Taming the Pro-Aging Factors and Pathways for Longevity and Improving Health-span

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Abstract

Biology of Aging and Longevity: The unicellular and multicellular organisms, both, age chronologically with their life span representing the average age of the organisms at death. At the cellular level, the chronological aging is mediated in part by ROS generated by mitochondria and attended by loss of mitochondrial function. Aging is, also, a major risk factor for disease-states such as atherosclerosis and cardiovascular disease, degenerative disorders including neurodegenerative diseases and disorders due to altered cellular physiology and mitosis like cancer.

Identifying Pro-Aging Pathways: There is an established role of IGF-1 and Insulin signaling in the regulation of the life span. Apart from this, the Akt and Ras pathways playing an important role in IGF-1 signaling, appear to regulate aging in mammals. Ras, Akt and Serine/threonine-protein kinase (also known as serum and glucocorticoid-regulated kinase - Sgk-1, encoded by the SGK1 gene) plays an essential role in cellular functions such as growth and metabolism and may accelerate aging in some tissues and organs.

Proaging and Longevity Mechanisms: There are similarities between the pathways that regulate stress resistance, cellular protection and aging in lower animals like C. elegans, yeast and Drosophila as well as in mammals. Insulin and insulin-like signaling factors regulate survival and lifespan and play a significant role in longevity in various animal species, from nematodes and Drosophila to higher vertebrates. In the regulation of replicative aging, the role of Ras pathway appears to be complex and the Ras and PI3K/Akt pathways are major intracellular mediators of the effect of IGF-1. The Ras pathway activates the RAF/MEK/ERK as well as the SEK/p38 and other pathways through IGF-1, which are associated with various cellular processes like growth, differentiation and apoptosis.

Dealing with Pro-Aging Factors: Understanding biological basis of the aging process has led to insights that can potentially identify measures to slow down the aging process. The aging, thus, becomes a modifiable risk factor and there are expectant possibilities to extend the lifespan and improve health-span. Through targeted lifestyle changes and potentially effective interventions through nutraceuticals and pharmaceuticals, it is possible to reduce the age-related chronic and debilitating morbidities and improve the health-span.

Keywords: *Akt Pathway; FOXO; Health Span; IGF-1 Signaling; Insulin Signaling; Lifespan; Longevity; mTOR Signaling; Nutraceuticals; Ras Pathway; Sirtuins*

Biology of aging and longevity

Both, the unicellular and multicellular organisms age chronologically with their life span representing the average age of the organism at death. In case of unicellular organisms like yeast (*Saccharomyces*), the number of times a yeast mother cell divides to produce daughter cells in its lifetime represents the replicative life span [1]. With cell survival and growth, there occurs progressive accumulation of

ribosomal DNA circles in the nucleolus, which appears an important etiological factor for the replicative aging. On the other hand, the chronological aging of an organism is related to cellular injury by ROS generated by the mitochondria and resultant altered metabolism and loss of mitochondrial function [2].

Aging is a major risk factor for disease-states such as atherosclerosis and cardiovascular disease, degenerative disorders including neurodegenerative diseases and disorders due to altered cellular physiology and mitosis like cancer. Understanding the aging phenomenon leads to biological insights that identify promising interventions to slow down the aging process. The aging, thus, becomes a modifiable risk factor and leads to the expectant possibility to extend the lifespan. Through targeted lifestyle changes and potentially effective interventions through nutraceuticals and pharmaceuticals it may be possible to reduce the age-related chronic and debilitating morbidities and improve the health-span.

Identifying pro-aging pathways

Regulation of stress and longevity

The discovery of the role of Ras (Ras proteins), adenylate cyclase, PKA (protein kinase A), and Sod (Superoxide dismutase) in the regulation of stress resistance and longevity, together with the discovery of the role of the IGF-1-like pathway in aging both in *Saccharomyces* and *C. elegans* have outlined the mechanisms and pathways that control chronological aging in simpler organisms. In organisms like *Saccharomyces* and *C. elegans*, the longevity regulatory pathways and mechanisms share significant similarities and include members of the PKB (Protein kinase B, also known as Akt), family of serine-threonine kinases as well as stress resistance transcription factors controlling the antioxidant enzymes and heat shock proteins. Similar pathways and mechanisms including the IGF-1-like receptor, Akt and a stress resistance transcription factor are also responsible for longevity regulation in *Drosophila*. Further, the lifespan-extending mutations affecting these pathways in *Saccharomyces*, *C. elegans* and *Drosophila* highlight that these pathways are activated during periods of starvation as well as during dietary calorie restriction.

