

Insulin/insulin-like growth factor-1 signalling (IIS) based regulation of lifespan across species

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Abstract An organism's well-being is facilitated by numerous molecular and biochemical pathways that ensure homeostasis within cells and tissues. Aging causes a gradual let-down in the maintenance of homeostasis due to various endogenous and environmental challenges, leading to amassing of damages, functional deterioration of different tissues and vulnerability to ailments. Nutrient sensing pathways that maintain glucose homeostasis in body are involved in regulation of aging. Insulin/insulin-like growth factor-1 (IGF-1) signalling (IIS) pathway was the first nutrient sensing pathway discovered to affect the aging process. This pathway is highly conserved and the most studied among different organisms. Epigenetic machineries that include DNA and histone modifying enzymes and various non-coding RNAs have been identified as important contributors to nutrition-related longevity and aging control. In this report, we present the homology and differences in IIS pathway of various organisms including worm, fly, rodent and human. We also discuss how epigenome

remodelling, chromatin based strategies, small and long non-coding RNA are involved to regulate multiple steps of aging or age-related insulin homeostasis. Enhanced study of the role of IIS pathway and epigenetic mechanisms that regulate aging may facilitate progressive prevention and treatment of human age-related diseases.

Keywords Insulin signalling · FOXO · Longevity · DNA methylation · Histone modification · Non-coding RNA

Introduction

An organism's lifespan is stated as the sum of damaging changes that occur in its body and the repair and maintenance mechanisms that respond to damage (Johnson et al. 1999). Both environmental and genetic components determine the lifespan of an organism (Paaby and Schmidt 2009). After discoveries were made in the worm *Caenorhabditis elegans* (Kenyon et al. 1993), it was found that many genetic pathways that govern lifespan are highly conserved from nematode to human (Garofalo 2002; Holzenberger et al. 2003; Nakae et al. 2002). Loss or gain of function mutations were employed for the identification of gerontogenes (that affect aging and lifespan) and hundreds of these genes were identified (Guarente and Kenyon 2000). Insulin/IGF-1(IIS), PI3K, TOR, MAPK, AMPK, PKC, NF- κ B, TGF- β , Notch and

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WNT are some of the signalling pathways that regulate aging (Moskalev et al. 2014). They maintain energy levels, cellular pliability, homeostasis, growth and reproduction of an organism under favourable conditions (Barzilai et al. 2012). Under stressful conditions, these pathways repress growth and activate stress-resistance proteins (Koubova and Guarente 2003; Tatar et al. 2003). Insulin/insulin-like growth factor-1 (IGF-1) signalling (IIS) pathway was the first pathway to be established for aging and was identified in *C. elegans* through mutations of the genes *age-1* encoding phosphoinositide 3-kinase (PI3K) and *daf-2* encoding IGF-1 receptor (Kenyon et al. 1993). This pathway is greatly conserved among *C. elegans*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, rodents and humans and regulates nutrient homeostasis, growth, aging and longevity (Barbieri et al. 2003).

The common denominators of aging in different organisms include genomic instability (Moskalev et al. 2013), telomere attrition (Blackburn et al. 2006), mitochondrial dysfunction (Harman 1965), stem cell depletion (Shaw et al. 2010), loss of proteostasis (Powers et al. 2009), cellular senescence (Campisi and di Fagagna 2007), altered intercellular communications (Russell and Kahn 2007), deregulated IIS pathway (Barzilai et al. 2012) and epigenetic alterations (Talens et al. 2012). Epigenetic modifications play numerous key roles in regulatory events that alter gene expression and hence impact other hallmarks of aging (Jung and Pfeifer 2015). The inherited epigenetic status undergoes numerous changes by environmental stimuli or stochastic errors that either benefit the organism or accelerate aging (Bennett-Baker et al. 2003; Cedar and Bergman 2012; Salpea et al. 2012).

In this report, we discuss the role of IIS pathway in aging of worm, fly, rodent and human, and its epigenetic regulation through different epigenetic machineries including non-coding RNA. We focus on non-coding RNA and the chromatin for reasons that regulatory RNA and epigenomic modulations are involved in transcriptional changes related to aging.

Insulin/insulin-like growth factor-1 signalling circuit and longevity

IIS pathway acts through the PI3-kinase (PI3K)/AKT kinase signalling cascade (explained in detail by (De Meyts 2016)) and is initiated by insulin-like peptides.

These peptides are secreted in response to food or the sensory perception of food (Fig. 1) and bind to insulin/IGF tyrosine kinase receptor (Wolkow et al. 2002). Activated receptor transduces the signal to the phosphatidylinositol 3-kinase (PI-3K) which in turn converts phosphatidylinositol 4,5-bisphosphate (PIP₂) into phosphatidylinositol 3,4,5-bisphosphate (PIP₃) (Morris et al. 1996). Increased levels of PIP₃ activate the protein kinases, leading to phosphorylation and retention of the forkhead box O (FOXO) transcription factors in the cytoplasm (Ogg et al. 1997). Under attenuated insulin signalling conditions, unphosphorylated FOXO gets transported to the nucleus to aid in the transcription of genes involved in organism longevity, of which shall be discussed in the next section.

A few other pathways also interact with IIS pathway to regulate lifespan. They are the target of rapamycin (TOR) signalling pathway which responds to nutrient levels (Hafen 2004; Partridge et al. 2011), the Jun-NH₂-terminal kinase (JNK) pathway (Karpac and Jasper 2009; Wang et al. 2005) which responds to stress levels and the transforming growth factor- β (TGF- β) signalling pathway (Narasimhan et al. 2011). In addition, the IIS pathway also controls lifespan through secondary hormones such as the Juvenile hormone (JH) and Ecdysone hormone (Richard et al. 2005). Central nervous system (CNS) plays a significant part in the longevity of varied organisms. Though lowered insulin signalling in the CNS can extend lifespan, IIS has been documented as being neuroprotective and vital for the development and survival of neurons. Hence, manipulation of the right signalling component in the right tissue is required to extend organism lifespan with less damaging effects or with even better health and function (Broughton and Partridge 2009).

Forkhead box O (FOXO)

FOXO proteins play the most pivotal role in IIS/PI3K/Akt signalling pathway (Brunet et al. 1999; Dong et al. 2008). They act as “master-switch” for cells to adapt and show metabolic stability under conditions of food shortage and stress (Horst and Burgering 2007). They belong to the Forkhead family of transcription factors, specified by a conserved DNA binding domain called the ‘Forkhead box’ (FOX). Forkhead gene was

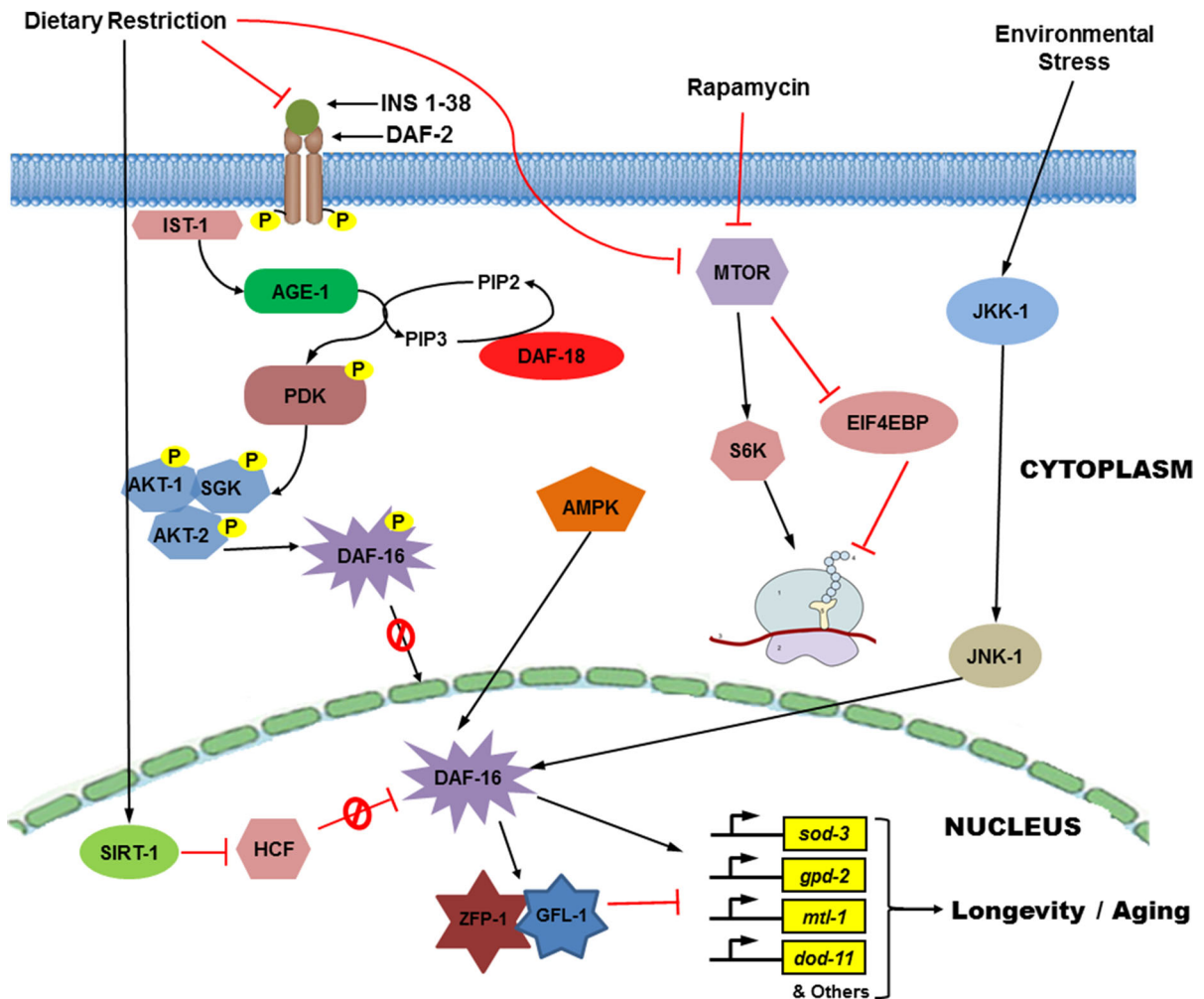


Fig. 1 Pathways that regulate aging in *C. elegans*. Activation of insulin/IGF signalling (IIS) pathway (see text for names of genetic homologs found in *D. melanogaster*, rodents and human) prevents translocation of DAF-16/FOXO transcription factor to the nucleus. Blocking of IIS pathway causes FOXO activation or repression of target genes that promote longevity. FOXO is also regulated by AMPK, JNK and SIRT1. Dietary restriction (DR) obstructs IIS pathway and TOR pathway whereas it activates SIRT1. TOR pathway results in an increase in autophagy and suppression of translation. Stress stimulates

JNK pathway, which involves the activation of FOXO. Chromatin modifier zinc finger protein 1 (ZFP-1) acts as a common mediator of IIS and DR mediated lifespan regulation. ZFP-1 interacts with glioma amplified sequence-41 (GFL-1 in *C. elegans*). DAF-16/FOXO directly binds and regulates the expression of the different isoforms of zfp-1 as well as gfl-1. ZFP-1/GFL-1 in turn negatively regulates the expression of DAF-16/FOXO target genes such as *sod-3*, *gpd-2*, *mtl-1*, *dod-11*, by forming feed-forward loops that control the amplitude and duration of target gene expression.

initially discovered in fly whose alteration lead to abnormal head-like structures that resembled a fork (Carter and Brunet 2007). Currently, they are much researched for their many roles involved in organism survival and lifespan and have been reviewed in detail by (Eijkelenboom and Burgering 2013; Kim et al. 2015; Martins et al. 2015; Proshkina et al. 2015). FOXO proteins respond to both nutritional and stress signals. Dietary restriction (DR) or stress conditions

cause nuclear translocation of FOXO, to promote the expression of target genes that induce stress resistance, damage repair, and cell cycle arrest (Greer and Brunet 2008; Shimokawa et al. 2015).

The mechanisms by which the amplified activity of FOXO leads to lifespan extension are still unclear (Papatheodorou et al. 2014). FOXO increases insulin sensitivity by prompting the expression of insulin receptor and insulin receptor substrate 2 (IRS2)

through feedback control (Puig and Tjian 2005). In addition, FOXO activation enables cell survival under conditions of food shortage by induction of cell cycle arrest and quiescence, reminiscent of the dauer phase induced in *C. elegans* (van der Horst and Burgering 2007). FOXO also delivers a metabolic shift from glucose to lipid oxidation, suppression of inflammation and enhanced mitochondrial biogenesis (van Heemst 2010). Identification of FOXO transcriptional targets has revealed a second tier of transcription factors that regulate a variety of other downstream responses (Alic et al. 2011). However, depending on the cell type, quality and strength of the stress, FOXO activity can also shift the cellular response from survival towards apoptosis (van Heemst 2010). FOXO activity in response to stress prevents aging and age-dependent impairments such as cancer, neurodegenerative diseases and diabetes (Calnan and Brunet 2008) and advocate organism longevity (Demontis and Perrimon 2010; Soerensen et al. 2015; Sun et al. 2015; Zeng et al. 2015).

