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Review

# *Caenorhabditis elegans* DAF-16/FOXO transcription factor and its mammalian homologs associate with age-related disease

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ABSTRACT

The insulin/IGF-1 signaling pathway is evolutionarily conserved and its function is mediated largely by FOXO transcription factors. Reduced insulin/IGF-1 signaling leads to translocation of FOXO proteins from the cytoplasm to the nucleus where they activate a set of genes that mediate oxidative stress, heat shock, and xenobiotic responses, innate immunity, metabolism, and autophagy. Disruptions in the insulin/IGF-1 signaling pathway affect lifespan in many species. Over the past two decades, the function of these FOXO proteins in age-related diseases has been extensively studied, in the model organism *Caenorhabditis elegans* as well as in humans. In this review we investigate the mechanisms by which FOXO proteins influence the development of age-related disease pathways in both species.



**Contents**



## 1. Insulin signaling, stress, and disease

The process of aging and senescence is almost always accompanied by the expression of disease and disorder. This creates a multi-stress environment and positions aging research in the field of stress biology. Understanding the mechanisms underlying aging as well as disease progression requires a fundamental insight into the gene regulatory responses to multi-stressors across species, including humans. But invasive experimental genetic manipulations cannot be carried out in humans for ethical as well as practical reasons and therefore more tractable species offer promising opportunities for studying the mechanisms of aging and associated diseases. In this respect, the short-lived nematode worm *Caenorhabditis elegans* (Nematoda, Rhabditidae) has

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become a powerful model species over the past few decades (Box 1). Genetic investigations into the formation of dauer larvae have led to the discovery of genes in the Insulin/IGF-1 signaling (IIS) pathway affecting dauer stage formation that strongly affect lifespan. Among these genes are *daf-2*, encoding an insulin-like receptor (Kenyon et al., 1993; Kimura et al., 1997), *daf-16*, that encodes a FOXO-transcription factor (Lin et al., 1997; Ogg et al., 1997), and *age-1*, the catalytic subunit of class-I phosphatidylinositol 3-kinase (PI(3)K) (Friedman and Johnson, 1988; Johnson, 1990). *Daf-16* can be regarded as a master regulator. It regulates the transcription of a large number of genes involved in abiotic and biotic stress resistance, and dauer formation, some of which also affect longevity (Tullett, 2015). PI(3)K inhibits FOXO activity through stimulation of Akt (AKT-1/2 in *C. elegans*) (Henderson and Johnson, 2001).

Murakami and Johnson (1996) were among the first to propose that stress resistance and lifespan are together negatively regulated by a set of gerontogenes, such as the *daf*-genes. DAF-16/FOXO plays a role in oxidative and heat stress and starvation (Henderson and Johnson, 2001) and was found to be homologous to four FOXO transcription factors found in humans, FOXO1, FOXO3a, FOXO4, and FOXO6 (Tzivion et al., 2011). One of these four proteins, FOXO3a, has been directly associated with prolonged lifespan in humans (Willcox et al., 2008; Anselmi et al., 2009; Flachsbart et al., 2009; Soerensen et al., 2010). Apart from having lifespan effects, FOXO transcription factors also influence age-related diseases, such as cancer (Pinkston et al., 2006; Paik et al., 2007; Pinkston-Gosse and Kenyon, 2007), osteoporosis (Iyer et al., 2013), Alzheimer's disease (AD), and diabetes mellitus (DM) type II (Erol, 2007; Manolopoulos et al., 2010).

Here we review the DAF-16/FOXO discoveries made in *C. elegans* and discuss how they are instrumental in the analysis of complex age-related disease pathways in humans.