Further up along the evolution, the role of IGF-1 and Insulin signaling in the regulation of the mouse life span, together with the central role of the Akt and Ras pathways in IGF-1 signaling, indicates that similar factors may regulate stress resistance and aging in mammals. In fact, Ras, Akt and Serine/threonine-protein kinase (also known as serum and glucocorticoid-regulated kinase - Sgk-1, encoded by the SGK1 gene) may accelerate age in various cells but at the same time play an essential role in functions such as cell growth and division, and metabolism. Various studies document that the mice lacking serum IGF-1, *Drosophila* deficient in IGF-1-like signaling, and *Saccharomyces* lacking the Akt/PKB homolog Sch9 live longer but smaller in size. On the other hand, *Saccharomyces* lacking *RAS2* (a gene encoding Ras) and *C. elegans* with mutations involving *daf-2* (the gene encoding the insulin-like/IGF-1 tyrosine kinase receptor) are able to attain normal size and live longer.

Insulin/IGF-1 and allied pathways

The studies in *C. elegans*, *Drosophila* and mice point to the insulin/insulin-like growth factor-1 (IGF-1)/phosphatidylinositol 3-kinase (PI3K)/Akt-like pathway being the prime regulator of aging and longevity. The insulin/IGF-1 pathway, which is a complex signal transduction pathway, includes an IGF-1-like receptor, PI3K, members of the Akt/protein kinase B (PKB) kinases and Forkhead transcription factor. The studies indicate that the components of an IGF-1-like pro-aging pathway are conserved from *Saccharomyces* to mammals. Further, in *C. elegans* serum- and glucocorticoid-inducible kinase (SGK-1), playing an important role in longevity regulation, has 55% sequence analogy to Akt [3]. In *Saccharomyces*, a similar pathway, which includes Sch9, a serine-threonine kinase homologous to mammalian Akt/protein kinase B, appears to regulate lifespan and longevity [4].

Further, in *Saccharomyces*, *C. elegans* and *Drosophila*, the partially conserved glucose or insulin/IGF-1-like pathways down-regulate antioxidant activity, reduce the accumulation of glycogen or fat, and improve growth and decrease longevity. It has been shown that the mutations impairing activity of these pathways extend longevity. In mammals, IGF-1 appears to activate the signal transduction pathways analogous to the longevity regulatory pathways in *C. elegans* and *Drosophila* and decrease longevity (Figure 1). In humans, alterations resulting in a deficiency of plasma GH or IGF-1 cause dwarfism and obesity and may negatively the chronological lifespan.
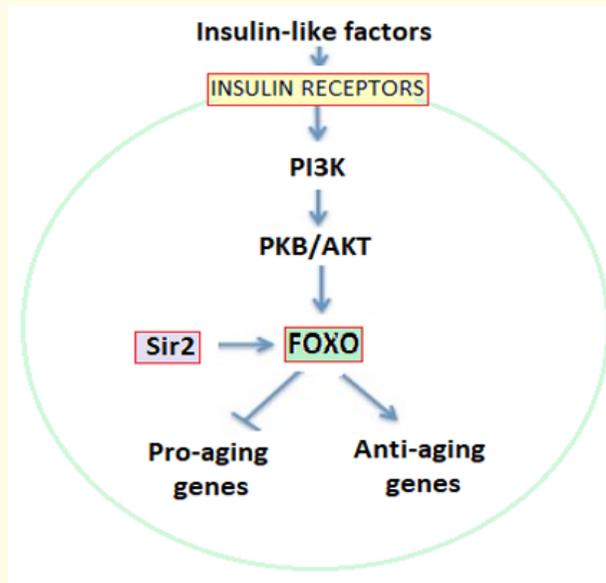
Figure 1: *Insulin Receptors are initially activated by binding of insulin-like peptides. The activated receptor phosphorylates the enzyme phosphoinositide 3-kinase (PI3K), a lipid kinase, which then recruits the PH-domain-containing protein kinase B (PKB/AKT). PKB signals downstream by inhibiting the FOXO, allowing FOXO to negatively regulate the expression of pro-aging genes and positively affect the expression of anti-aging genes. The overexpression of Sir2 or Sirtuin increases longevity by influencing FOXO.*

Ras dependent pathways

The chronological aging in *Saccharomyces*, in addition, is also regulated by a second pathway that includes Ras, adenylate cyclase, protein kinase A, the transcription factors - Msn2 and Msn4 (multicopy suppressor of *SNF1* mutation proteins 2 and 4) and Sod2. This signal transduction pathway, regulated by Ras2 and partially influenced by Sch9, has been shown to have an impact on stress resistance and life span in *Saccharomyces*. Ras and Sch9 function in an overlapping manner to stimulate growth to mediate via glucose-dependent signaling but impair stress resistance. This is proved by the reversal of slow growth in *Saccharomyces* phenotype lacking Sch9 by the expression of human Akt, indicating that human Akt is analogous and a functional substitute for Sch9 in *Saccharomyces*.

