF-1 α regulation and aging will be more complicated to discern in mammals than in *C. elegans*.

Despite the complexities involved, there are signs that HIF-1 α may play an important role in mammalian aging. Studies have shown that HIF-1 α protein levels increase with age, perhaps due to increased hypoxia or increased oxidative stress, but that this increase is mitigated by DR (Kim et al. 2002; Kang et al. 2005). Interestingly, one of these studies showed increased HIF-1 α binding to HIF-1 responsive elements (HREs) in young dietary restricted mice, which was stable over time, suggesting that preemptive HIF-1 α increases may help prevent age-associated damage. Another study examining PHD3 in rats found that PHD3 levels increase with age in liver, heart and skeletal muscle, and that this increase correlates with a decrease in HIF-1 α activity (Rohrbach et al. 2008). Dietary restriction was able to mitigate the increase PHD3 and maintain HIF-1 α activity in aged rats, raising the possibility this activity may be involved in DR's protective effects.

A separate study showing that HIF-1 α activity improves the inotropic responses of aged cardiac myocytes (Tan et al. 2010) further suggests a role for HIF-1 α in resistance to age-related disease. Many other age-related diseases, including Alzheimer's disease, Parkinson's disease, and diabetes, involve hypoxic conditions that can worsen the disease. Studies examining A β accumulation in Alzheimer's disease suggest that increased HIF-1 α tends to be protective in disease free states, but that increased HIF-1 α levels are also a sign of advanced disease progression (Ogunshola & Antoniou 2009). This seemingly contradictory data may stem from the role of HIF-1 α in stress response, in that its up-regulation can be either protective or reactive in nature, and the context of measurement is important (Ogunshola & Antoniou 2009).

Recent studies have linked mammalian HIF to another aging-related group of proteins, the sirtuins. Sirtuins are a family of NAD-dependent protein deacetylases named for their founding member yeast Sir2. Overexpression of Sir2 was first shown to increase replicative life span in yeast (Kaerberlein et al. 1999), and Sir2-orthologs have been subsequently shown to similarly modulate longevity in worms (Tissenbaum & Guarente 2001) and flies (Rogina & Helfand 2004). Although activation of mammalian sirtuins has not been found to increase life span, the mouse Sir2 ortholog SIRT1 has been linked to multiple age-related diseases (Finkel et al. 2009), and knockout of a second Sir2 homolog SIRT6 results in genomic instability and phenotypes resembling accelerated aging (Mostoslavsky et al. 2006). Dioum et al. (Dioum et al. 2009) reported that SIRT1 can bind and deacetylate HIF-2 α in order to promote HIF activation in response to hypoxia. The authors suggest that HIF-2 α is more responsive to subtle changes in oxygen than HIF-1 α , and that SIRT1 is necessary for its activation and "fine-tuning" of the hypoxic response. A separate study by Zhong *et al.* (Zhong et al. 2010) suggests that HIF-1 α is regulated by SIRT6. The authors contend that SIRT6 deacetylates histones near HIF-1 α target genes, deactivating their promoters. They provide evidence that the extreme hypoglycemia of SIRT6 knockout mice results from cellular glucose uptake caused in large part by HIF-1 α activation. This work not only links HIF to an important aging-related protein family, but suggests another potential therapeutic role for HIF-1 α activation by enhancing glucose clearance in age-related diseases such as type II diabetes.

In sum, the data to date indicate that the hypoxic response is a promising new avenue for aging research. In just a year since the first published paper on HIF-1 and aging in *C.*

elegans, multiple studies have confirmed and extended the initial work and have suggested that HIF-1 plays a central role in worm aging. While the role of HIF in mammalian aging and age-related disease will surely have increased complexity, we are optimistic that the lessons learned from *C. elegans* will provide insight into this problem. Further studies on the mechanism(s) by which HIF-1 acts as both a positive and negative regulator of longevity in worms should provide a better understanding of the pathways involved and a potential starting point for more detailed mammalian studies and, perhaps, therapeutic modulation of HIF activity.

Acknowledgments

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Figure 1. Models of HIF-1 in aging

Five papers published in 2009 describe a role for HIF-1 as a longevity regulator in *C. elegans*. Three of these papers (Mehta et al, Muller et al, and Zhang et al.) define the hypoxic response as an important longevity-promoting pathway that is distinct from insulin-like signaling and dietary restriction. Three papers (Zhang et al., Chen et al., Bellier et al.) also identify a longevity-limiting role for HIF-1 (deletion increases life span), but differ on the downstream mechanism. Zhang et al. report that life span extension from loss of HIF-1 requires DAF-16, while Chen et al. report that life span extension from loss of HIF-1 is DAF-16-independent and acting through IRE-1. Bellier et al. do not address potential downstream mechanisms of life span modulation by HIF-1.