## 2. FOXO transcription factors

### 2.1. Regulation involved in multi-stress response

FOXO transcription factors play an important role in the signal transduction pathway associated with stress and aging phenotypes. This is clearly illustrated by the fact that FOXO activity is strongly regulated by cellular stress signals, especially oxidative stress (Fig. 1) which ultimately modulates lifespan. Cellular stress signals lead to the induction of specific stress-related kinases that activate FOXO by phosphorylation, such as JUN kinase (JNK), that is part of the oxidative stress response

**Box 1**
*C. elegans* as a model organism for studying age-related diseases

*Caenorhabditis elegans* is a small saprophytic nematode of about 1 mm in length in the adult stage. It is a transparent self-fertilizing hermaphrodite, producing both sperm and eggs. *C. elegans* worms have a life cycle of about 3 days and an average lifespan of 18 to 20 days when cultivated in vitro on *Escherichia coli* at 20 °C. The postembryonic life cycle of *C. elegans* consists of four larval stages, L1–L4, and a reproductive stage. Under unfavorable conditions, the L2 stage can enter the dauer larval stage, instead of developing into the regular L3 stage (Brenner, 1974). Since the early 70s, many studies with *C. elegans* have led to new discoveries in neuroscience, signal transduction, cell death, RNA interference, environmental toxicology and biomedical science, among others (Kenyon, 2011). Since the discovery of a genetic pathway that appeared to greatly influence aging and stress resistance in *C. elegans* (Tullet, 2015), it has also been used extensively as a model organism in studies aiming to unravel lifespan determination.

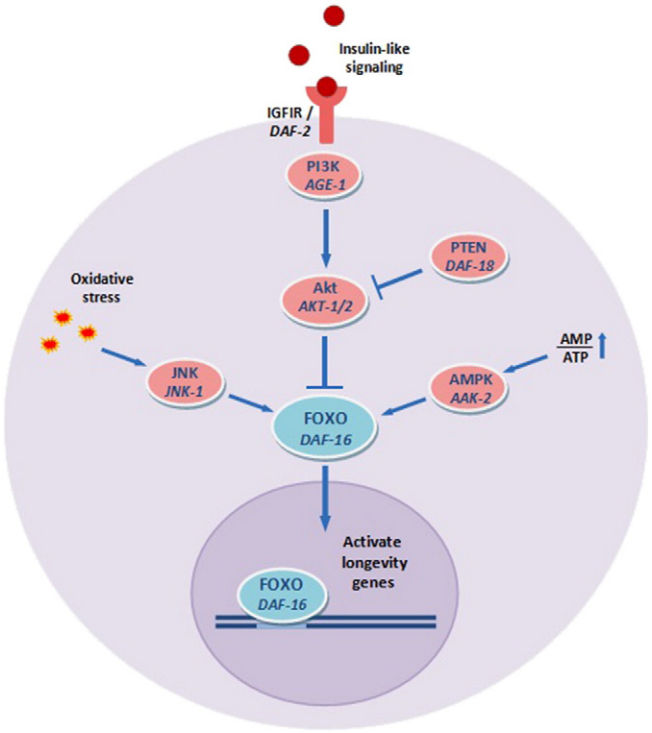


**Fig. 1.** A schematic overview of the regulation of FOXO activity. Insulin-like signaling leads to PI(3)K activation (Friedman and Johnson, 1988; Johnson, 1990), which induces Akt to inhibit FOXO by phosphorylation (Henderson and Johnson, 2001). The human tumor suppressor PTEN inhibits Akt activity, possibly by phosphorylation of PIP3 (not shown) (Christensen et al., 2011). When cells are under oxidative stress, JNK activity increases (Oh et al., 2005), while AMPK is activated by high AMP/ATP ratios (Apfeld et al., 2004; Greer et al., 2007). Both kinases activate FOXO by phosphorylation (Apfeld et al., 2004; Oh et al., 2005; Greer et al., 2007). When FOXO is active, it relocates to the nucleus and promotes the expression of genes that promote longevity (Henderson and Johnson, 2001). *C. elegans* homologs of mammalian proteins are shown in *italics*.