A major role of Ras proteins in mammalian IGF-1 signaling, which have not been implicated in longevity regulation in worms or flies, raises the possibility that homologs of yeast Ras2 probably accelerate aging in mammals [5]. Thus, Ras signaling has been found to shorten life span and promote cellular senescence in *Saccharomyces* and mammals, whereas in the long-lived *C. elegans daf-2* mutants, the Ras signaling promotes longevity. In experiments, the Ras inactivation extends life span in *Saccharomyces* and Ras1 and Ras2, which function directly upstream of adenylate cyclase, catalysing the production of cAMP from adenosine triphosphate in the Ras/cAMP/PKA pathway, play overlapping roles in cell growth, development and stress resistance [6].

The Ras is highly conserved in many organisms from *Saccharomyces* to humans and constitutive activation of Ras causes a major decrease in life span. Whereas, the inactivation of Ras2 functioning upstream of adenylate cyclase, favourably alters life span. Further, the long-surviving ras2 and cyr1 mutants display the need for stress resistance transcription factors Msn2 and Msn4 and the mitochondrial Sod2, highlighting the increased investment in cell protection and repair to slow down aging. There are indications that Ras activation promotes growth and aging by shifting energy investment from cell protection and turn-over. It is held that the pro-aging effect of the Ras/cAMP/PKA pathway through activation of PKA leads to decreased lifespan. Whereas the Ras inactivation via increasing Sod activity, leads to survival benefit and increased lifespan in *Saccharomyces*, *C. elegans* and *Drosophila*, as well as mammalian neuronal cells, *in vitro* [7].

Proaging and longevity mechanisms

Ras, stress resistance and apoptosis

There are similarities in the pathways regulating oxidative stress and longevity in *Saccharomyces*, *C. elegans* and *Drosophila*. It appears that similar mechanisms influence cell survival and aging in mammals. The four *Saccharomyces* genes, *SOD1, SOD2, RAS2* and *SCH9* have been found to have profound effect on its chronological lifespan and are conserved from *Saccharomyces* to *Homo sapiens* [8]. Further, in addition to promoting cell growth, the Ras activation may promote or prevent apoptosis, dependant on the activity of other signal transduction pathways and the relative activity of downstream signaling agents such as mitogen-activated protein kinase (MAPK), Rac1 and PKB.

The Ras exhibits contradictory pro- and anti-aging effects on cellular physiology. Thus, depending on ROS and other factors, Ras may either prevent apoptosis to promote cell growth and function or induce cellular damage and senescence. There are studies to suggest that Ras activity might contribute to aging in mammalian cells. The chronic Ras-dependent increase in the generation of oxidants appears to increase cellular appendicular damage, accelerate aging and contribute to age-related disorders [9]. The Ras has also been shown to mediate apoptosis in T cells and epithelial cells and induce replicative senescence in diploid fibroblasts by augmenting intracellular oxidant injury.

Interrelationship between insulin/IGF-1 and Ras pathways

The similarities between the glucose- or IGF-1-like signaling pathways that regulate life span in *Saccharomyces*, *C. elegans* and *Drosophila* suggest that the IGF-1 pathway may play a central role in the aging in mammals, as well. The Ras and PI3K/Akt pathways are major intracellular mediators of the effect of IGF-1 on longevity, considering their primary role in IGF-1 signaling. Following IGF-1 binding to its receptor, Ras binds to GTP and activates the RAF/MEK/ERK, the SEK/p38 and other pathways involved in cellular functions like growth, differentiation and apoptosis. A possible link between Ras activity and aging in mammals is through p66[shc], which is tyrosine-phosphorylated and binds to Ras guanine nucleotide exchange factor, Grb2. The role of Ras pathway in the regulation of replicative aging appears to be intricate.

The CR through NAD and SIR2 has been shown to down-regulate the Ras/cAMP/PKA pathway and extend replicative life span in *Saccharomyces* [10]. Sir2 is a conserved deacetylase that modulates life span in *C. elegans* and *Drosophila* also, and stress response in mammals. In *Saccharomyces* Sir2 is required for maintaining replicative life span and increasing Sir2 dosage can delay replicative aging [11].