Homologs of FOXO are DAF-16 in worm, dFOXO in fly (Puig et al. 2003; Tatar et al. 2003) (discussed in the next section) and FOXO1 (FKHR), FOXO3 (FKHRL1), FOXO4 (AFX) and FOXO6 in mammals (Papanicolaou et al. 2008). In mammals, a higher FOXO1 activity is observed in organs that control glucose homeostasis such as liver, pancreas, adipose tissue and skeletal muscle where, FOXO1 gets activated under low glucose conditions (Eijkelenboom and Burgering 2013; Zhang et al. 2011). High FOXO4 levels are exhibited in skeletal muscles, while FOXO3 is expressed abundantly in the brain, heart, kidney and spleen (Morris et al. 2015b). FOXO6 is observed in the brain, both during development and in adulthood (Eijkelenboom and Burgering 2013; Webb and Brunet 2014). FOXO3 presents an exciting avenue for clinical investigation of aging in human as it has been identified as the strongest example of longevity gene (Morris et al. 2015b). The association of FOXO3 with human longevity has been researched by many individual groups on different long-lived subjects (Anselmi et al. 2009). Studies of single-nucleotide polymorphisms (SNPs) associated with human FOXO3 gene in a group of American men of Japanese ancestry (known to live long) as well in other diverse human populations revealed the association of FOXO3 SNPs with longer lifespan (Murabito et al. 2012; Willcox et al. 2008).

Along with organism aging, FOXOs are also highly investigated for other intrinsic roles such as modulation of neuronal activities under low-insulin signalling conditions (Kaletsky et al. 2016), maintenance of germline progenitor cells in *C. elegans* (Qin and Hubbard 2015) and the maintenance of lower blood pressure and essential hypertension (Morris et al. 2015a).

IIS circuit across species

In *Caenorhabditis elegans*

C. elegans was among the first and the most intensively researched organisms to study longevity. It has 38 insulin-like ligands (Broughton and Partridge 2009) that initiate insulin signalling. IIS pathway in *C. elegans* includes proteins coded by *daf-2*, *age-1*, *akt-2*, *daf-16* and *daf-18* and they mediate regulation of dauer diapause, reproduction and adult lifespan (Claeys et al. 2002). Aging in *C. elegans* is influenced in a varied manner by signals from different tissues, with the most important signal from the CNS (Wolkow et al. 2000). These signals coordinate the aging rate between different tissues to create homeostasis, thereby affecting general aging of the whole organism. Communication between the tissues is intermediated by insulin-like peptide INS-7, which is regulated by *daf-16* activity in the intestine. The *daf-16* activity in the intestine in turn influences *daf-16* activity in other tissues (Murphy et al. 2007). Mutations in the *daf-2* gene extend worm lifespan along with a high release of enzymes involved in antioxidant activity (Barbieri et al. 2003). Working synergistically with TGF- β signalling pathway, *daf-16* also cooperates with nematode SMAD proteins to mediate the activation of genes that impact nematode metabolism and development (Ogg et al. 1997). Reduced IIS also delays *C. elegans* aging through the activation of dauer-related processes during adulthood (McElwee et al. 2004; Partridge and Harvey 1993). However, certain reduced IIS conditions have been found to confer robust lifespan extension without dauer formation (Arantes-Oliveira et al. 2003; Gems et al. 1998) but through the involvement of NF-E2-related factor orthologue SKN-1. SKN-1 acts similar to DAF-16. It upturns the expression of collagens and other extracellular matrix genes that mediate adulthood

extracellular matrix remodelling, which is an essential feature for longevity assurance (Ewald et al. 2015).

Of various environmental methods, DR is the most effective in increasing longevity through insulin signalling pathway. Various DR methods such as genetic mutations that lower pharyngeal pumping rate, changes in dilution of bacteria in liquid culture, changes in components of liquid media and the use of ‘DR mimetics’ have been employed to increase survivorship in *C. elegans* (Greer and Brunet 2009; Mair and Dillin 2008). In worms, *daf-16* has been shown to be required for lifespan extension through DR (Greer and Brunet 2009). However, the use of many other model organisms have exposed DR to also affect longevity independent of *daf-16* (Kenyon 2010). Along with DAF-16, transcription factor PHA-4 also responds to certain DR methods in *C. elegans* (Mair and Dillin 2008).

IIS pathway is also regulated by sirtuins (Burnett et al. 2011). In worm, overexpression of *sirt-2.1* activates *daf-16* by interacting with 14-3-3 proteins where, SIRT-2.1 and 14-3-3 act in parallel to IIS pathway to activate *daf-16* and extend lifespan (Berdichevsky et al. 2006). *sirt-2.1* can also turn on *daf-16* directly by deacetylation (Kenyon 2005). The finding that sirtuins can deacetylate FOXO proteins directly, and the fact that IIS pathway mutants do not require *sirt-2.1* for longevity, demonstrates that sirtuins in worm can impact FOXO and lifespan independent of IIS pathway (Kenyon 2010).

In *Drosophila melanogaster*

The IIS pathway in *Drosophila* is similar to that of *C. elegans* and consists of insulin/IGF receptor (INR), insulin receptor substrate (CHICO), PI3K and PI3K target protein PKB (Weinkove and Leivers 2000). There are eight insulin-like peptides (DILPs) in *Drosophila* (Brogiolo et al. 2001; Garelli et al. 2012; Gronke et al. 2010). The *dilp* genes show divergent temporal expression patterns (Broughton et al. 2005) and are expressed in varied sites (Nassel et al. 2015). DILPs are majorly secreted in the median neurosecretory cells of the brain called as insulin-producing cells (IPCs) (Ikeya et al. 2002). Removal of neurosecretory cells from *Drosophila* brain was found to increase organism lifespan and exhibit resistance to oxidative stress and starvation (Broughton et al. 2005). Among the eight DILPs encoded, DILP2 is

homologous to human insulin and has been widely studied for lifespan regulation (Broughton et al. 2008). The *dilp2* null-mutant flies live longer (Gronke et al. 2010). DILP6, which is largely produced in the fat body, also plays an important role as lifespan regulator (Okamoto et al. 2009). Overexpression of *dilp6* in the abdominal fat body represses *dilp2* in the brain, suggesting synergistic effects of dInR antagonist DILP6 and agonist DILP2 for longevity (Bai et al. 2012). On the whole, cross talk between DILPs of the brain and fat body influence IIS mediated longevity. Hypomorphic mutation (*teq⁰¹⁷⁹²*) or downregulation of the gene Tequila in fly neurons, drastically increases longevity through reduced circulation of DILP2, reduced Akt phosphorylation, small body size and changed glucose homeostasis (Huang et al. 2015). Genetic inhibition of negative regulators of dFOXO such as DILPs (Broughton et al. 2005), dInR (Tatar et al. 2001) and CHICO (Clancy et al. 2001) extend the lifespan of *Drosophila*. On the other hand, overexpression of IIS negative regulators such as dPTEN or dFOXO extend the fly lifespan (Demontis and Perrimon 2010). Mutation of *chico* regulates aging by interacting with eukaryotic translation initiation factor 4E binding protein (4E-BP) (Bai et al. 2015). Tissue-specific overexpression of IIS components also regulate the fly lifespan in a cell non-autonomous way such as, through upregulation of dFOXO in the adult head fat body (Hwangbo et al. 2004), muscle-specific overexpression of either dPTEN or dFOXO (Demontis and Perrimon 2010) and the ablation of IPCs (Haselton et al. 2010). Stress responsive JNK pathway also requires FOXO to promote longevity in *Drosophila*. JNK counteracts IIS and translocates FOXO to the nucleus (Wang et al. 2005). Fly adult diapause results in a drastically prolonged lifespan with no senescence, increased stress resistance, somatic maintenance, developmental arrest and reallocated energy resources. IIS is one among the many pathways to get downregulated during diapause (Kucerova et al. 2016).

In response to DR, FOXO does not play any essential role for lifespan extension as flies knocked out of *foxo* live long under DR. But, it is suggested that the presence of an active FOXO affects the normal response to DR by changing the expression of FOXO target genes (Giannakou et al. 2008). Overexpression of *foxo* under DR is also associated with a reduction in DILP2 (Min et al. 2008).

Studies on *dsirt2* (*Drosophila sirtuin 2*) show that SIRT2 and its orthologs play a crucial role in metabolic homeostasis and IIS (Liang et al. 2009; Schenk et al. 2011). *dSIRT2* present in the fat body of fly is crucial for maintaining the metabolic regulatory network across the organism and for survival during starvation (Banerjee et al. 2012a, b).

In rodents

Altering genes involved in IIS pathway and the growth hormone (GH) cause longevity in mice (Bartke et al. 2013; Longo and Finch 2003). Deficits in GH and IGF-1 levels show reduced body size, delay in sexual maturation and age-related diseases, whereas elevated plasma levels of GH lead to increased body mass, early onset of reproductive maturity and senescence, high frequency of cancer occurrence and diminished lifespan in mice (Bartke et al. 2013). The GH-deficient mice also show high levels of antioxidant enzymes and increased stress resistance in muscle cells and fibroblasts (Brown-Borg 2009). Similar to GH-deficient mice, mice that do not have GH receptor-binding protein (GHR-BP) show 50% lifespan extension with high insulin sensitivity and lesser incidence of age-dependent diseases (Ikeno et al. 2009). Reduced occurrence of tumours in kidney and small intestine and protection against β -amyloid toxicity were found in GH-deficient mice (Cohen et al. 2009; Garcia et al. 2008; Patel et al. 2005). Decrease in IGF-1 delays the onset of cancer and immune decline and helps to maintain youthful cognitive ability (Anisimov and Bartke 2013). Mice carrying fat-specific insulin receptor knockout (FIRKO) genotype live 18% longer (Bluhner et al. 2003). Reduction of insulin receptor signalling in the mouse brain extends lifespan by 18% (Taguchi et al. 2007).

DR in rodents reduced the levels of GH and IGF-1 (Lee and Longo 2011), but the link between DR, GH and aging is yet to be uncovered. The long-lived GH receptor knock-out (GHRKO) mice did not show extended lifespan or the health benefits associated with DR (Bonkowski et al. 2006; Westbrook et al. 2014), whereas the long-lived GH-deficient Ames mice responded to DR (Bartke et al. 2001). This suggests a complex involvement of the GH/IGF-1 axis and periphery pathways in response to DR. Starvation also protects mice from high dosage chemotherapy

treatments by decreasing IGF-1 signalling in the serum. This suggests that starvation or severe DR can be used for disease treatment to a certain extent. However, this does not help in the treatment of cancer cells as the oncogenes block turning on of stress resistance during decline of nutrient levels (Raffaello et al. 2008).

In worms and flies, a positive role for sirtuins in prolonging lifespan is well established. In mammals, certain sirtuins have been identified which are associated with different aging pathways. Alterations in their balance have proved beneficial in different models of age-associated diseases. Experiments with rodents are yet to examine lifespan extension from overexpression of the *sirt* gene, although resveratrol mediated activation of *sirt1* was found to reverse partially the life-shortening effect of high fat diet (Kenyon 2010). Under DR, SIRT1 helps in stress tolerance by deacetylating FOXO1 (Brunet et al. 2004). SIRT1 also deacetylates FOXO4 along with p53 for stress response and cell survival (Outeiro et al. 2008) and FOXO3 to promote DR induced renal protection against hypoxia (Kume et al. 2010). *Sirt6* global knockout mice were reported dead by 4 weeks of age due to severe hypoglycemia (Mostoslavsky et al. 2006). This attributes to cell-autonomous roles of SIRT6 in promoting mitochondrial respiration via suppression of HIF-1 α (hypoxia-inducible factor 1- α) function and in reducing cellular insulin signalling, through mechanisms unknown (Xiao et al. 2010; Zhong et al. 2010).

In human

Human aging is more complicated than worm, fly and rodents and the relevance of IIS pathway to human longevity has generated more controversy. In humans, insulin insensitivity increases with age and this constitutes a risk factor for various conditions like hypertension, atherosclerosis, obesity that cause ill health, frailty and death among the elderly (Barbieri et al. 2003). Although the role of IIS pathway in humans is poorly understood, the genetic variants and/or combinations of small-nucleotide polymorphisms (SNPs) in the human components of the IIS pathway, including various FOXO SNPs have been found to correlate with low IGF-1 plasma levels present in centenarians (van Heemst et al. 2005). Overexpression of phosphatase and tensin homolog (PTEN) which

dephosphorylates PIP₃ to PIP₂, causes a high antioxidant activity and longevity in human umbilical vein endothelial cell (HUVEC) cultures (Tait et al. 2015). Low IGF-1 levels have been predicted as the cause for long-lived individuals (Milman et al. 2014; Vitale et al. 2012). Female Ashkenazi Jewish centenarians show overrepresentation of loss-of-function mutations in the IGF1R (Suh et al. 2008) while, Laron dwarves, who are normally overweight and long lived, show reduced levels of IGF-1 and reduced incidence of type-2 diabetes and cancer (Guevara-Aguirre et al. 2011; Steurman et al. 2011). Studies conducted on people with acromegaly (caused by elevated levels of GH and IGF-1) showed increased mortality rates (Clayton 2003). These findings suggest that “IGF-related longevity genes” do exist in the human population. When trying to establish a relationship between IIS signalling, tallness and longevity in humans, women having lower IIS scores exhibited shorter height with increased old age survival. Genetically induced IIS inactivation can thus be advantageous to women than men for survival at old age (van Heemst et al. 2005).