(Oh et al., 2005), MST, that is involved in growth control (Lehtinen et al., 2006), AMP activated protein kinase (AMPK in mammals, AAK-2 in *C. elegans*) responding to high AMP/ATP ratios (Apfeld et al., 2004; Greer et al., 2007), and the p38 MAP kinase pathway, that influences innate immunity and stress response in *C. elegans* (Troemel et al., 2006). When activated, DAF-16/FOXO relocates to the nucleus, upregulating genes involved in stress response and cell survival and downregulating developmental genes (Murphy et al., 2003). Another transcription factor, PQM-1, activates the developmental genes that are negatively regulated by DAF-16/FOXO. Both transcription factors work as antagonists to one another to maintain the balance between cell survival and development (Tepper et al., 2013). SMK-1 (SMEK in mammals) is a nuclear co-factor or co-regulator of the transcription of the target genes of FOXO proteins under oxidative stress, in response to UV, and in innate immunity (Wolff et al., 2006). By priming the stress response machinery of the worm, increased lifespan can be achieved in which DAF-16/FOXO plays a key role in combating stress and subsequent age-related disease (Table 1).

### 2.2. DAF-16/FOXO also affects age-related disease

DAF-16/FOXO not only affects lifespan but is also involved in age-related disease progression. For instance DAF-16/FOXO is linked to germline tumorigenesis in *C. elegans*. Since there are no dividing somatic cells in the *C. elegans* adult, only germline tumors occur in this organism. Mutations in the tumor suppressor gene known as *gld-1* lead to germline tumors that are lethal (Pinkston et al., 2006). In *gld-1* mutants, germline cells re-enter the mitotic cell cycle early in the process of oogenesis, and start to proliferate rapidly. Eventually the cells will

**Table 1**
Age-related diseases influenced by DAF-16/FOXO and/or mammalian FOXO proteins.

| Category | Disease | Pathology | Inhibition | Stimulation | Evidence |
|---|---|---|---|---|---|
| Cancer | Germline tumorigenesis (*C. elegans*) | Mutation *gld-1* gene leads to uncontrolled proliferation of germline cells. | DAF-16/FOXO upregulates nuclear pore proteins which are required for p53 induced apoptosis of germline cells. | | Pinkston et al. (2006), Pinkston-Gosse and Kenyon (2007) |
| | Tumorigenesis (mammals) | Activation/mutation of proto-oncogenes and/or inhibition/mutation of tumor suppressor genes leads to uncontrolled proliferation of the affected cells. More often than not, multiple genes need to be affected before tumorigenesis occurs. | *mTOR*: FOXO activity inhibits the function of mTORC1 by upregulation of TSC-1. This prevents mTORC1 from stimulating increased glycolysis, which leads to apoptosis resistance and tumor cell survival. | | Khatri et al. (2010), Johnson et al. (2013) |
| | | | *HIF-1α*: FOXO inhibits HIF-1α activity. The HIF-1α transcription factor promotes VEGF-A activity, which leads to blood vessel growth towards solid tumors and can promote tumor growth as well as metastasis. | | Bakker et al. (2007), Dansen and Burgering (2008), Chiacchiera and Simone (2009), Chiacchiera et al. (2009) |
| | | | *ERβ*: This receptor increases transcription of the FOXO3a gene. FOXO3a activates PUMA, which leads to p53-independent apoptosis in human prostate cancer cells. | | Yu and Zhang (2008), Dey et al. (2014) |
| Metabolic disease | Diabetes mellitus II (mammals) | Insulin resistance in peripheral tissues and decreased insulin production by pancreatic beta cells lead to high blood glucose levels and altered fat metabolism. | | FOXO is activated by JNK under oxidative stress conditions and in turn inhibits insulin-like signaling. This can promote the development of insulin resistance which further promotes degeneration of pancreatic beta cells and thus the onset of DM II. It also increases production of ROS, reactivating JNK. | Erol (2007), Manolopoulos et al. (2010) |
| Protein aggregation and polyglutamine | Alzheimer's disease (mammals) | Formation of β-amyloid plaques and τ protein phosphorylation in neurons causes neurodegeneration and decreased cognitive function. | | FOXO binds to β-catenin when activated by JNK, inhibiting Wnt-signaling. This leads to increased deposition of β-amyloid plaques and τ protein phosphorylation, resulting in neurodegeneration. It also causes an increase in ROS, reactivating JNK. | Erol (2007), Manolopoulos et al. (2010) |
| | Duchenne muscular dystrophy (*C. elegans*) | As a result of a loss-of-function mutation in the *dys-1* gene in humans, muscle necrosis and degeneration caused by dystrophin induced polyQ protein aggregations occur. | In *C. elegans*, increased DAF-16/FOXO activity protects from the polyQ protein aggregation caused by mutation of the *C. elegans* ortholog of the *dys-1* gene. | | Oh and Kim (2013) |
| | Huntington's disease (mammals) | Expanding polyQ tracts in the huntingtin protein cause protein aggregation resulting in neurodegeneration. This leads to chorea, cognitive decline, and psychiatric disorders. | Possibly depending on the stage of the disease, FOXO3a activity has been shown to be capable of stimulating the disease progression of HD. | | Mojsilovic-Petrovic et al. (2009), Jiang et al. (2011) |
| | | | | Possibly depending on the stage of the disease, FOXO3a activity has been shown to be capable of protecting against the disease progression of HD. | Davila et al. (2012), Davila and Torres-Aleman (2008) |
| Skeletal disease | Osteoporosis (mammals) | Decrease of bone formation during aging causes bones to become more brittle. | | Inhibition of Wnt-signaling by the binding of β-catenin by FOXO leads to a decrease in bone formation by osteoblasts, promoting osteoporosis. | Almeida et al. (2007), Essers et al. (2005), Manolagas and Almeida (2007), Iyer et al. (2013) |