IGF and insulin like factors

Insulin and insulin-like signaling regulate survival and lifespan and play a significant role in longevity in a variety of animal species, from *C. elegans* and *Drosophila* to higher vertebrates and mammals [12]. The IGF-I receptor knockdown mice are fertile, slightly smaller and resist oxidative stressors more effectively when compared with normal littermate mice [13]. The brain IGF-I receptor and brain IRS2 control mammalian lifespan through neuroendocrine mechanisms, control of energy metabolism and modified stress resistance. Further, insulin receptor substrate molecules are implicated downstream of insulin and IGF receptors in the extension of lifespan. Several genes of the somatotropic axis act as longevity determinants, and the variants of FOXO3A, downstream signaling molecule in the insulin/IGF pathway, are associated with extreme longevity in humans. Finally, several functional mutations of the human IGF-IR have been discovered in centenarians.

Complex interplay of key proteins and longevity pathways

Several key proteins in the evolutionary conserved pathways have been linked to regulation of lifespan [14]. These compounds belong to the sirtuin family of NAD-dependent enzymes, various components of the insulin/IGF [2] pathway and the mechanistic target and downstream effectors of mTOR kinase.

Sirtuins - the family of NAD-dependent enzymes

The sirtuins catalyse various deacylation reactions including demalonylation, desuccinylation and deproprionylation at cellular level [15]. The link of these enzymes to aging was noted when it was observed that overexpression of *Sir2* extended replicative lifespan in *S. cerevisiae* [16]. In an important development, the life-extending benefit of caloric restriction in *Saccharomyces* has been linked to *Sir2* [17]. Further, similar to higher organisms, the overexpression of *Sir2* in both *C. elegans* and *Drosophila* is able to extend lifespan. The lifespan extending effects of sirtuins has been linked to favourable alterations in genomic stability, mitochondrial function and biogenesis, and suppression of inflammation [18].

Biochemically, the Sirtuins are class III histone deacetylases. They are able to remove acetyl groups from modified lysine in the histone N-terminal tails in presence of NAD^+ as a cofactor. Through this process, namely, removal of histone acetyl groups the sirtuins appear play a central role in regulatory networks linked with aging and longevity favourably alter the lifespan [19,20]. There are seven sirtuins, SIRT1 to 7, existing in various cellular compartments in mammals. The SIRT1, SIRT6 and SIRT7 are predominantly nuclear forms, whereas SIRT3, SIRT4 and SIRT5 are present in mitochondria.

Experimentally, the overexpression of SIRT1 results in an appreciable increase in median lifespan in both female and male mice. Whereas, only the SIRT6-overexpressing transgenic male mice show approximately 15% extension in median lifespan. Other mouse sirtuin overexpression experiments fail to alter lifespan, though certain interventions may improve health span. The SIRT1 overexpression has been shown to protect from metabolic syndrome, cardiovascular disease and heart failure as well as decreasing tendency for neurodegenerative disorders and certain malignancies. The SIRT6 overexpression has also shown to favourably alter the health span. Apart from, gain-of-function models involving overexpression, the sirtuin knock-out models also elucidate the importance of SRT in age-related pathologies. As shown in mice, the absence of SIRT3 predisposes mice to early onset of various aging disorders. Whereas, mice experiments outline the ability of caloric restriction in preventing the age-related hearing loss linked to SIRT3 expression [21].

A polyphenol, resveratrol is the sirtuin-activating molecule that has been documented to improve the lifespan in *Saccharomyces*, *C. elegans* and *Drosophila* [22]. The evidence from mice models and clinical studies in humans shows that resveratrol may protect from metabolic disorders and various age-related diseases associated with physiological aging and diet-induced obesity [23]. It has cardiovascular protective effects in high-fat-fed non-human primates, as well. The administration of resveratrol in obese human subjects, has shown some measurable short-term benefits [24]. The STACs, thus, have potential to protect against various age-related disorders [25]. Further, two STACs (SRT1720 and SRT2104) have been shown experimentally to improve health span in mice fed on a standard diet, with modest positive effects on mean lifespan [26].

The genetic manipulation of sirtuin activity is, thus, helpful in outlining and defining certain age-related alterations as well as developing sirtuin-activating compounds - STACs (Figure 2).
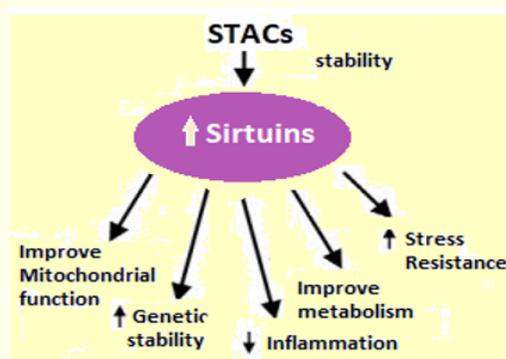

Figure 2: The STACs and their role in cellular physiology and dysfunction.