DR offers a likely route for longevity in humans (Cava and Fontana 2013; Zhao et al. 2014). The discovery of the positive effects of DR in various animal models led to the search for possibilities in middle aged humans. In humans, suppression of IGF-1 depends more on reducing protein consumption rather than decreasing calorie intake (Redman et al. 2010). Long-term DR in humans inhibits IIS pathway in skeletal muscle, with a transcriptional profile resembling those who are young in age. This indicates that humans, like rats, share similar transcriptional responses to DR for enhanced health and survival. However, this association cannot be implied as the exact causative for increased longevity (Mercken et al. 2013). It is yet to be resolved whether DR can extend lifespan in humans in addition to decreasing disease specific mortality risk.

The life-prolonging effects of *sirt* genes and the emerging evidences of link between sirtuins and DR in lower organisms and rodents prompted a number of investigations in humans to identify *sirt* genes associated with exceptional longevity. Human genome encodes seven *sirt* genes of which, *sirt1* and *sirt3* have been investigated for their role in longevity (Polito et al. 2010). *sirt1* variation cannot be linked to reduced human mortality rate (Flachsbart et al. 2006; Kuningas et al. 2007; Willcox et al. 2008) but *sirt3* has been

found to play a role in modulating human longevity (Bellizzi et al. 2005; Lescai et al. 2009).

Epigenetic mechanisms of aging

Various cellular processes face age-dependent changes in gene expression regulated by different epigenetic machineries. Epigenetic regulation of aging is mediated through methylation of regulatory DNA sequences, histone protein modification and the expression of regulatory non-coding RNAs (Esteller 2002). With time, regulation of epigenetic marks affect the expression of specific genes involved in longevity (Han and Brunet 2012; Kouzarides 2007) and create physiological changes associated with aging (Thompson et al. 2010). While we discuss the role of different epigenetic mechanisms that impact aging, we also examine evidences for the influence of epigenetics on aging through IIS pathway.

DNA methylation

The extent of DNA methylation is inversely proportional to the activation of a gene. As an organism ages, DNA methylation affects in two ways. One, it brings a general decrease in global gene expression mediated by changes in the distribution of 5-methylcytosine (5-mC) across the genome (Waki et al. 2003) and two, it causes promoters of specific genes to likely shift from unmethylated to methylated status, leading to gene turnoff (examples—promoters of tumor, aging related genes such as *runx3* and *tig1*) (Kim et al. 2004; Waki et al. 2003). A link has been observed in humans among 5-mC DNA methylation, chronological and biological age suggesting that DNA methylation patterns could be used as an aging clock (Benayoun et al. 2015; Hannum et al. 2013; Horvath 2013; Johnson et al. 2012; Mitteldorf 2016; Weidner et al. 2014).

There are many reports that establish DNA methylation regulation of aging (Ben-Avraham et al. 2012; Bennett-Baker et al. 2003; Lin et al. 2005; Tra et al. 2002). DR influences organism aging through ways other than IIS pathway. DR causes reversal of aberrant DNA methylation by aiming at specific loci rather than on a global level (Munoz-Najar and Sedivy 2011). Studies show that DR increases methylation levels of proto-oncogene *ras* (Hass et al. 1993), transcription factor RUNX3 (Kim et al. 2004) and tumor suppressor

gene *p16^{INK4a}* (Lichtenberg 2011) (Fig. 2). Hypermethylated gene promoters are recognized by transcriptional repressors, leading to silencing of the gene. This contributes to DR mediated cancer prevention (Li et al. 2011c). Global decrease of DNA methylation with aging causes a decrease in DNMT1 and DNMT3a activity significantly (Casillas et al. 2003). DR contributes to a significant increase in DNMT1 activity to correct the decreased methylation level caused by aging (Li et al. 2010).

Effect of DNA methylation on aging through IIS pathway remains uncovered. However, a recent study

shows that the expression of *Dnmt1* and *Dnmt3a* is influenced by GH, suggesting an association between DNA methylation and the IGF-1/FOXO pathway (Armstrong et al. 2014).

Histone modification

Gene expression is also controlled by various histone modifications catalyzed by acetyltransferases, deacetylases, methyltransferases and demethylases (Kouzarides 2007; Mosammamarast and Shi 2010). These enzymes regulate the expression of several gerontogenes and

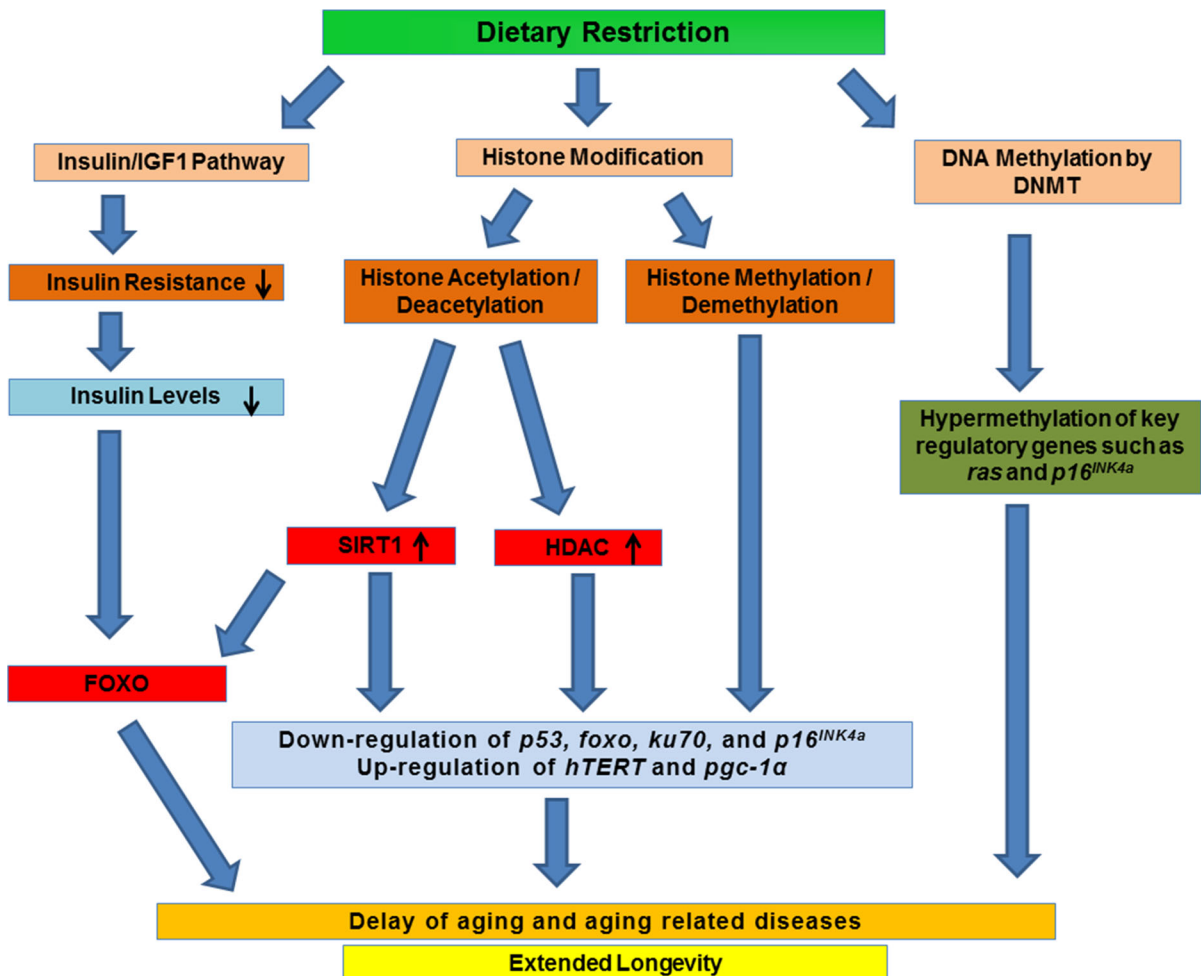


Fig. 2 DR contributes to extended lifespan by delay of aging and aging-related diseases. It regulates aging through metabolic pathway and epigenetic processes of DNA methylation and histone modification. Reduced food intake affects IIS pathway to cause nuclear translocation and transcription of FOXO. FOXO activates or represses genes important for promoting

longevity. DNA methylation regulation during dietary restriction involves DNMT activation and silencing of target genes such as *ras*. DR-induced histone modification involves up and downregulation of target genes through histone acetylation and methylation. DR-associated epigenetic regulation reverses aberrant gene expression and maintains chromatin stability.

pose as therapeutic targets that help avert or treat age-dependent impairments. Histone methylation, facilitated by histone methyl transferases and histone demethylases, impacts organism aging (Greer et al. 2011; Pu et al. 2015; Sen et al. 2015; Siebold et al. 2010). Histone acetylation, mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) influences metabolic health and longevity in a conserved manner and its pattern changes during normal aging (Sato et al. 2013; Toiber et al. 2013). Changes in activities of HATs and HDACs by genetic manipulation or by targeting with chemicals, natural polyamines (Eisenberg et al. 2009) and drugs show extensive changes in longevity across taxa (Benayoun et al. 2015).

DR-associated epigenetic regulation of lifespan extension also includes histone remodelling (Fig. 2). DR-induced stimulation of SIRT1 and HDAC1 leads to deacetylation changes in the expression of key genes such as *foxo*, *p53*, *ku70*, *pgc-1a* and *p16^{INK4a}* and DR-induced histone methylation regulates genes such as hTERT and *p16^{INK4a}* (Li et al. 2011b).

Various studies conducted prove the involvement of histone modifications in IIS pathway regulation of aging. Both DNA methylation and histone modification in regions of IGF-1 promoter alter the expression of *Igf-1* gene (Yang et al. 2015). High glucose (HG) induced epigenetic modifications of IGF1R is found during development of diabetic cardiomyopathy (Yu et al. 2010). HG also increases the connection between p53 and HDAC subtype 1 and reduces the association between acetylated histone 4 and IGF1R promoter. This shows that HG induced suppression of IGF1R is arbitrated by the association of p53 with the IGF1R promoter, and also by the involvement of HDAC1 to the IGF1R promoter-p53 complex (Yu et al. 2010). Activation of methionine synthase by IGF-1 and dopamine has shown to fuel PI3K and MAPK-dependent pathways that amplify DNA methylation (Waly et al. 2004). UTX-1 has been identified as one of the epigenetic regulators of the IIS pathway (Jin et al. 2011; Maures et al. 2011) (Fig. 3). UTX-1 is the structural homolog of mammalian demethylases that target H3 K27me3 (Jin et al. 2011). In *C. elegans*, age-related increase of UTX-1 level leads to deletion of the repressive H3 K27me3 mark on genes *daf-2*, *akt-1*, and *akt-2* in the IIS pathway. This removal leads to increased transcription of these genes and down-regulation of DAF-16 activity leading to cell aging (Jin et al. 2011). FOXO/DAF-16 transcription factor

also acts through the use of SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeler (Riedel et al. 2013). While SWI/SNF modifies chromatin in an ATP-dependent manner and not through epigenetic change, the recruitment of SWI/SNF is not totally dependent on DAF-16 suggesting the possibility of involvement of epigenetic mechanism (Riedel et al. 2013). Chromatin modifier zinc finger protein 1 (ZFP-1) acts as a common facilitator of IIS and DR mediated lifespan regulation (Fig. 1). ZFP-1 interacts with Glioma amplified sequence-41 (GFL-1 in *C. elegans*) and DAF-16/FOXO regulates the expression of the different isoforms of *zfp-1* and *gfl-1*. ZFP-1/GFL-1 negatively regulates the expression of DAF-16/FOXO target genes through feed-forward loops. This suggests that the knock down of *zfp-1* or *gfl-1* would lead to suppression of DAF-16 target gene expression under low insulin signalling conditions (Singh et al. 2016).

Non-coding RNA surveillance of organism longevity

Non-coding RNAs include small regulatory RNAs such as microRNAs (miRNAs), siRNAs, piwi-interacting RNAs, QDE-2-interacting RNAs (qiRNAs) and a wide range of long non-coding RNAs (lncRNAs). They influence varied biological events by controlling gene expression and maintaining stability of the genome. Although the role of these regulatory RNAs is well studied in different biological processes, their role in aging mediated through IIS pathway remains largely unknown.