accumulate, leading to an early death (Pinkston et al., 2006). It was observed that the germline cells and lifespan of *daf-2* mutants, in which DAF-16/FOXO is active, were not influenced by mutation of *gld-1*. In addition, it was found that DAF-16/FOXO, when activated, interacts with p53 to induce germline apoptosis. In *C. elegans*, p53 only induces apoptosis in germline cells in which DNA damage has occurred (genotoxic stress), and not under normal circumstances. However, the *C. elegans* p53 gene, *cep-1*, is not a DAF-16/FOXO target gene. Instead, it is postulated that DAF-16/FOXO induces the same response in cells as if they were under genotoxic stress, enabling p53 to stimulate apoptosis. Nuclear pore proteins, which are upregulated by DAF-16/FOXO, are necessary for DAF-16/p53-dependent apoptosis. It has been suggested that these proteins modify nuclear pores in such a way that a protein specifically required for p53-dependent cell death can be transported through them (Pinkston-Gosse and Kenyon, 2007). The induction of cell death by DAF-16/FOXO is restricted to the germline tumor and does not affect healthy tissue (Pinkston et al., 2006). In fact, normal germline cell death is completely independent of DAF-16/FOXO as well as of p53 (Pinkston-Gosse and Kenyon, 2007). These findings underline that a strong correlation exists between aging and the susceptibility to cancer development. This suggests that tumorigenesis is influenced by genes that regulate aging, such as *daf-16* and its homologs (Pinkston et al., 2006).