Components of insulin/insulin-like growth factor signaling (IIS) pathway

The IIS pathway includes insulin and insulin-like peptides, the transmembrane receptors and their substrates, and the downstream effectors. There is considerable evidence that insulin and insulin-like peptides modulate cellular growth and development. insulin signaling is primarily involved in nutrient regulation, whereas IGF-1 modulates growth. Further, various studies have linked the IIS pathway to the aging process.

The studies in model organisms have highlighted that the genetic alterations modestly reducing IIS signaling positively influence the lifespan. The factors, namely, phosphatidylinositol-3-OH kinase (PI3K) and insulin-like receptor through *age-1* and *daf-2* gene products regulate lifespan in *C. elegans* [27]. There occurs an increase in the lifespan following reduction in insulin or IGF-1 signaling, which involves *daf-2* and *daf-16* genes. These genes are regulators of dauer, the stress-resistant and alternative developmental state, to which the nematodes can alter when exposed to adverse environmental conditions. The dauer state nematodes with the altered IIS pathway are resistant to environmental stresses including oxidative, osmotic, heat and hypoxic stress and have a longer lifespan [28]. In addition, in *C. elegans* the Forkhead box O (FOXO) transcription factor Daf-16 functions as an important transcriptional target of the IIS pathway and involved regulating expression of genes involved in metabolism, stress resistance and immune function [29]. These functions are conserved in higher species and the FOXO homologs appear to regulate antioxidant levels in mammals.

The production of IGF-1 occurs mainly in the liver in mammals and modulated by growth hormone (GH). In mice experiments, reduced circulating IGF-1 levels contributed to a 16% increase in median lifespan of female GH mutant mice, but a similar deletion in male mice did not alter lifespan. The underlying physiology of this sexual dimorphic response, however, is not well understood. The reduced level of circulating IGF-1 or reduced expression of IGF-1R, is attended by altered degradation of IGF-1-binding proteins.

The long-lived IIS mutants have been shown to possess an increased stress resistance and various other attributes responsible for their increased lifespan. The GH mutant mice, in animal studies, have altered mitochondrial function and oxygen consumption with a beneficial increased metabolic dependence on fatty acid oxidation. These alterations in the IIS pathway also leads to reduced mTOR signaling, which may have a major influence on lifespan. A corollary evidence comes from the clinical studies in humans indicating a correlation between increased circulating IGF-1 levels and the development of a wide range of malignancies. The patients with Laron syndrome, an autosomal recessive disorder caused by mutation in the growth hormone receptor, have extremely low levels of circulating IGF-1, which results in short stature but increased metabolic fitness and low incidence of diabetes and malignancy.

mTOR (mechanistic target of rapamycin) signaling

The mTOR kinase is a serine/threonine protein kinase belonging to the PI3K-related family, inhibited by rapamycin, an immunosuppressive drug. Working as an energy sensor in the simple organisms like *C. elegans*, it is activated by surplus nutrients supply. On activation, mTOR inhibits catabolic processes like autophagy and promotes anabolic processes like protein synthesis and ribosomal biogenesis to promote cellular growth and proliferation. Whereas, limited availability of nutrients or forced caloric restriction inhibits mTOR and promotes autophagy and halts proliferation.

In mammals, mTOR exists as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The upstream signals like nutrients, growth factors, energy and stress act to tune the activity of mTORC1. Whereas, downstream effectors of mTORC1 include regulation of mitochondrial metabolism, ribosomal biogenesis and protein, pyrimidine and lipid synthesis. In addition, mTORC1 modulates autophagy and the senescence-associated secretory phenotype [30]. It also regulates various key transcription factors like sterol regulatory element-binding protein (SREBP) 1, peroxisome proliferator-activated receptor γ (PPARγ), HIF-1α, NF-κB and transcription factor EB (TFEB) associated respectively with lipogenesis, adipogenesis, metabolism, inflammation and autophagy. As compared to mTORC1, the mTORC2 is less sensitive to rapamycin and associated with has been linked to cellular metabolism and modulation of cytoskeleton dynamics and cell cycle.

The studies in *C. elegans* and *Drosophila*, have indicated that the inhibition of mTOR pathway significantly increased longevity. Inhibiting S6K, involved in protein translation and downstream effector for mTOR, in *Drosophila*, extended the lifespan. The studies in mice have highlighted a similar relationship between S6K and lifespan. The mouse models lacking one allele of mTOR had reduced mTORC1 activity, and there was significant increase in median survival of female mice. Similarly, in a mouse model of hypomorphic mTOR expression, mice live longer than controls and have slower age-dependent decline in various tissues and some organs.