MicroRNAs

MicroRNA (miRNA) maturation and function requires argonaute like gene-1 (*alg-1*). Loss of *alg-1* in adult worm has been found to affect lifespan, thus signifying the need of miRNAs in adulthood for proper aging (Kato et al. 2011). *lin-4* of *C. elegans* was the first miRNA to be discovered, followed by the successive identification of other miRNAs (Lee et al. 1993). Many miRNAs have now been discovered in plants, animals and human, with over 2588 human miRNAs noted in the miRBase (<http://www.mirbase.org>) (Kozomara and Griffiths-Jones 2014). In *C. elegans*, miRNAs affect lifespan in both positive and negative manner and significant expression changes are observed in multiple miRNAs during aging (de

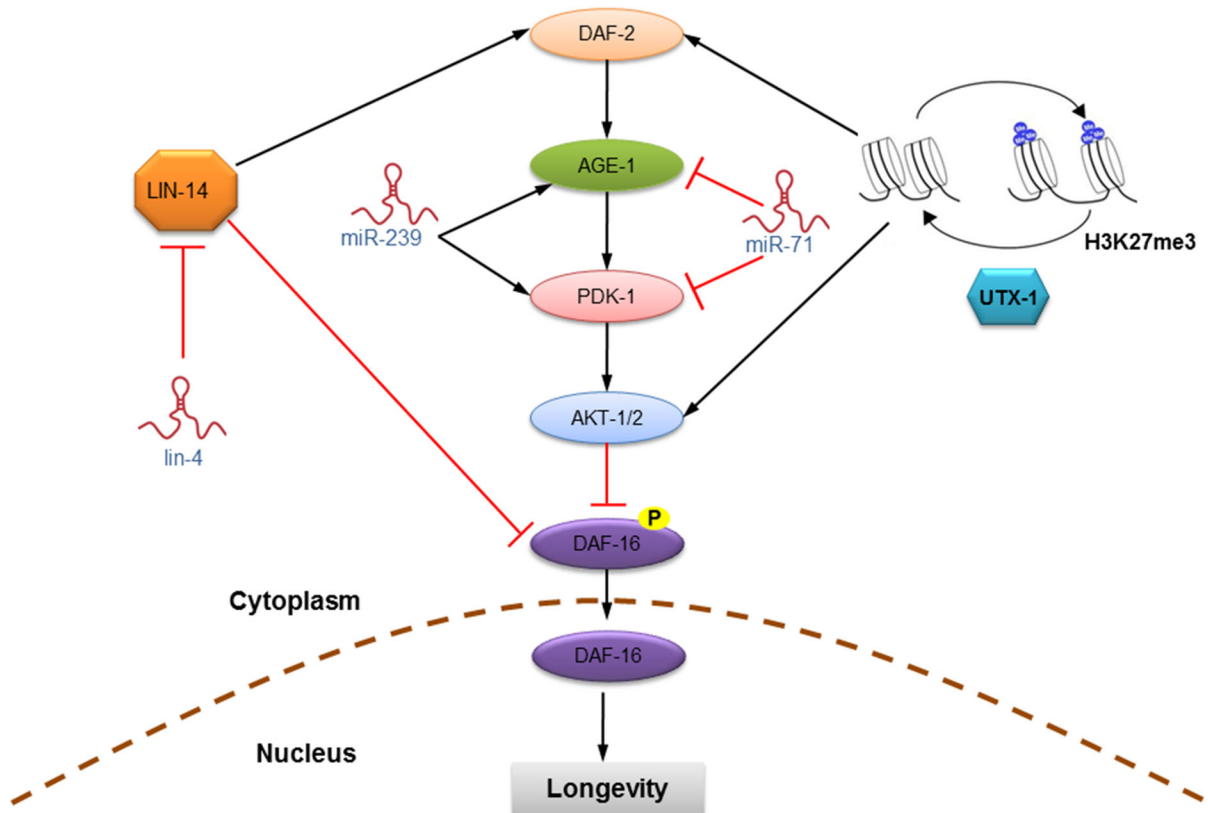


Fig. 3 Schematic representation of the role of miRNAs and UTX-1 in IIS pathway to regulate *C. elegans* aging. miR-71, 238, 239 and 246 regulate worm lifespan. Targets have been established for some of the miRNAs. miRNA lin-4 downregulates LIN-14 expression by forming RNA duplex with 3'-UTR of lin-14 mRNA. LIN-14 causes direct repression of DAF-16 or

indirect repression of DAF-16 through DAF-2 activation. miR-71 and miR-239 have opposite effects on AGE-1 and PDK-1 to regulate lifespan. During aging, UTX-1 levels increase and lead to the removal of the repressive H3K27me3 mark on genes *daf-2*, *akt-1*, and *akt-2* leading to increased transcription of these genes and in turn cause downregulation of DAF-16 activity.

Lencastre et al. 2010; Grillari et al. 2010; Kato et al. 2011). miRNA lin-4 targets 3'-UTR of lin-14 mRNA to form RNA duplex and suppresses the expression of lin-14 in *C. elegans*. LIN-14 blocks the activity of *daf-16* and promotes the activity of *daf-2* (Fig. 3). Loss of function of lin-4 or gain of function of lin-14 reduces the worm lifespan. Contrariwise, overexpression of lin-4 or knockdown of lin-14 results in extended lifespan (Baugh and Sternberg 2006; Boehm and Slack 2005). miR-71, miR-238, and miR-246 are the most overexpressed in aged animal and hence considered to increase longevity in *C. elegans*. miR-239 limits worm lifespan (de Lencastre et al. 2010). miR-71 and miR-246 act as biomarkers of aging in *C. elegans* (Pincus et al. 2011). miR-71 and miR-239 of *C. elegans* function through IIS pathway and show opposite effects on AGE-1 and PDK-1 to regulate lifespan. In

addition to it, miR-71 also interacts with the DNA damage response pathway by knocking down two check point proteins, CDC-25.1 and CHK-1 (de Lencastre et al. 2010). miR-34 gets upregulated during aging, dauer phase and early dormancy of *C. elegans* (de Lencastre et al. 2010; Karp et al. 2011) and target the aging genes (Zhao et al. 2010) when compared to other miRNAs that get normally downregulated with time (de Lencastre et al. 2010; Ibanez-Ventoso et al. 2006). This is in contrary to the general upregulation of miRNA expression reported in mice during aging. Thus, variances can be seen in global and tissue-specific miRNA expression between worm and rodents (Li et al. 2011b).

Fly miR-8 (homolog of human miR-200) targets USH (ZFPM2 or FOG2 in humans), which obstructs PI3K of IIS pathway (Hyun et al. 2009). Also, miR-8

null flies were observed small in size with faulty insulin signalling in the fat body (Hwangbo et al. 2004; Hyun et al. 2009). This suggests miR-8/miR-200 to be vital for governing fly and human aging respectively (Hyun et al. 2009).

Genotype-by-age interaction (GbA) refers to the genotype-specific changes found in circulating levels of 21 miRNAs of young and old Ames dwarf mice during aging. Genotype-by-age miRNAs (GbAmiRNAs) target transcripts involved in tumor suppression, anti-inflammatory response and modulate Wnt, insulin, mTOR, and MAPK signaling pathways. Comparison of circulating GbAmiRNAs in Ames mice with DR-mediated circulating miRNAs in another long-lived mouse proved that both DR-independent and DR-dependent mechanisms increase lifespan in the Ames mouse (Victoria et al. 2015). Mouse model of Hutchinson-Gilford progeria syndrome (Enhanced aging due to improper maturation of lamin A in the nuclear envelope) shows higher levels of miR-1 in the liver, kidney and muscle when compared to wild-type animals (Marino et al. 2010). miR-1 suppresses IGF1 in progeroid mice, indicating that it functions through the IIS pathway (Marino et al. 2010). Aging mammals show miRNA expression to be tissue-specific such as in liver (Bates et al. 2010; Li et al. 2011a), brain (Khanna et al. 2011; Li et al. 2011b; Liang et al. 2011) and skeletal muscles (Drummond et al. 2011; Hamrick et al. 2010). This suggests for aging related tissue-specific functions of signalling pathways. miR-470, miR-669b and miR-681 in mammals suppress the expression of IGF1R and AKT to result in diminished phosphorylation of FOXO3 (Liang et al. 2011). The miR-17-92 cluster along with its paralogous clusters miR-106a-363 and miR-106b-25 that regulate cellular senescence, are reduced in several aging models and upregulated in some cancers (Faraonio et al. 2012; Grillari et al. 2010). These miRNAs generally target PTEN and their downregulation during aging elevates PTEN levels, which in turn suppresses the IIS pathway (Grillari et al. 2010).

Expression of most sirtuins is regulated by multiple miRNAs. Interaction between them helps in the regulation of multiple pathways. Interplay between SIRT1 and miR-181a regulates hepatic insulin sensitivity (Zhou et al. 2012) and the crosstalk between SIRT6 and miR-33a/b aids in fatty acid metabolism and insulin signalling (Davalos et al. 2011).

Long non-coding RNAs

Long non-coding RNAs (lncRNAs) control different facets of aging. LncRNAs are involved in various cellular activities, proliferation, and differentiation, quiescence, senescence and stress response related to aging (Grammatikakis et al. 2014; Jain et al. 2016). These include *TERC* and *TERRA* that control telomere length, *Aim*, *PTENpg1-AS* and *H19* involved in epigenetic changes, *lncRNA-p21* involved in translation repression process and *MALAT1*, *ANRIL*, *eRNAs* and *7SL* that control cell division (Grammatikakis et al. 2014; Kim et al. 2016).

lncRNA transcribed telomeric sequence 1 (tts-1) are found on ribosomes of the worm *daf-2* mutants. Deletion of *tts-1* in *daf-2* mutants increases ribosome levels and significantly decreases their lifespan. This suggests the expression of *tts-1* in different longevity affecting signalling pathways to reduce ribosome levels and promote life extension (Essers et al. 2015). The *H19* long intergenic non-coding RNA (lincRNA) is one of the most abundant and conserved transcripts expressed during mammalian development. Overexpression of miR-675 which is found in *H19*'s first exon upregulates *Igf1r* (growth-promoting insulin-like growth factor 1 receptor) gene. *H19*'s main physiological role is chartered by the controlled processing of miR-675. The controlled release of miR-675 from *H19* might inhibit cell proliferation in response to cellular stress or oncogenic signals (Keniry et al. 2012).

Other regulatory non-coding RNAs

Endo-siRNAs are a class of siRNAs first identified in the germline and soma of flies (Chung et al. 2008; Czech et al. 2008; Ghildiyal and Zamore 2009). They were later identified in worm and mammals (Okamura and Lai 2008). Endo-siRNAs of *Drosophila* are produced in a Dicer-2 (*Dcr-2*)-dependent manner. Loss of *Dcr-2* function reduced fly lifespan, making them more vulnerable to oxidative, endoplasmic reticulum, starvation and cold stresses along with abnormal lipid and carbohydrate metabolism (Lim et al. 2011). This suggests the involvement of endo-siRNA pathway in defence against stress and aging. However, the mechanism of involvement of endo-siRNAs in age-related processes is yet to be uncovered.

Piwi-interacting RNAs (piRNAs) were first discovered in germ line cells of organisms ranging from worm (Batista et al. 2008; Wang and Reinke 2008) to mammals (Aravin and Hannon 2008). They were identified to stabilise germ line cells by silencing transposons. Recent findings have identified piRNAs to be operative other than in germline cells (Malone et al. 2009; Rajasethupathy et al. 2012). The association of aging with changes in chromatin structure and methylation status (Liu et al. 2009) and the discovery of piRNAs to form complexes with RecQ1, (DNA helicase necessary for genomic stability) (Lau et al. 2006; Sharma and Brosh 2007), it opens ways for piRNAs to be explored in the context of aging.

QDE-2-interacting RNAs (qiRNAs), first reported in the fungus *Neurospora crassa*, interact with the Argonoute protein QDE-2 and hence their name. DNA damage initiates the transcription of qiRNAs for repair mechanisms (Lee et al. 2009). Dicer, QDE-1 (an RNA-dependent RNA polymerase) and QDE-3 are needed for qiRNA transcription. QDE-3 is the homologue of Werner (WRN) and Bloom (BLM) DNA helicase of human RecQ family (Lee et al. 2009). The discovery of alterations in human WRN or BLM genes leading to premature aging (Martin and Oshima 2000) and the identification of telomerase as the mammalian RNA-dependant RNA polymerase (Maida et al. 2009), it offers a wide area of research in the field of biogerontology and qiRNA biogenesis in mammals.

Concluding remarks

Although evolutionarily conserved, IIS pathway is more complicated in higher organisms. IIS pathway controls life expectancy in many organisms, but despite recent research, its molecular mechanisms remain largely unknown. Disruption of IIS pathway in worm or fly causes significant increase in lifespan but it does not do so in higher animals as mammals have different receptors for insulin and IGFs with distinct pathways and diverse functions. Mammals have insulin/IGF-1 receptors in many organs whereas lower organisms have this signalling mainly through nervous system. IIS pathway in humans is responsible for approximately 5% of lifespan, but the kinds of dramatic increases seen from IIS in worms and flies are unlikely from similar manipulations in humans. On a molecular level, human longevity is mysterious.