#### 2.2.1. Protein aggregation and polyglutamine

In addition to tumorigenesis, DAF-16/FOXO is a key player in the formation of diseases due to protein aggregation and polyglutamine (*polyQ*). In humans, neurodegenerative diseases, such as Huntington's disease (HD) and AD, are associated with polyQ peptide chains in proteins. These chains are formed because of expanding stretches of the codon CAG in genes, encoding glutamine, leading to long stretches of polyglutamines in the affected proteins. Generally, if the glutamine chain lengths in the proteins expand beyond 35 to 40 residues, they become cytotoxic in the nerve cells leading to cell death (Morley et al., 2002; Khare et al., 2005; Morimoto, 2008). The onset of polyQ-associated pathology correlates with the lifespan of *C. elegans* and this is linked to DAF-16 (Morley et al., 2002). PolyQ-mediated protein aggregation occurs rapidly in *C. elegans* in which the heat shock factor 1 gene, *hsf-1* is knocked down (Nollen et al., 2004). DAF-16/FOXO and heat shock factor-1 (HSF-1) work partly together to promote longevity in the absence of DAF-2 insulin-like signaling (Hsu et al., 2003). Together DAF-16/FOXO and HSF-1 promote the expression of four small heat-shock protein (sHSP) genes, *hsp-16.1*, *hsp-16.49*, *hsp-12.6*, and *sip-1*. This suggests that DAF-16/FOXO is involved in heat-shock response and that it also delays the formation of polyQ aggregations through these four sHSP genes. The hypothesis that sHSPs influence protein aggregation (Clark and Muchowski, 2000) was confirmed by showing that sHSPs bind to unfolded proteins and prevent them from aggregating (Hsu et al., 2003; Fu, 2014). In the case of AD endoproteolysis of an amyloid precursor protein (APP) leads to the formation of certain peptides, Aβ1–42 in particular, that are prone to aggregation. Aβ1–42 aggregation is reduced in long-lived *C. elegans* mutants in which the insulin/insulin growth factor-1-like signaling is knocked-down. DAF-16/FOXO activity was involved in the reduction of SOD1 aggregate neurotoxicity (Boccitto et al., 2012), also suggesting a protective role of *daf-16* in neurodegenerative diseases.

In addition to HD and AD, Duchenne muscular dystrophy is a fatal muscle degenerative disease with no cure available at this time, which is caused by loss-of-function mutations in the *dys-1* gene. The *dys-1* gene encodes dystrophin, which forms a complex with other proteins known as dystrophin associated protein complex (DAPC) (Oh and Kim, 2013). The result of a loss-of-function mutation in the dystrophin gene in humans is muscle necrosis caused by proteotoxicity of dystrophin induced polyQ protein aggregations. Mutation of the *C. elegans* ortholog of the dystrophin gene (also named *dys-1*) causes mild muscle degeneration as well as muscle contractility dysfunction. Reduced insulin/IGF-1 signaling prevents protein aggregation and muscle cell death in a manner dependent on DAF-16/FOXO (Oh and Kim, 2013). Whether the mechanism by which this effect is mediated is the same as in other polyQ-associated diseases still needs to be elucidated. A combination of mutations in *dys-1* with a *daf-2* protects the *dys-1* mutant from muscle cell death. Additionally, the lifespan of the *daf-2;dys-1* double mutants was extended to a level similar to that of the *daf-2* mutant (Oh and Kim, 2013).

From these findings it can be concluded that degenerative diseases and disorders of the neuronal system in *C. elegans* are strongly linked to DAF-16/FOXO activity. This illustrates that research into lifespan and aging associated DAF-16/FOXO provides important insights into complex aging-related diseases of the nervous system.

## 3. Mammalian FOXO proteins

### 3.1. Cancer

While FOXO3A has been associated with long life in humans (Willcox et al., 2008; Anselmi et al., 2009; Flachsbart et al., 2009; Soerensen et al., 2010) it also, together with other FOXO transcription factors, has been shown to play a role in tumor progression. Deletion of FOXO1, FOXO3a, and FOXO4 in mice immediately resulted in tumor growth (Paik et al., 2007). Notably, in most types of cancer, FOXO activity is inhibited by the overexpression of phosphoinositide 3-kinase (PI(3)K) and protein kinase B (PKB or Akt) (Hu et al., 2004) which promote cytoplasmic retention and degradation of FOXO transcription factors. Although PI(3)K and Akt activities are very important, there are also factors that regulate FOXO activity independently of these kinases.