Similar to IIS signaling, the precise physiology of reduced mTOR signaling effect on lifespan is unclear. The benefit probably occurs through reduced mRNA translation and protein synthesis, and reduced activities related to protein folding, repair and degradation. These effects appear to be mediated by two downstream effectors of mTOR, namely S6K and eukaryotic translation initiation factor 4E-binding protein (4E-BP). The caloric restriction in *Drosophila* up-regulates d4E-BP (the *Drosophila* equivalent of 4E-BP) and increases lifespan. In mice studies, an increase in 4E-BP in skeletal muscle has been shown to prevent certain age-related metabolic alterations.

Activation of autophagy appears to be another potential explanation for the effect of mTORC1 inhibition on longevity. In general, the autophagic activity declines with age, resulting in the accumulation of protein aggregates and dysfunctional mitochondria. In addition, the mTOR inhibition improves stem cell self-renewal both in the hematopoietic system as well as in the intestines and suppresses the secretion of inflammatory cytokines by senescent cells associated with age-related disorders. The mTOR inhibition has been linked to the ability of the cell or tissue to withstand energetic, oxidative and hypoxic stresses, and improve mitochondrial biogenesis and physiology.

Interactions between longevity pathways

In general, the three pathways - sirtuins, mTOR and the IIS responding to nutrient availability, modulate the lifespan. The major interconnection between these pathways is through AMP-activated protein kinase (AMPK). It has been observed that mTOR activity declines and sirtuin activity increases on exposure to starvation or caloric restriction. The activated AMPK influences intracellular metabolism and upregulates NAD+ levels and SIRT1 activity. The SIRT1 in turn deacetylate and regulate FOXO transcriptional activity, which is a downstream effector of the IIS pathway [31]. The interaction between AMPK and the sirtuins is bidirectional. The SIRT1 deacetylates liver kinase B1 (LKB1), an upstream activator of AMPK. In turn, the mTOR activity is regulated by AMPK. There exists, thus, a network of complex interactions between sirtuins, mTOR and Insulin/IGF-1 signaling. In CR models in *C. elegans*, *Drosophila* and mammals, AMPK phosphorylates FOXO proteins.

The three longevity pathways, sirtuins, IIS and mTOR, also converge to regulate autophagy through multiple interdependent effectors. The ISS inhibits autophagy through activating PI3K/AKT signaling. ThePI3K/AKT in turn stimulates mTOR. The mTOR shuts off autophagosome through phosphorylating ATG1 in *Saccharomyces* or ULK1/2 in mammals. The AKT activation has a negatively impact on FOXO transcriptional activity, whereas FOXO regulates the expression of various autophagic genes. The sirtuins influence the FOXO activity through deacetylation and modulate autophagic flux, similar to IIS and mTOR [32]. In addition, SIRT1 also deacetylate autophagy genes and regulate autophagic flux. The SIRT1/FOXO pathway is critical for the CR-induced autophagy response.

In addition, there lies a synergy for the positive feedback loop involving AMPK and FOXO proteins, suggesting that the complex interactions between these pathways are dynamic. Further, these pathways are not the only regulators of lifespan, there is also a role for mitochondrial physiology and metabolism in aging [33]. Various mitochondrial pathways appear to intersect with the factors like NAD and AMPK and simultaneously exert influence on aging via activation of such distinct signaling mechanisms as the mitochondrial unfolded protein response.

Mechanisms and hallmarks of aging

Theorising aging mechanisms

Aging involves complex physiology and characterized by reduced general health and fitness, resulting in the accumulation increased prevalence of age-related diseases and increased risk of dying. The hallmarks of aging at the cellular level are telomere attrition and epigenetic alterations, which lead to deregulated nutrient sensing, mitochondrial dysfunction, loss of genomic stability, cellular senescence

and altered intercellular communication, and stem cell exhaustion. Various theories for aging, broadly are - Program Theories, Damage Theories or Combined Theories.

Program Theories are based on the concept that aging is genetically programmed. The theory of replicative senescence is based on the Hayflick phenomenon highlighting the loss of the shelter-in complex with cell divisions. The cellular senescence is held to be triggered by factors such as oxidative stress and oncogene overexpression. In a biological system, the cellular senescence is an anti-tumor mechanism resulting in the accumulation prevents the damaged or deranged cells from dividing any further. Senescent cells undergo phenotypic changes and start secreting various proteins including cytokines and chemokines, which attract immune cells aimed at removal of the senescent cells. With increasing age, the senescent cells accumulate and trigger pathological changes in the particular organ. The theory of antagonistic pleiotropy conceptualizes that the mutations during evolution provide an advantage for early stages of life but are detrimental late in life. Thus, in the biological systems the damaged cells become senescent to prevent tumor formation but are responsible for various physiological adverse effects. Another program theory, the disposable soma theory is also a program theory stating that that resources for an organism are limited and they have to be allocated between maintenance of homeostasis and reproduction. The neuroendocrine theory of aging is also a kind of program theory, asserting that aging is regulated by hormones similar to physiological phases of like puberty and menopause. In its support are the facts that animals reaching sexual maturity in a short period of time have also a short lifespan and the parabiosis experiments demonstrating that blood from young mice has a rejuvenating effect on old mice. Further, the changes occur in the regulation of gene expression during aging which is responsible for altered growth and maldevelopment.