Further research is needed to throw light on the distinct molecular mechanisms of human longevity and their evolutionary trends. FOXO transcriptional factors play a crucial and diversified role in the IIS pathway. Activation of FOXO under low IIS activity leads to diverse effects on various tissues to aid in lifespan increase. Studies on FOXO in both lower and higher organisms open broader avenues for the investigation of lifespan extension. Interaction between diet and nutrient-sensing pathways plays a vital role in affecting organism physiology and health.

DR has attracted much attention as it extends lifespan and prevents or postpones a number of age-related diseases without causing irreversible developmental or reproductive flaws. DR increases lifespan by reducing IIS. DR also increases lifespan by stimulation of sirtuins, some of which in turn act through IIS. Exploration of DR coupled with IIS pathway and sirtuins holds a promising future for organism longevity.

Additionally, we show epigenomic and non-coding RNA-mediated manipulation of aging. DNA methylation and histone modifications are known to influence aging. Recent research shows their role in aging through IIS. Regulatory RNA-based epigenomics appears to be a novel understanding of aging regulatory pathway. Non-coding RNA induced epigenetic instability during lifespan forms an integral driver of the aging process. Among non-coding RNAs, the role of miRNAs in aging has been most widely researched. Components of IIS pathway are involved in miRNA-mediated regulation of aging in *C. elegans*. However, with both beneficial and antagonising roles of miRNAs on the lifespan of *C. elegans*, it opens a whole new arena of research both in lower and developed organisms. LncRNAs show diverse roles in regulating lifespan and age-related diseases. Their involvement in aging through IIS pathway is yet to be established. We suggest the importance of different regulatory non-coding RNAs in the pursuit of various factors for healthy aging.

Despite recent progress, a complete mapping of the insulin signalling pathway, role of DR in relation to IIS and the multiple roles of regulatory RNAs including microRNAs and lncRNAs that impact aging through nutrient sensing pathway is still elusive. It will be of great interest to further explore the association between epigenetic and non-epigenetic factors that influence the lifespan of an organism. This will help in the advancement of strategies to reverse different aging mechanisms in humans.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Alic N et al (2011) Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. *Mol Syst Biol* 7:502. doi:[10.1038/msb.2011.36](https://doi.org/10.1038/msb.2011.36)
- Anisimov VN, Bartke A (2013) The key role of growth hormone-insulin-IGF-1 signaling in aging and cancer. *Crit Rev Oncol Hematol* 87:201–223. doi:[10.1016/j.critrevonc.2013.01.005](https://doi.org/10.1016/j.critrevonc.2013.01.005)
- Anselmi CV et al (2009) Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res* 12:95–104. doi:[10.1089/rej.2008.0827](https://doi.org/10.1089/rej.2008.0827)
- Arantes-Oliveira N, Berman JR, Kenyon C (2003) Healthy animals with extreme longevity. *Science* 302:611. doi:[10.1126/science.1089169](https://doi.org/10.1126/science.1089169)
- Aravin AA, Hannon GJ (2008) Small RNA silencing pathways in germ and stem cells. *Cold Spring Harb Symp Quant Biol* 73:283–290. doi:[10.1101/sqb.2008.73.058](https://doi.org/10.1101/sqb.2008.73.058)
- Armstrong VL, Rakoczy S, Rojanathammanee L, Brown-Borg HM (2014) Expression of DNA methyltransferases is influenced by growth hormone in the long-living Ames dwarf mouse in vivo and in vitro. *J Gerontol A Biol Sci Med Sci* 69:923–933. doi:[10.1093/gerona/glt133](https://doi.org/10.1093/gerona/glt133)
- Bai H, Kang P, Tatar M (2012) *Drosophila* insulin-like peptide-6 (dilp6) expression from fat body extends lifespan and represses secretion of *Drosophila* insulin-like peptide-2 from the brain. *Aging Cell* 11:978–985. doi:[10.1111/accel.12000](https://doi.org/10.1111/accel.12000)
- Bai H, Post S, Kang P, Tatar M (2015) *Drosophila* longevity assurance conferred by reduced insulin receptor substrate chico partially requires d4eBP. *PLoS ONE* 10:e0134415. doi:[10.1371/journal.pone.0134415](https://doi.org/10.1371/journal.pone.0134415)
- Banerjee KK, Ayyub C, Ali SZ, Mandot V, Prasad NG, Kolthur-Seetharam U (2012a) dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner. *Cell Rep* 2:1485–1491. doi:[10.1016/j.celrep.2012.11.013](https://doi.org/10.1016/j.celrep.2012.11.013)
- Banerjee KK, Ayyub C, Sengupta S, Kolthur-Seetharam U (2012b) dSir2 deficiency in the fatbody, but not muscles, affects systemic insulin signaling, fat mobilization and starvation survival in flies. *Aging* 4:206–223
- Barbieri M, Bonafe M, Franceschi C, Paolisso G (2003) Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. *Am J Physiol Endocrinol Metab* 285:E1064–E1071. doi:[10.1152/ajpendo.00296.2003](https://doi.org/10.1152/ajpendo.00296.2003)
- Bartke A, Wright JC, Mattison JA, Ingram DK, Miller RA, Roth GS (2001) Extending the lifespan of long-lived mice. *Nature* 414:412. doi:[10.1038/35106646](https://doi.org/10.1038/35106646)
- Bartke A, Sun LY, Longo V (2013) Somatotrophic signaling: trade-offs between growth, reproductive development, and longevity. *Physiol Rev* 93:571–598. doi:[10.1152/physrev.00006.2012](https://doi.org/10.1152/physrev.00006.2012)
- Barzilai N, Huffman DM, Muzumdar RH, Bartke A (2012) The critical role of metabolic pathways in aging. *Diabetes* 61:1315–1322. doi:[10.2337/db11-1300](https://doi.org/10.2337/db11-1300)
- Bates DJ et al (2010) MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging. *Aging Cell* 9:1–18. doi:[10.1111/j.1474-9726.2009.00529.x](https://doi.org/10.1111/j.1474-9726.2009.00529.x)
- Batista PJ et al (2008) PRG-1 and 21U-RNAs interact to form the piRNA complex required for fertility in *C. elegans*. *Mol Cell* 31:67–78. doi:[10.1016/j.molcel.2008.06.002](https://doi.org/10.1016/j.molcel.2008.06.002)
- Baugh LR, Sternberg PW (2006) DAF-16/FOXO regulates transcription of cki-1/Cip/Kip and repression of lin-4 during *C. elegans* L1 arrest. *Curr Biol* 16:780–785. doi:[10.1016/j.cub.2006.03.021](https://doi.org/10.1016/j.cub.2006.03.021)
- Bellizzi D et al (2005) A novel VNTR enhancer within the SIRT3 gene, a human homologue of SIR2, is associated with survival at oldest ages. *Genomics* 85:258–263. doi:[10.1016/j.ygeno.2004.11.003](https://doi.org/10.1016/j.ygeno.2004.11.003)
- Ben-Avraham D, Muzumdar RH, Atzmon G (2012) Epigenetic genome-wide association methylation in aging and longevity. *Epigenomics* 4:503–509. doi:[10.2217/epi.12.41](https://doi.org/10.2217/epi.12.41)
- Benayoun BA, Pollina EA, Brunet A (2015) Epigenetic regulation of ageing: linking environmental inputs to genomic stability. *Nat Rev Mol Cell Biol* 16:593–610. doi:[10.1038/nrm4048](https://doi.org/10.1038/nrm4048)
- Bennett-Baker PE, Wilkowski J, Burke DT (2003) Age-associated activation of epigenetically repressed genes in the mouse. *Genetics* 165:2055–2062
- Berdichevsky A, Viswanathan M, Horvitz HR, Guarente L (2006) *C. elegans* SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span. *Cell* 125:1165–1177. doi:[10.1016/j.cell.2006.04.036](https://doi.org/10.1016/j.cell.2006.04.036)
- Blackburn EH, Greider CW, Szostak JW (2006) Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med* 12:1133–1138. doi:[10.1038/nm1006-1133](https://doi.org/10.1038/nm1006-1133)
- Bluher M, Kahn BB, Kahn CR (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299:572–574. doi:[10.1126/science.1078223](https://doi.org/10.1126/science.1078223)
- Boehm M, Slack F (2005) A developmental timing microRNA and its target regulate life span in *C. elegans*. *Science* 310:1954–1957. doi:[10.1126/science.1115596](https://doi.org/10.1126/science.1115596)
- Bonkowski MS, Rocha JS, Masternak MM, Al Regaiey KA, Bartke A (2006) Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. *Proc Natl Acad Sci USA* 103:7901–7905. doi:[10.1073/pnas.0600161103](https://doi.org/10.1073/pnas.0600161103)
- Broggiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E (2001) An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr Biol* 11:213–221
- Broughton S, Partridge L (2009) Insulin/IGF-like signalling, the central nervous system and aging. *Biochem J* 418:1–12. doi:[10.1042/BJ20082102](https://doi.org/10.1042/BJ20082102)

- Broughton SJ et al (2005) Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc Natl Acad Sci USA* 102:3105–3110. doi:[10.1073/pnas.0405775102](https://doi.org/10.1073/pnas.0405775102)
- Broughton S et al (2008) Reduction of DILP2 in *Drosophila* triages a metabolic phenotype from lifespan revealing redundancy and compensation among DILPs. *PLoS ONE* 3:e3721. doi:[10.1371/journal.pone.0003721](https://doi.org/10.1371/journal.pone.0003721)
- Brown-Borg HM (2009) Hormonal control of aging in rodents: the somatotrophic axis. *Mol Cell Endocrinol* 299:64–71. doi:[10.1016/j.mce.2008.07.001](https://doi.org/10.1016/j.mce.2008.07.001)
- Brunet A et al (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96:857–868
- Brunet A et al (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303:2011–2015. doi:[10.1126/science.1094637](https://doi.org/10.1126/science.1094637)
- Brunet C et al (2011) Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and *Drosophila*. *Nature* 477:482–485. doi:[10.1038/nature10296](https://doi.org/10.1038/nature10296)
- Calnan DR, Brunet A (2008) The FoxO code. *Oncogene* 27:2276–2288. doi:[10.1038/onc.2008.21](https://doi.org/10.1038/onc.2008.21)
- Campisi J, di Fagagna FDA (2007) Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol* 8:729–740. doi:[10.1038/nrm2233](https://doi.org/10.1038/nrm2233)
- Carter ME, Brunet A (2007) FOXO transcription factors. *Curr Biol* 17:R113–R114. doi:[10.1016/j.cub.2007.01.008](https://doi.org/10.1016/j.cub.2007.01.008)
- Casillas MA Jr, Lopatina N, Andrews LG, Tollefsbol TO (2003) Transcriptional control of the DNA methyltransferases is altered in aging and neoplastically-transformed human fibroblasts. *Mol Cell Biochem* 252:33–43
- Cava E, Fontana L (2013) Will calorie restriction work in humans? *Aging* 5:507–514
- Cedar H, Bergman Y (2012) Programming of DNA methylation patterns. *Annu Rev Biochem* 81:97–117. doi:[10.1146/annurev-biochem-052610-091920](https://doi.org/10.1146/annurev-biochem-052610-091920)
- Chung WJ, Okamura K, Martin R, Lai EC (2008) Endogenous RNA interference provides a somatic defense against *Drosophila* transposons. *Curr Biol* 18:795–802. doi:[10.1016/j.cub.2008.05.006](https://doi.org/10.1016/j.cub.2008.05.006)
- Claeys I, Simonet G, Poels J, Van Loy T, Vercammen L, De Loof A, Vanden Broeck J (2002) Insulin-related peptides and their conserved signal transduction pathway. *Peptides* 23:807–816
- Clancy DJ et al (2001) Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292:104–106. doi:[10.1126/science.1057991](https://doi.org/10.1126/science.1057991)
- Clayton RN (2003) Cardiovascular function in acromegaly. *Endocr Rev* 24:272–277. doi:[10.1210/er.2003-0009](https://doi.org/10.1210/er.2003-0009)
- Cohen E et al (2009) Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* 139:1157–1169. doi:[10.1016/j.cell.2009.11.014](https://doi.org/10.1016/j.cell.2009.11.014)
- Czech B et al (2008) An endogenous small interfering RNA pathway in *Drosophila*. *Nature* 453:798–802. doi:[10.1038/nature07007](https://doi.org/10.1038/nature07007)
- Davalos A et al (2011) miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc Natl Acad Sci USA* 108:9232–9237. doi:[10.1073/pnas.1102281108](https://doi.org/10.1073/pnas.1102281108)
- de Lencastre A, Pincus Z, Zhou K, Kato M, Lee SS, Slack FJ (2010) MicroRNAs both promote and antagonize longevity in *C. elegans*. *Curr Biol* 20:2159–2168. doi:[10.1016/j.cub.2010.11.015](https://doi.org/10.1016/j.cub.2010.11.015)
- De Meys P (2016) The insulin receptor and its signal transduction network. In: De Groot LJ, Chrousos G, Dungan K, et al., (eds) *Endotext* [Internet]. South Dartmouth (MA): MDText.com Inc, Available from: <https://www.ncbi.nlm.nih.gov/books/NBK378978/>
- Demontis F, Perrimon N (2010) FOXO/4E-BP signaling in *Drosophila* muscles regulates organism-wide proteostasis during aging. *Cell* 143:813–825. doi:[10.1016/j.cell.2010.10.007](https://doi.org/10.1016/j.cell.2010.10.007)
- Dong XC, Copps KD, Guo S, Li Y, Kollipara R, DePinho RA, White MF (2008) Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. *Cell Metab* 8:65–76. doi:[10.1016/j.cmet.2008.06.006](https://doi.org/10.1016/j.cmet.2008.06.006)
- Drummond MJ, McCarthy JJ, Sinha M, Spratt HM, Volpi E, Esser KA, Rasmussen BB (2011) Aging and microRNA expression in human skeletal muscle: a microarray and bioinformatics analysis. *Physiol Genom* 43:595–603. doi:[10.1152/physiolgenomics.00148.2010](https://doi.org/10.1152/physiolgenomics.00148.2010)
- Eijkelenboom A, Burgering BM (2013) FOXOs: signalling integrators for homeostasis maintenance. *Nat Rev Mol Cell Biol* 14:83–97. doi:[10.1038/nrm3507](https://doi.org/10.1038/nrm3507)
- Eisenberg T et al (2009) Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* 11:1305–1314. doi:[10.1038/ncb1975](https://doi.org/10.1038/ncb1975)
- Essers PB et al (2015) A long noncoding RNA on the ribosome is required for lifespan extension. *Cell Rep*. doi:[10.1016/j.celrep.2014.12.029](https://doi.org/10.1016/j.celrep.2014.12.029)
- Esteller M (2002) CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. *Oncogene* 21:5427–5440. doi:[10.1038/sj.onc.1205600](https://doi.org/10.1038/sj.onc.1205600)
- Ewald CY, Landis JN, Porter Abate J, Murphy CT, Blackwell TK (2015) Dauer-independent insulin/IGF-1-signalling implicates collagen remodelling in longevity. *Nature* 519:97–101. doi:[10.1038/nature14021](https://doi.org/10.1038/nature14021)
- Faraonio R et al (2012) A set of miRNAs participates in the cellular senescence program in human diploid fibroblasts. *Cell Death Differ* 19:713–721. doi:[10.1038/cdd.2011.143](https://doi.org/10.1038/cdd.2011.143)
- Flachsbar F, Croucher PJ, Nikolaus S, Hampe J, Cordes C, Schreiber S, Nebel A (2006) Sirtuin 1 (SIRT1) sequence variation is not associated with exceptional human longevity. *Exp Gerontol* 41:98–102. doi:[10.1016/j.exger.2005.09.008](https://doi.org/10.1016/j.exger.2005.09.008)
- Garcia AM et al (2008) Effect of Ames dwarfism and caloric restriction on spontaneous DNA mutation frequency in different mouse tissues. *Mech Ageing Dev* 129:528–533. doi:[10.1016/j.mad.2008.04.013](https://doi.org/10.1016/j.mad.2008.04.013)
- Garelli A, Gontijo AM, Miguela V, Caparros E, Dominguez M (2012) Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and maturation. *Science* 336:579–582. doi:[10.1126/science.1216735](https://doi.org/10.1126/science.1216735)
- Garofalo RS (2002) Genetic analysis of insulin signaling in *Drosophila*. *Trends Endocrinol Metab* 13:156–162
- Gems D et al (1998) Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* 150:129–155
- Ghildiyal M, Zamore PD (2009) Small silencing RNAs: an expanding universe. *Nat Rev Genet* 10:94–108. doi:[10.1038/nrg2504](https://doi.org/10.1038/nrg2504)