#### 3.1.1. FOXO proteins and mTOR under hypoxia

The mechanistic target of rapamycin (mTOR) is a key modulator of aging and age-related disease and works antagonistically to FOXO proteins. mTOR is incorporated in two cellular complexes, mTOR complex 1 (mTORC1) and mTORC2. The latter acts downstream of insulin signaling components PI(3)K and Akt and is involved in the direct inhibition of FOXO activity by insulin/IGF-1 signaling (Johnson et al., 2013). mTORC1 is also activated by the PI(3)K/Akt kinase cascade and is a key regulator of protein synthesis, lipid metabolism, autophagy, inflammation, and glycolysis. mTOR also activates hypoxia-inducible factor 1 alpha (HIF-1α), a transcription factor that negatively influences FOXO activity (Johnson et al., 2013). FOXO proteins also upregulate the tuberous sclerosis protein 1 (TSC-1) gene (*tsc-1*), which functions as a tumor suppressor and which is an upstream inhibitor of mTORC1 (Khatri et al., 2010; Johnson et al., 2013). However, the interaction between TSC-1, mTORC1 and FOXO proteins is more intricate. Upon activation by Akt, mTORC1 increases glycolysis in cells in which FOXO3a has been knocked-down, by downregulating the expression of TSC-1. This strongly suggests that FOXO3a prevents the effect of Akt-mediated mTORC1-dependent glycolysis by controlling the transcription of *tsc-1* (Khatri et al., 2010). Since increased glycolysis leads to apoptosis resistance and thus tumor cell survival, this suggests another important role for FOXO proteins in the prevention of tumorigenesis.

#### 3.1.2. HIF-1α

FOXO proteins also suppress the development of new blood vessels in tumor, and possibly even metastasis, by down-regulating the transcription factor HIF-1α (Bakker et al., 2007). One of the main transcriptional targets of this transcription factor is an attractant for endothelial cells known as secreted vascular endothelial growth factor-A (VEGF-A). This pathway stimulates growth of new blood vessels towards solid tumors. HIF-1α is activated by mTORC1 (Khatri et al., 2010; Johnson et al., 2013) at low levels of oxygen in cells as well as by high levels of mitochondrial reactive oxygen species (ROS) (Simon, 2006; Dansen and Burgering, 2008). FOXO activity upregulates the expression of CITED2 that associates with the p300 transcriptional co-activator. In doing so, it prevents the binding of p300 to HIF-1α and thus the

expression of HIF-1α's target genes (Bakker et al., 2007; Dansen and Burgering, 2008). It is possible that FOXO proteins also prevent HIF-1α activity by upregulating the expression of MnSOD and other enzymes that can scavenge ROS, since ROS can activate HIF-1α (Kops et al., 2002; Simon, 2006). HIF-1α is also crucial for tumor cell survival because it upregulates the transcription of genes that mediate aerobic glycolysis. Tumor cells rely on constitutive aerobic glycolysis for their ATP production (Chiacchiera and Simone, 2009; Chiacchiera et al., 2009). It seems plausible that through this mechanism mTORC1 increases glycolysis, since mTORC1 promotes HIF-1α activity. However, this has not yet been confirmed.

#### 3.1.3. Apoptosis and the estrogen receptor β

Although FOXO transcription factors can prevent cells from entering apoptosis (i.e. programmed cell-death) they are also capable of promoting apoptosis (Dijkers et al., 2000; Tang et al., 2002; Dansen and Burgering, 2008; Chapuis et al., 2010). They can promote the expression of diverse apoptosis regulator genes such as Bcl-2-like protein (BIM) (Dijkers et al., 2000), tumor necrosis factor ligand 6 (Fas ligand or FasL) (Chapuis et al., 2010) and B-cell lymphoma 6 (BCL6) (Tang et al., 2002). Which pathway FOXO transcription factors promote depends on their binding to co-activators as well as other gene regulators (Dansen and Burgering, 2008; van der Vos and Coffer, 2008).

Estrogen receptor β (ERβ) plays a crucial role in the induction of apoptosis in human prostate cancer cells. This effect is mediated by the p53-upregulated modulator of apoptosis (PUMA) and is dependent on FOXO3a, but not dependent on p53 (Yu and Zhang, 2008; Dey et al., 2014). Interestingly, ERβ does not influence FOXO3a phosphorylation, but it increases the transcription of the FOXO3a gene. This leads to higher PUMA levels and consequently to apoptosis of the cancer cells via caspase-9 (Dey et al., 2014).

Taken together, evidence is accumulating that mammalian FOXO proteins are not only associated with long life but also with carcinogenic phenotypes, very often related with hypoxia conditions.