The damage theories include the free radical theory of aging. The reactive oxygen species (ROS) are by-product of oxidative phosphorylation in mitochondria, which damage DNA, attack unsaturated fatty acids in the cell membranes producing chemically hyper-active aldehydes which in turn react with DNA and proteins resulting in irreversible modifications including neurodegeneration. In addition, proteins are also attacked by ROS and oxidatively damaged proteins have the tendency to denature and form aggregates which the cell which may cause cell death eventually as is the case in Alzheimer's disease. The theory of Inflamm-Aging is a modified damage theory visualizing the aging as a result of the ongoing low level sterile inflammatory processes. In this respect, the inflammatory process plays a role in various aging-related degenerative diseases such as Alzheimer's, Parkinson's, arteriosclerosis, arthritis, multiple sclerosis, osteoporosis and T2DM. Various inflammatory reactions are spear-headed by debris of dead cells and undegradable protein aggregates which form the basis of Garb-Aging (garbage and aging) theory. In this perspective, the aging can be seen as a progressive decline in fitness due to the rising deleteriome [34]. Corollary to this, is the concept that in biological systems neither the synthesis of biomolecules nor the repair systems can work flawlessly. In addition, there are chemical reactions between various biomolecules leading to accumulation of unwanted reaction products and unrepaired damage, that is, the deleteriome which increases as cost of living and exacerbates the aging process.

The hallmarks of biological aging

1. **DNA damage:** The aging is attended by genomic instability due to DNA damage caused by factors like ROS, lipid peroxidation products, ultra-violet irradiation, hydrolytic reactions and environmental mutagens. Usually, the repair systems can deal with the DNA damage which has far-reaching effects. But, the efficiency of repair systems declines with aging.

2. **Telomere attrition:** The telomeres get shorter with every cell division and reach a critical length to halt the cell division, leading to replicative senescence. The degree of shortening has been related to immuno-senescence and various aging diseases, including osteoporosis, bone marrow failure, premature onset of emphysema and pulmonary and hepatic fibrosis.

3. **Epigenetic alterations:** There occur epigenetically modifications with age, comprising of alterations in histones, DNA methylation, nucleosome and non-coding RNAs. The alterations in histones and methylation patterns of CpG ligands influence gene expression leading to a set of changes termed as the epigenetic clock [35]. The epigenetic clock has been related to the biological age and age-related functional decline [36]. The lifestyle factors like diet, exercise and social factors such as economic class and education have an influence on this epigenetic clock [37].

4. **Loss of Proteostasis:** The proteostasis includes synthesis and degradation of proteins as well as folding and conformational maintenance. The deranged proteostasis is a major cause of aging [38]. With aging, there occurs decline of chaperones, proteasomal activity and autophagy leading to accumulation of denatured proteins which form aggregates detrimental to the cell and cause apoptosis [39]. The activity of the proteasome is significantly high in the longer-living animals.

5. **Dysregulated nutrient sensing:** The major nutrient sensing pathways that are also longevity pathways are IGF-1 and insulin signaling pathway, mTOR pathway, AMP-activated protein kinase (AMPK) pathway and NAD$^+$ dependent sirtuins. The IGF-1 is a growth factor, akin to insulin, acting through insulin and IGF-1 signaling (IIS) pathway. Down-regulation of the IIS pathway has been shown to improve the lifespan. Whereas, the mTOR pathway promotes protein synthesis and growth, and down-regulates autophagy. AMPK is sensor and regulator pathway for energy metabolism and homeostasis. It has been related favourably to lifespan in *Saccharomyces*, *C. elegans* and *Drosophila*, whereas in mice experiments it improves the health-span. The NAD-dependent sirtuins not only deacetylate histones but modify various non-histone proteins and prevent diseases and certain aspects of aging [40]. There is a reduced synthesis of the sensor proteins as well as dysregulation of nutrient sensing signal transduction pathways with age.