- Giannakou ME, Goss M, Partridge L (2008) Role of dFOXO in lifespan extension by dietary restriction in *Drosophila melanogaster*: not required, but its activity modulates the response. *Aging Cell* 7:187–198. doi:[10.1111/j.1474-9726.2007.00362.x](https://doi.org/10.1111/j.1474-9726.2007.00362.x)
- Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M (2014) Long noncoding RNAs (lncRNAs) and the molecular hallmarks of aging. *Aging* 6:992–1009. doi:[10.18632/aging.100710](https://doi.org/10.18632/aging.100710)
- Greer EL, Brunet A (2008) Signaling networks in aging. *J Cell Sci* 121:407–412. doi:[10.1242/jcs.021519](https://doi.org/10.1242/jcs.021519)
- Greer EL, Brunet A (2009) Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*. *Aging Cell* 8:113–127. doi:[10.1111/j.1474-9726.2009.00459.x](https://doi.org/10.1111/j.1474-9726.2009.00459.x)
- Greer EL et al (2011) Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* 479:365–371. doi:[10.1038/nature10572](https://doi.org/10.1038/nature10572)
- Grillari J, Hackl M, Grillari-Voglauer R (2010) miR-17-92 cluster: ups and downs in cancer and aging. *Biogerontology* 11:501–506. doi:[10.1007/s10522-010-9272-9](https://doi.org/10.1007/s10522-010-9272-9)
- Gronke S, Clarke DF, Broughton S, Andrews TD, Partridge L (2010) Molecular evolution and functional characterization of *Drosophila* insulin-like peptides. *PLoS Genet* 6:e1000857. doi:[10.1371/journal.pgen.1000857](https://doi.org/10.1371/journal.pgen.1000857)
- Guarente L, Kenyon C (2000) Genetic pathways that regulate ageing in model organisms. *Nature* 408:255–262. doi:[10.1038/35041700](https://doi.org/10.1038/35041700)
- Guevara-Aguirre J et al (2011) Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. *Sci Transl Med* 3:70ra13. doi:[10.1126/scitranslmed.3001845](https://doi.org/10.1126/scitranslmed.3001845)
- Hafen E (2004) Cancer, type 2 diabetes, and ageing: news from flies and worms. *Swiss Med Wkly* 134:711–719
- Hamrick MW et al (2010) The adipokine leptin increases skeletal muscle mass and significantly alters skeletal muscle miRNA expression profile in aged mice. *Biochem Biophys Res Commun* 400:379–383. doi:[10.1016/j.bbrc.2010.08.079](https://doi.org/10.1016/j.bbrc.2010.08.079)
- Han S, Brunet A (2012) Histone methylation makes its mark on longevity. *Trends Cell Biol* 22:42–49. doi:[10.1016/j.tcb.2011.11.001](https://doi.org/10.1016/j.tcb.2011.11.001)
- Hannum G et al (2013) Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 49:359–367. doi:[10.1016/j.molcel.2012.10.016](https://doi.org/10.1016/j.molcel.2012.10.016)
- Harman D (1965) The free radical theory of aging: effect of age on serum copper levels. *J Gerontol* 20:151–153
- Haselton A, Sharmin E, Schrader J, Sah M, Poon P, Fridell YW (2010) Partial ablation of adult *Drosophila* insulin-producing neurons modulates glucose homeostasis and extends life span without insulin resistance. *Cell Cycle* 9:3063–3071. doi:[10.4161/cc.9.15.12458](https://doi.org/10.4161/cc.9.15.12458)
- Hass BS, Hart RW, Lu MH, Lyn-Cook BD (1993) Effects of caloric restriction in animals on cellular function, oncogene expression, and DNA methylation in vitro. *Mutat Res* 295:281–289
- Holzenberger M et al (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421:182–187. doi:[10.1038/nature01298](https://doi.org/10.1038/nature01298)
- Horvath S (2013) DNA methylation age of human tissues and cell types. *Genome Biol* 14:R115. doi:[10.1186/gb-2013-14-10-r115](https://doi.org/10.1186/gb-2013-14-10-r115)
- Huang CW et al (2015) Tequila regulates insulin-like signaling and extends life span in *Drosophila melanogaster*. *J Gerontol A Biol Sci Med Sci* 70:1461–1469. doi:[10.1093/gerona/glv094](https://doi.org/10.1093/gerona/glv094)
- Hwangbo DS, Gershman B, Tu MP, Palmer M, Tatar M (2004) *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* 429:562–566. doi:[10.1038/nature02549](https://doi.org/10.1038/nature02549)
- Hyun S et al (2009) Conserved microRNA miR-8/miR-200 and its target USH/FOG2 control growth by regulating PI3K. *Cell* 139:1096–1108. doi:[10.1016/j.cell.2009.11.020](https://doi.org/10.1016/j.cell.2009.11.020)
- Ibanez-Ventoso C, Yang M, Guo S, Robins H, Padgett RW, Driscoll M (2006) Modulated microRNA expression during adult lifespan in *Caenorhabditis elegans*. *Aging Cell* 5:235–246. doi:[10.1111/j.1474-9726.2006.00210.x](https://doi.org/10.1111/j.1474-9726.2006.00210.x)
- Ikeno Y et al (2009) Reduced incidence and delayed occurrence of fatal neoplastic diseases in growth hormone receptor/binding protein knockout mice. *J Gerontol A Biol Sci Med Sci* 64:522–529. doi:[10.1093/gerona/glp017](https://doi.org/10.1093/gerona/glp017)
- Ikeya T, Galic M, Belawat P, Nairz K, Hafen E (2002) Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr Biol* 12:1293–1300
- Jain S, Thakkar N, Chhatai J, Bhadra MP, Bhadra U (2016) Long non-coding RNA: functional agent for disease traits. *RNA Biol* 1–14. doi:[10.1080/15476286.2016.1172756](https://doi.org/10.1080/15476286.2016.1172756)
- Jin C et al (2011) Histone demethylase UTX-1 regulates *C. elegans* life span by targeting the insulin/IGF-1 signaling pathway. *Cell Metab* 14:161–172. doi:[10.1016/j.cmet.2011.07.001](https://doi.org/10.1016/j.cmet.2011.07.001)
- Johnson FB, Sinclair DA, Guarente L (1999) Molecular biology of aging. *Cell* 96:291–302
- Johnson AA, Akman K, Calimport SR, Wuttke D, Stolzing A, de Magalhaes JP (2012) The role of DNA methylation in aging, rejuvenation, and age-related disease. *Rejuvenation Res* 15:483–494. doi:[10.1089/rej.2012.1324](https://doi.org/10.1089/rej.2012.1324)
- Jung M, Pfeifer GP (2015) Aging and DNA methylation. *BMC Biol* 13:7. doi:[10.1186/s12915-015-0118-4](https://doi.org/10.1186/s12915-015-0118-4)
- Kaletsky R, Lakhina V, Arey R, Williams A, Landis J, Ashraf J, Murphy CT (2016) The *C. elegans* adult neuronal IIS/FOXO transcriptome reveals adult phenotype regulators. *Nature* 529:92–96. doi:[10.1038/nature16483](https://doi.org/10.1038/nature16483)
- Karp X, Hammell M, Ow MC, Ambros V (2011) Effect of life history on microRNA expression during *C. elegans* development. *Rna* 17:639–651. doi:[10.1261/rna.2310111](https://doi.org/10.1261/rna.2310111)
- Karpac J, Jasper H (2009) Insulin and JNK: optimizing metabolic homeostasis and lifespan. *Trends Endocrinol Metab* 20:100–106. doi:[10.1016/j.tem.2008.11.004](https://doi.org/10.1016/j.tem.2008.11.004)
- Kato M, Chen X, Inukai S, Zhao H, Slack FJ (2011) Age-associated changes in expression of small, noncoding RNAs, including microRNAs, in *C. elegans*. *RNA* 17:1804–1820. doi:[10.1261/rna.2714411](https://doi.org/10.1261/rna.2714411)
- Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, Reik W (2012) The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat Cell Biol* 14:659–665. doi:[10.1038/ncb2521](https://doi.org/10.1038/ncb2521)
- Kenyon C (2005) The plasticity of aging: insights from long-lived mutants. *Cell* 120:449–460. doi:[10.1016/j.cell.2005.02.002](https://doi.org/10.1016/j.cell.2005.02.002)
- Kenyon CJ (2010) The genetics of ageing. *Nature* 464:504–512. doi:[10.1038/nature08980](https://doi.org/10.1038/nature08980)

- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–464. doi:[10.1038/366461a0](https://doi.org/10.1038/366461a0)
- Khanna A, Muthusamy S, Liang R, Sarojini H, Wang E (2011) Gain of survival signaling by down-regulation of three key miRNAs in brain of calorie-restricted mice. *Aging* 3:223–236
- Kim HS, Choi ES, Shin JA, Jang YK, Park SD (2004) Regulation of Swi6/HP1-dependent heterochromatin assembly by cooperation of components of the mitogen-activated protein kinase pathway and a histone deacetylase Clr6. *J Biol Chem* 279:42850–42859. doi:[10.1074/jbc.M407259200](https://doi.org/10.1074/jbc.M407259200)
- Kim DH et al (2015) The roles of FoxOs in modulation of aging by calorie restriction. *Biogerontology* 16:1–14. doi:[10.1007/s10522-014-9519-y](https://doi.org/10.1007/s10522-014-9519-y)
- Kim J, Kim KM, Noh JH, Yoon JH, Abdelmohsen K, Gorospe M (2016) Long noncoding RNAs in diseases of aging. *Biochim Biophys Acta* 1859:209–221. doi:[10.1016/j.bbagr.2015.06.013](https://doi.org/10.1016/j.bbagr.2015.06.013)
- Koubova J, Guarente L (2003) How does calorie restriction work? *Genes Dev* 17:313–321. doi:[10.1101/gad.1052903](https://doi.org/10.1101/gad.1052903)
- Kouzarides T (2007) Chromatin modifications and their function. *Cell* 128:693–705. doi:[10.1016/j.cell.2007.02.005](https://doi.org/10.1016/j.cell.2007.02.005)
- Kozomara A, Griffiths-Jones S (2014) miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res* 42:D68–D73. doi:[10.1093/nar/gkt1181](https://doi.org/10.1093/nar/gkt1181)
- Kucerova L, Kubrak OI, Bengtsson JM, Strnad H, Nylin S, Theopold U, Nassel DR (2016) Slowed aging during reproductive dormancy is reflected in genome-wide transcriptome changes in *Drosophila melanogaster*. *BMC Genom* 17:50. doi:[10.1186/s12864-016-2383-1](https://doi.org/10.1186/s12864-016-2383-1)
- Kume S et al (2010) Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. *J Clin Invest* 120:1043–1055. doi:[10.1172/JCI41376](https://doi.org/10.1172/JCI41376)
- Kunigas M, Putters M, Westendorp RG, Slagboom PE, van Heemst D (2007) SIRT1 gene, age-related diseases, and mortality: the Leiden 85-plus study. *J Gerontol A Biol Sci Med Sci* 62:960–965
- Lau NC, Seto AG, Kim J, Kuramochi-Miyagawa S, Nakano T, Bartel DP, Kingston RE (2006) Characterization of the piRNA complex from rat testes. *Science* 313:363–367. doi:[10.1126/science.1130164](https://doi.org/10.1126/science.1130164)
- Lee C, Longo VD (2011) Fasting vs dietary restriction in cellular protection and cancer treatment: from model organisms to patients. *Oncogene* 30:3305–3316. doi:[10.1038/onc.2011.91](https://doi.org/10.1038/onc.2011.91)
- Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 75:843–854
- Lee HC, Chang SS, Choudhary S, Aalto AP, Maiti M, Bamford DH, Liu Y (2009) qiRNA is a new type of small interfering RNA induced by DNA damage. *Nature* 459:274–277. doi:[10.1038/nature08041](https://doi.org/10.1038/nature08041)
- Lescai F et al (2009) Human longevity and 11p15.5: a study in 1321 centenarians. *Eur J Hum Genet* 17:1515–1519. doi:[10.1038/ejhg.2009.54](https://doi.org/10.1038/ejhg.2009.54)
- Li Y, Liu L, Tollesbol TO (2010) Glucose restriction can extend normal cell lifespan and impair precancerous cell growth through epigenetic control of hTERT and p16 expression. *FASEB J* 24:1442–1453. doi:[10.1096/fj.09-149328](https://doi.org/10.1096/fj.09-149328)
- Li N, Bates DJ, An J, Terry DA, Wang E (2011a) Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. *Neurobiol Aging* 32:944–955. doi:[10.1016/j.neurobiolaging.2009.04.020](https://doi.org/10.1016/j.neurobiolaging.2009.04.020)
- Li N, Muthusamy S, Liang R, Sarojini H, Wang E (2011b) Increased expression of miR-34a and miR-93 in rat liver during aging, and their impact on the expression of *Mgst1* and *Sirt1*. *Mech Ageing Dev* 132:75–85. doi:[10.1016/j.mad.2010.12.004](https://doi.org/10.1016/j.mad.2010.12.004)
- Li Y, Daniel M, Tollesbol TO (2011c) Epigenetic regulation of caloric restriction in aging. *BMC Med* 9:98. doi:[10.1186/1741-7015-9-98](https://doi.org/10.1186/1741-7015-9-98)
- Liang F, Kume S, Koya D (2009) SIRT1 and insulin resistance. *Nat Rev Endocrinol* 5:367–373. doi:[10.1038/nrendo.2009.101](https://doi.org/10.1038/nrendo.2009.101)
- Liang R et al (2011) Post-transcriptional regulation of IGF1R by key microRNAs in long-lived mutant mice. *Aging Cell* 10:1080–1088. doi:[10.1111/j.1474-9726.2011.00751.x](https://doi.org/10.1111/j.1474-9726.2011.00751.x)
- Lichtenberg FR (2011) The quality of medical care, behavioral risk factors, and longevity growth. *Int J Health Care Finance Econ* 11:1–34. doi:[10.1007/s10754-010-9086-y](https://doi.org/10.1007/s10754-010-9086-y)
- Lim DH et al (2011) The endogenous siRNA pathway in *Drosophila* impacts stress resistance and lifespan by regulating metabolic homeostasis. *FEBS Lett* 585:3079–3085. doi:[10.1016/j.febslet.2011.08.034](https://doi.org/10.1016/j.febslet.2011.08.034)
- Lin MJ, Tang LY, Reddy MN, Shen CK (2005) DNA methyltransferase gene *dDnmt2* and longevity of *Drosophila*. *J Biol Chem* 280:861–864. doi:[10.1074/jbc.C400477200](https://doi.org/10.1074/jbc.C400477200)
- Liu L, van Groen T, Kadish I, Tollesbol TO (2009) DNA methylation impacts on learning and memory in aging. *Neurobiol Aging* 30:549–560. doi:[10.1016/j.neurobiolaging.2007.07.020](https://doi.org/10.1016/j.neurobiolaging.2007.07.020)
- Longo VD, Finch CE (2003) Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science* 299:1342–1346. doi:[10.1126/science.1077991](https://doi.org/10.1126/science.1077991)
- Maida Y et al (2009) An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature* 461:230–235. doi:[10.1038/nature08283](https://doi.org/10.1038/nature08283)
- Mair W, Dillin A (2008) Aging and survival: the genetics of life span extension by dietary restriction. *Annu Rev Biochem* 77:727–754. doi:[10.1146/annurev.biochem.77.061206.171059](https://doi.org/10.1146/annurev.biochem.77.061206.171059)
- Malone CD, Brennecke J, Dus M, Stark A, McCombie WR, Sachidanandam R, Hannon GJ (2009) Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary. *Cell* 137:522–535. doi:[10.1016/j.cell.2009.03.040](https://doi.org/10.1016/j.cell.2009.03.040)
- Marino G, Ugalde AP, Fernandez AF, Osorio FG, Fueyo A, Freije JM, Lopez-Otin C (2010) Insulin-like growth factor 1 treatment extends longevity in a mouse model of human premature aging by restoring somatotroph axis function. *Proc Natl Acad Sci USA* 107:16268–16273. doi:[10.1073/pnas.1002696107](https://doi.org/10.1073/pnas.1002696107)
- Martin GM, Oshima J (2000) Lessons from human progeroid syndromes. *Nature* 408:263–266. doi:[10.1038/35041705](https://doi.org/10.1038/35041705)
- Martins R, Lithgow GJ, Link W (2015) Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. *Aging Cell*. doi:[10.1111/accel.12427](https://doi.org/10.1111/accel.12427)

- Maures TJ, Greer EL, Hauswirth AG, Brunet A (2011) The H3K27 demethylase UTX-1 regulates *C. elegans* lifespan in a germline-independent, insulin-dependent manner. *Aging Cell* 10:980–990. doi:10.1111/j.1474-9726.2011.00738.x
- McElwee JJ, Schuster E, Blanc E, Thomas JH, Gems D (2004) Shared transcriptional signature in *Caenorhabditis elegans* Dauer larvae and long-lived *daf-2* mutants implicates detoxification system in longevity assurance. *J Biol Chem* 279:44533–44543. doi:10.1074/jbc.M406207200
- Mercken EM et al (2013) Calorie restriction in humans inhibits the PI3K/AKT pathway and induces a younger transcription profile. *Aging Cell* 12:645–651. doi:10.1111/accel.12088
- Milman S, Atzmon G, Huffman DM, Wan J, Crandall JP, Cohen P, Barzilai N (2014) Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity. *Aging Cell* 13:769–771. doi:10.1111/accel.12213
- Min KJ, Yamamoto R, Buch S, Pankratz M, Tatar M (2008) *Drosophila* lifespan control by dietary restriction independent of insulin-like signaling. *Aging Cell* 7:199–206. doi:10.1111/j.1474-9726.2008.00373.x
- Mitteldorf J (2016) An epigenetic clock controls aging. *Biogerontology* 17:257–265. doi:10.1007/s10522-015-9617-5
- Morris JZ, Tissenbaum HA, Ruvkun G (1996) A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* 382:536–539. doi:10.1038/382536a0
- Morris BJ et al (2015a) Association analysis of FOXO3 longevity variants with blood pressure and essential hypertension. *Am J Hypertens*. doi:10.1093/ajh/hpv171
- Morris BJ, Wilcox DC, Donlon TA, Willcox BJ (2015b) FOXO3: a major gene for human longevity—a mini-review. *Gerontology* 61:515–525. doi:10.1159/000375235
- Mosammaparast N, Shi Y (2010) Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. *Annu Rev Biochem* 79:155–179. doi:10.1146/annurev.biochem.78.070907.103946
- Moskalev AA, Shaposhnikov MV, Plyusnina EN, Zhavoronkov A, Budovsky A, Yanai H, Fraifeld VE (2013) The role of DNA damage and repair in aging through the prism of Koch-like criteria. *Ageing Res Rev* 12:661–684. doi:10.1016/j.arr.2012.02.001
- Moskalev AA, Aliper AM, Smit-McBride Z, Buzdin A, Zhavoronkov A (2014) Genetics and epigenetics of aging and longevity. *Cell Cycle* 13:1063–1077. doi:10.4161/cc.28433
- Mostoslavsky R et al (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 124:315–329. doi:10.1016/j.cell.2005.11.044
- Munoz-Najar U, Sedivy JM (2011) Epigenetic control of aging. *Antioxid Redox Signal* 14:241–259. doi:10.1089/ars.2010.3250
- Murabito JM, Yuan R, Lunetta KL (2012) The search for longevity and healthy aging genes: insights from epidemiological studies and samples of long-lived individuals. *J Gerontol A Biol Sci Med Sci* 67:470–479. doi:10.1093/gerona/gls089
- Murphy CT, Lee SJ, Kenyon C (2007) Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 104:19046–19050. doi:10.1073/pnas.0709613104
- Nakae J, Biggs WH 3rd, Kitamura T, Cavenee WK, Wright CV, Arden KC, Accili D (2002) Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1. *Nat Genet* 32:245–253. doi:10.1038/ng890
- Narasimhan SD, Yen K, Bansal A, Kwon ES, Padmanabhan S, Tissenbaum HA (2011) PDP-1 links the TGF-beta and IIS pathways to regulate longevity, development, and metabolism. *PLoS Genet* 7:e1001377. doi:10.1371/journal.pgen.1001377
- Nassel DR, Liu Y, Luo J (2015) Insulin/IGF signaling and its regulation in *Drosophila*. *Gen Comp Endocrinol* 221:255–266. doi:10.1016/j.ygcen.2014.11.021
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389:994–999. doi:10.1038/40194
- Okamoto N, Yamanaka N, Yagi Y, Nishida Y, Kataoka H, O'Connor MB, Mizoguchi A (2009) A fat body-derived IGF-like peptide regulates postfeeding growth in *Drosophila*. *Dev Cell* 17:885–891. doi:10.1016/j.devcel.2009.10.008
- Okamura K, Lai EC (2008) Endogenous small interfering RNAs in animals. *Nat Rev Mol Cell Biol* 9:673–678. doi:10.1038/nrm2479
- Outeiro TF, Marques O, Kazantsev A (2008) Therapeutic role of sirtuins in neurodegenerative disease. *Biochim Biophys Acta* 1782:363–369. doi:10.1016/j.bbadis.2008.02.010
- Paaby AB, Schmidt PS (2009) Dissecting the genetics of longevity in *Drosophila melanogaster*. *Fly* 3:29–38
- Papanicolaou KN, Izumiya Y, Walsh K (2008) Forkhead transcription factors and cardiovascular biology. *Circ Res* 102:16–31. doi:10.1161/CIRCRESAHA.107.164186
- Papatheodorou I, Petrovs R, Thornton JM (2014) Comparison of the mammalian insulin signalling pathway to invertebrates in the context of FOXO-mediated ageing. *Bioinformatics* 30:2999–3003. doi:10.1093/bioinformatics/btu493
- Partridge L, Harvey PH (1993) Gerontology. Methuselah among nematodes. *Nature* 366:404–405. doi:10.1038/366404a0
- Partridge L, Alic N, Bjedov I, Piper MD (2011) Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. *Exp Gerontol* 46:376–381. doi:10.1016/j.exger.2010.09.003
- Patel NV et al (2005) Caloric restriction attenuates Aβ-deposition in Alzheimer transgenic models. *Neurobiol Aging* 26:995–1000. doi:10.1016/j.neurobiolaging.2004.09.014
- Pincus Z, Smith-Vikos T, Slack FJ (2011) MicroRNA predictors of longevity in *Caenorhabditis elegans*. *PLoS Genet* 7:e1002306. doi:10.1371/journal.pgen.1002306
- Polito L, Kehoe PG, Forloni G, Albani D (2010) The molecular genetics of sirtuins: association with human longevity and age-related diseases. *Int J Mol Epidemiol Genet* 1:214–225
- Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem* 78:959–991. doi:10.1146/annurev.biochem.052308.114844
- Proshkina EN, Shaposhnikov MV, Sadritdinova AF, Kudryavtseva AV, Moskalev AA (2015) Basic