### 3.2. Diabetes mellitus type II and Alzheimer's disease: FOXO under oxidative stress

FOXO transcription factors play a central role in the pathophysiological processes of DM and AD. It seems that both diseases are affected by the same positive feedback loop that starts with high levels of oxidative stress. Additionally, the onset of one disease stimulates that of the other and vice versa (Erol, 2007; Manolopoulos et al., 2010). In reaction to oxidative stress, JNK activates FOXO proteins by phosphorylation. FOXO activity inhibits Akt and thereby insulin/IGF-1 signaling, which can lead to a lower responsiveness of cells to insulin. Increased activity of FOXO1 has been shown to directly decrease insulin sensitivity in liver, pancreatic, and adipose cells (Nakae et al., 2002). This insulin resistance leads to an increase in blood glucose levels and subsequently higher levels of ROS, resulting in further activation of FOXO by JNK (Erol, 2007; Manolopoulos et al., 2010). Additionally, FOXO1 activity was found to inhibit pancreatic β-cell differentiation by decreasing the expression of the pancreatic transcription factor pancreas/duodenum homeobox gene-1 (*Pdx1*) (Kitamura et al., 2002). This would further promote the development of DM II by reducing the production of insulin. Constitutive JNK activity and FOXO activity are capable of inhibiting Wnt signaling, leading to deposition of β-amyloid plaques, τ protein phosphorylation, and subsequent neurodegeneration by apoptosis. This process is strongly associated with AD pathogenesis (Manolopoulos et al., 2010) and will also lead to the formation of more ROS, completing the circle. These pathological processes in turn lead to more oxidative stress and activate JNK again (Fig. 2) (Erol, 2007; Manolopoulos et al., 2010). It can be concluded that under oxidative stress conditions, FOXO transcription factors play a central role in complex diseases such as DM and AD.

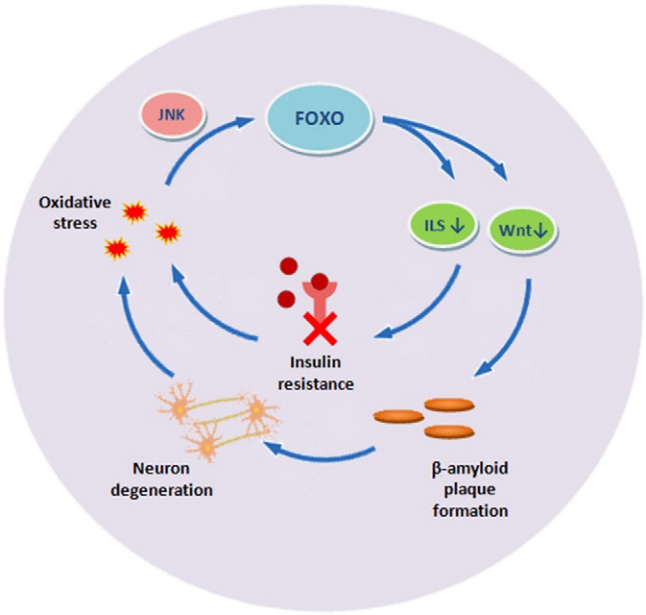


**Fig. 2.** Schematic overview of the influence of FOXO on development of DM and AD. Oxidative stress activates JNK, which leads to increased FOXO activity. FOXO reduces both insulin-like signaling (ILS) and Wnt signaling. Reduced ILS eventually leads to insulin resistance, hyperglycemia and thus development of DMII (Erol, 2007; Manolopoulos et al., 2010). The result of reduced Wnt signaling is the formation of β-amyloid plaques as well as protein aggregation (not shown), that causes degeneration of neurons. This is associated with the pathogenesis of AD (Manolopoulos et al., 2010). Development of both diseases will eventually cause an increase in ROS, activating JNK and completing the cycle (Erol, 2007; Manolopoulos et al., 2010).

### 3.3. Huntington's disease

HD is an autosomal dominant disease which is caused by an expanding polyQ tract in the huntingtin (HTT) protein. It is a neurodegenerative disorder marked by abnormal involuntary movements (chorea), cognitive decline, and psychiatric disorders (Huntington's Disease Collaborative Research Group, 1993). In contrast to the protective role of *daf-16* in neurodegenerative diseases the functional effects of FOXO3A on disease progression can be counteracting. On the one hand FOXO3a has been shown to stimulate HD progression (Davila and Torres-Aleman, 2008; Bahia et al., 2012; Davila et al., 2012) whereas in other studies HD development was alleviated (Mojsilovic-Petrovic et al., 2009; Jiang et al., 2011). It has been suggested that the influence of FOXO3a activity differs between the early/mild stage of HD than in the late/severe stage (Neri, 2012). More research is necessary to determine if FOXOs can be targeted for treatment of HD, and whether they should be up- or downregulated to achieve this goal, possibly depending on the stage of the disease.

### 3.4. Osteoporosis

During the aging process, the formation of new bone tissue declines resulting in more brittle bones in a process known as osteoporosis. Osteoporosis has been linked to elevated activity of FOXO transcription factors triggered by oxidative stress in combination with reduced production of growth factors (Manolagas and Almeida, 2007; Iyer et al., 2013). When cells are under oxidative stress, FOXO proteins physically associate with β-catenin, which is then prevented from fulfilling its role in Wnt signal transduction (Essers et al., 2005; Manolagas and Almeida, 2007). Since Wnt signaling is involved the stimulation of bone formation by osteoblasts, this leads to a decrease in bone formation (Almeida et al., 2007; Manolagas and Almeida, 2007; Iyer et al., 2013). Bipotential progenitor cells (i.e. adipocyte or osteoblast) of

mice lacking FOXO1, FOXO3a, and FOXO4 activity more often differentiate into osteoblasts than wild type controls. Furthermore, bone tissue in these mutant mice remained denser with increasing age than controls, and there was less adipose tissue formed in the bone marrow (Iyer et al., 2013).

## 4. Perspectives

Research on components of the insulin-like signaling pathway and *daf-16* in *C. elegans* has facilitated the discovery of homologs of these proteins in humans. These human homologs are not only key regulators of aging, but also of age-related diseases. DAF-16/FOXO influences germline tumorigenesis (Pinkston et al., 2006; Pinkston-Gosse and Kenyon, 2007) and protein aggregation diseases (Clark and Muchowski, 2000; Morley et al., 2002; Khare et al., 2005; Morimoto, 2008; Oh and Kim, 2013) in *C. elegans* while human FOXO proteins are very important in protection against cancer development (Dijkers et al., 2000; Hu et al., 2004; Bakker et al., 2007; Paik et al., 2007; Dansen and Burgering, 2008; van der Vos and Coffer, 2008; Yu and Zhang, 2008; Chiacchiera and Simone, 2009; Chiacchiera et al., 2009; Chapuis et al., 2010; Khatri et al., 2010; Johnson et al., 2013; Dey et al., 2014). However, these proteins also seem to promote the development of other age-related diseases such as Alzheimer's, diabetes mellitus type II (Erol, 2007; Manolopoulos et al., 2010) and osteoporosis (Almeida et al., 2007; Manolagas and Almeida, 2007; Iyer et al., 2013) and seem to have conflicting effects on the progression of Huntington's disease (Davila and Torres-Aleman, 2008; Mojsilovic-Petrovic et al., 2009; Jiang et al., 2011; Bahia et al., 2012; Davila et al., 2012; Neri, 2012). Because FOXO proteins are such key regulators, increasing or decreasing their activity for therapeutic purposes is likely to have many unwanted side effects if not manipulated either very specifically, or very locally. The fact that AD, DM type II, and osteoporosis are less localized than most tumors complicates the use of FOXO proteins as molecular targets in treatment of these diseases. Besides their potential for therapeutic application, further study of FOXO transcription factors and their functions could lead to a better understanding of how increased human life expectancies and healthy aging could be achieved.

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