- mechanisms of longevity: a case study of *Drosophila* pro-longevity genes. *Ageing Res Rev* 24:218–231. doi:10.1016/j.arr.2015.08.005
- Pu M et al (2015) Trimethylation of Lys36 on H3 restricts gene expression change during aging and impacts life span. *Genes Dev* 29:718–731. doi:10.1101/gad.254144.114
- Puig O, Tjian R (2005) Transcriptional feedback control of insulin receptor by dFOXO/FOXO1. *Genes Dev* 19:2435–2446. doi:10.1101/gad.1340505
- Puig O, Marr MT, Ruhf ML, Tjian R (2003) Control of cell number by *Drosophila* FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev* 17:2006–2020. doi:10.1101/gad.1098703
- Qin Z, Hubbard EJ (2015) Non-autonomous DAF-16/FOXO activity antagonizes age-related loss of *C. elegans* germline stem/progenitor cells. *Nat Commun* 6:7107. doi:10.1038/ncomms8107
- Raffaghello L, Lee C, Safdie FM, Wei M, Madia F, Bianchi G, Longo VD (2008) Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. *Proc Natl Acad Sci USA* 105:8215–8220. doi:10.1073/pnas.0708100105
- Rajasethupathy P, Antonov I, Sheridan R, Frey S, Sander C, Tuschl T, Kandel ER (2012) A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. *Cell* 149:693–707. doi:10.1016/j.cell.2012.02.057
- Redman LM, Veldhuis JD, Rood J, Smith SR, Williamson D, Ravussin E, Pennington CT (2010) The effect of caloric restriction interventions on growth hormone secretion in nonobese men and women. *Ageing Cell* 9:32–39. doi:10.1111/j.1474-9726.2009.00530.x
- Richard DS et al (2005) Insulin signaling is necessary for vitellogenesis in *Drosophila melanogaster* independent of the roles of juvenile hormone and ecdysteroids: female sterility of the chico1 insulin signaling mutation is autonomous to the ovary. *J Insect Physiol* 51:455–464. doi:10.1016/j.jinsphys.2004.12.013
- Riedel CG et al (2013) DAF-16 employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. *Nat Cell Biol* 15:491–501. doi:10.1038/ncb2720
- Russell SJ, Kahn CR (2007) Endocrine regulation of ageing. *Nat Rev Mol Cell Biol* 8:681–691. doi:10.1038/nrm2234
- Salpea P et al (2012) Postnatal development- and age-related changes in DNA-methylation patterns in the human genome. *Nucleic Acids Res* 40:6477–6494. doi:10.1093/nar/gks312
- Satoh A et al (2013) Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab* 18:416–430. doi:10.1016/j.cmet.2013.07.013
- Schenk S et al (2011) Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. *J Clin Invest* 121:4281–4288. doi:10.1172/JCI58554
- Sen P et al (2015) H3K36 methylation promotes longevity by enhancing transcriptional fidelity. *Genes Dev* 29:1362–1376. doi:10.1101/gad.263707.115
- Sharma S, Brosh RM Jr (2007) Human RECQ1 is a DNA damage responsive protein required for genotoxic stress resistance and suppression of sister chromatid exchanges. *PLoS ONE* 2:e1297. doi:10.1371/journal.pone.0001297
- Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM (2010) Aging of the innate immune system. *Curr Opin Immunol* 22:507–513. doi:10.1016/j.coi.2010.05.003
- Shimokawa I et al (2015) The life-extending effect of dietary restriction requires Foxo3 in mice. *Ageing Cell* 14:707–709. doi:10.1111/accel.12340
- Siebold AP, Banerjee R, Tie F, Kiss DL, Moskowitz J, Harte PJ (2010) Polycomb repressive complex 2 and trithorax modulate *Drosophila* longevity and stress resistance. *Proc Natl Acad Sci USA* 107:169–174. doi:10.1073/pnas.0907739107
- Singh A, Kumar N, Matai L, Jain V, Garg A, Mukhopadhyay A (2016) A chromatin modifier integrates insulin/IGF-1 signalling and dietary restriction to regulate longevity. *Ageing Cell*. doi:10.1111/accel.12477
- Soerensen M, Nygaard M, Dato S, Stevnsner T, Bohr VA, Christensen K, Christiansen L (2015) Association study of FOXO3A SNPs and aging phenotypes in Danish oldest-old individuals. *Ageing Cell* 14:60–66. doi:10.1111/accel.12295
- Steuerman R, Shevah O, Laron Z (2011) Congenital IGF1 deficiency tends to confer protection against post-natal development of malignancies. *Eur J Endocrinol* 164:485–489. doi:10.1530/EJE-10-0859
- Suh Y et al (2008) Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc Natl Acad Sci USA* 105:3438–3442. doi:10.1073/pnas.0705467105
- Sun L et al (2015) FOXO3 variants are beneficial for longevity in Southern Chinese living in the Red River Basin: a case-control study and meta-analysis. *Sci Rep* 5:9852. doi:10.1038/srep09852
- Taguchi A, Wartschow LM, White MF (2007) Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* 317:369–372. doi:10.1126/science.1142179
- Tait IS, Li Y, Lu J (2015) Effects of PTEN on the longevity of cultured human umbilical vein endothelial cells: the role of antioxidants. *Int J Mol Med* 35:277–284. doi:10.3892/ijmm.2014.1999
- Talens RP et al (2012) Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. *Ageing Cell* 11:694–703. doi:10.1111/j.1474-9726.2012.00835.x
- Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS (2001) A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110. doi:10.1126/science.1057987
- Tatar M, Bartke A, Antebi A (2003) The endocrine regulation of aging by insulin-like signals. *Science* 299:1346–1351. doi:10.1126/science.1081447
- Thompson RF, Atzmon G, Gheorghe C, Liang HQ, Lowes C, Greally JM, Barzilai N (2010) Tissue-specific dysregulation of DNA methylation in aging. *Ageing Cell* 9:506–518. doi:10.1111/j.1474-9726.2010.00577.x
- Toiber D et al (2013) SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. *Mol Cell* 51:454–468. doi:10.1016/j.molcel.2013.06.018
- Tra J, Kondo T, Lu Q, Kuick R, Hanash S, Richardson B (2002) Infrequent occurrence of age-dependent changes in CpG island methylation as detected by restriction landmark genome scanning. *Mech Ageing Dev* 123:1487–1503

- van der Horst A, Burgering BM (2007) Stressing the role of FoxO proteins in lifespan and disease. *Nat Rev Mol Cell Biol* 8:440–450. doi:[10.1038/nrm2190](https://doi.org/10.1038/nrm2190)
- van Heemst D (2010) Insulin, IGF-1 and longevity. *Aging Dis* 1:147–157
- van Heemst D et al (2005) Reduced insulin/IGF-1 signalling and human longevity. *Aging Cell* 4:79–85. doi:[10.1111/j.1474-9728.2005.00148.x](https://doi.org/10.1111/j.1474-9728.2005.00148.x)
- Victoria B, Dhahbi JM, Nunez Lopez YO, Spinel L, Atamna H, Spindler SR, Masternak MM (2015) Circulating micro-RNA signature of genotype-by-age interactions in the long-lived Ames dwarf mouse. *Aging Cell* 14:1055–1066. doi:[10.1111/accel.12373](https://doi.org/10.1111/accel.12373)
- Vitale G et al (2012) Low circulating IGF-I bioactivity is associated with human longevity: findings in centenarians' offspring. *Aging* 4:580–589
- Waki T, Tamura G, Sato M, Motoyama T (2003) Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. *Oncogene* 22:4128–4133. doi:[10.1038/sj.onc.1206651](https://doi.org/10.1038/sj.onc.1206651)
- Waly M et al (2004) Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal. *Mol Psychiatry* 9:358–370. doi:[10.1038/sj.mp.4001476](https://doi.org/10.1038/sj.mp.4001476)
- Wang G, Reinke V (2008) A *C. elegans* Piwi, PRG-1, regulates 21U-RNAs during spermatogenesis. *Curr Biol* 18:861–867. doi:[10.1016/j.cub.2008.05.009](https://doi.org/10.1016/j.cub.2008.05.009)
- Wang MC, Bohmann D, Jasper H (2005) JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121:115–125. doi:[10.1016/j.cell.2005.02.030](https://doi.org/10.1016/j.cell.2005.02.030)
- Webb AE, Brunet A (2014) FOXO transcription factors: key regulators of cellular quality control. *Trends Biochem Sci* 39:159–169. doi:[10.1016/j.tibs.2014.02.003](https://doi.org/10.1016/j.tibs.2014.02.003)
- Weidner CI et al (2014) Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol* 15:R24. doi:[10.1186/gb-2014-15-2-r24](https://doi.org/10.1186/gb-2014-15-2-r24)
- Weinkove D, Leivers SJ (2000) The genetic control of organ growth: insights from *Drosophila*. *Curr Opin Genet Dev* 10:75–80
- Westbrook R, Bonkowski MS, Arum O, Strader AD, Bartke A (2014) Metabolic alterations due to caloric restriction and every other day feeding in normal and growth hormone receptor knockout mice. *J Gerontol A Biol Sci Med Sci* 69:25–33. doi:[10.1093/gerona/glt080](https://doi.org/10.1093/gerona/glt080)
- Willcox BJ et al (2008) FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA* 105:13987–13992. doi:[10.1073/pnas.0801030105](https://doi.org/10.1073/pnas.0801030105)
- Wolkow CA, Kimura KD, Lee MS, Ruvkun G (2000) Regulation of *C. elegans* life-span by insulin like signaling in the nervous system. *Science* 290:147–150
- Wolkow CA, Munoz MJ, Riddle DL, Ruvkun G (2002) Insulin receptor substrate and p55 orthologous adaptor proteins function in the *Caenorhabditis elegans* daf-2/insulin-like signaling pathway. *J Biol Chem* 277:49591–49597. doi:[10.1074/jbc.M207866200](https://doi.org/10.1074/jbc.M207866200)
- Xiao C et al (2010) SIRT6 deficiency results in severe hypoglycemia by enhancing both basal and insulin-stimulated glucose uptake in mice. *J Biol Chem* 285:36776–36784. doi:[10.1074/jbc.M110.168039](https://doi.org/10.1074/jbc.M110.168039)
- Yang Q, Sun M, Ramchandran R, Raj JU (2015) IGF-1 signaling in neonatal hypoxia-induced pulmonary hypertension: role of epigenetic regulation. *Vasc Pharmacol* 73:20–31. doi:[10.1016/j.vph.2015.04.005](https://doi.org/10.1016/j.vph.2015.04.005)
- Yu XY, Geng YJ, Liang JL, Lin QX, Lin SG, Zhang S, Li Y (2010) High levels of glucose induce apoptosis in cardiomyocyte via epigenetic regulation of the insulin-like growth factor receptor. *Exp Cell Res* 316:2903–2909. doi:[10.1016/j.yexcr.2010.07.004](https://doi.org/10.1016/j.yexcr.2010.07.004)
- Zeng Y et al (2015) Interaction between FOXO1A-209 genotype and tea drinking is significantly associated with reduced mortality at advanced ages. *Rejuvenation Res*. doi:[10.1089/rej.2015.1737](https://doi.org/10.1089/rej.2015.1737)
- Zhang X, Tang N, Hadden TJ, Rishi AK (2011) Akt, FoxO and regulation of apoptosis. *Biochim Biophys Acta* 1813:1978–1986. doi:[10.1016/j.bbamcr.2011.03.010](https://doi.org/10.1016/j.bbamcr.2011.03.010)
- Zhao T, Li J, Chen AF (2010) MicroRNA-34a induces endothelial progenitor cell senescence and impedes its angiogenesis via suppressing silent information regulator 1. *Am J Physiol Endocrinol Metab* 299:E110–E116. doi:[10.1152/ajpendo.00192.2010](https://doi.org/10.1152/ajpendo.00192.2010)
- Zhao G, Guo S, Somel M, Khaitovich P (2014) Evolution of human longevity uncoupled from caloric restriction mechanisms. *PLoS ONE* 9:e84117. doi:[10.1371/journal.pone.0084117](https://doi.org/10.1371/journal.pone.0084117)
- Zhong L et al (2010) The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1 α . *Cell* 140:280–293. doi:[10.1016/j.cell.2009.12.041](https://doi.org/10.1016/j.cell.2009.12.041)
- Zhou B et al (2012) Downregulation of miR-181a upregulates sirtuin-1 (SIRT1) and improves hepatic insulin sensitivity. *Diabetologia* 55:2032–2043. doi:[10.1007/s00125-012-2539-8](https://doi.org/10.1007/s00125-012-2539-8)