6. **Mitochondrial dysfunction:** The ROS are important signaling molecules regulating various pathways in mitochondria. The quality of mitochondria has a bearing on mitochondrial dynamics. The important external determinants of mitochondrial dynamics are availability of nutrients and physical activity. The excess supply of nutrients may lead to fragmentation of mitochondria, whereas a poor availability leads to mitochondrial fusion and elongation. The mTOR and AMP-activated kinase pathways and sirtuins play an important role in TCA cycle, fatty acid metabolism, electron transport chain and regulation of metabolic plasticity. The quality of mitochondrial function has an impact on the aging process and the disordered mitochondrial dynamics potentiates inflammatory response and inflamm-aging and promotes sarcopenia, tissue damage and fibrosis in various organs, and harbinger of age-related diseases metabolic, cardiovascular and neurodegenerative diseases [41,42].

7. **Cellular senescence:** The cellular senescence is marked by an irreversible arrest of the cell cycle, which is attended by oxidative stress, mitochondrial dysfunction, proteotoxicity and nucleic acid damage, oncogene expression and telomeric erosions. The senescent cells change their morphology, get larger with massive changes in organization of chromatin [43]. Further, they start secreting a number of proteins called the senescence-associated secretory phenotype (SASP). The SASPs include pro-inflammatory chemokines and cytokines and contribute to inflamm-aging. The increase of senescent cells with aging occurs due to suppression of apoptosis associated with decline in immune function. The senescent cells are detrimental to the organ function and culminate as age-dependent degenerative diseases.

8. **Stem cell exhaustion:** The stem cells are important for tissue homeostasis and regeneration and their exhaustion is a potential etiological factor for aging at the tissue level. The regenerative capacity of stem cells is affected by various intrinsic and extrinsic factors. At the tissue level, factors like telomere attrition, DNA damage, epigenetic alterations, defective nutrient sensing and disturbed proteostasis lead to senescence and premature exhaustion of the stem cells, which is apparent in age-related diseases. Another intrinsic factor is autophagy and the failure of maintenance of autophagy is an important precursor of cellular senescence.

9. **Altered intercellular communication:** There occurs an elevated concentration of Wnt-proteins in muscle stem cells with aging, attended by an altered intercellular signaling between muscle stem cells and the environment with aging. The protein, Klotho is essential for the homeostasis of mineral metabolism including phosphate and modulates the signaling pathways of IGF-1 and Wnt. It acts as an antagonist of Wnt/β-catenin signaling, decreases during aging. Various pro-inflammatory cytokines secreted by the senescent cells also affect the intercellular communication. Simultaneously, the DAMPs (damage-associated molecular patterns) comprising of necrotic cell debris, amyloid fibers, HMGB1, heat shock proteins, cholesterol components and uric acid activate the inflammasomes in the innate immune system, which lead to release of interleukins, such as IL-1β and IL-18. The altered intercellular communication and inflammatory reactions have been cited as harbinger of various age-related disorders like Alzheimer´s disease.

The cells secrete lipid vesicles containing proteins and functional RNAs, called exosomes. The exosomes are relayed locally or via circulation and contribute to intercellular communication, in which miRNAs play an important role. During aging, their amount remains more or less constant, but their content becomes pro-inflammatory and plays a role in cellular senescence and aging [44].

1. **Genetic control of aging:** The recent research continues to provide clues about the molecular pathways linked with aging [45]. Various experiments have documented that *C. elegans* with a particular single-gene mutation has twice the lifespan as compared to other members of the species lacking the mutation [46]. This finding has strengthened the concept that aging may be under some sort of genetic control rather than physiological deterioration with course of time. Further, it has opened the possibility to modify life span through genetic manipulation. The responsible gene is *daf-2* and is encoded by DAF-2 protein, which resembles the receptor protein in humans that responds to the insulin - being a primitive form of human insulin receptors.

The *daf-2* gene and DAF-2 protein are conserved evolutionarily in various animals. In *C. elegans* and *Drosophila*, the gene codes for a receptor protein that is activated by an insulin-like growth factor and the signaling pathway is analogous to the mammalian insulin pathway [47]. It has been documented that *daf-2* controls various other genes, which in turn regulate a variety of physiological processes at different stages by encoding for proteins that can extend lifespan by regulating metabolism and modifying favourably the antioxidant and antibacterial defence. In this respect, a specific gene is *daf-16*, which encodes DAF-2 protein. DAF-16 is not phosphorylated in the mutant *C. elegans* but retained in an active form, which appears to be a necessary step toward lifespan extension.

The transcription factor DAF-16 controls the expression of various genes having effects on lifespan either promoting aging or longevity in *C. elegans*. The pro-longevity genes include the genes involved in encoding antioxidant enzymes and others encoding heat-shock proteins. The latter are important for restoring misfolded proteins to their active conformations. The genes that promote aging include a pro-ageing protein which is similar to the insulin-like INS-7 and by binding to the insulin/IGF-1 receptor (DAF-2), may repress DAF-16 to modulate DAF-2 activity through a feedback loop (Figure 3).
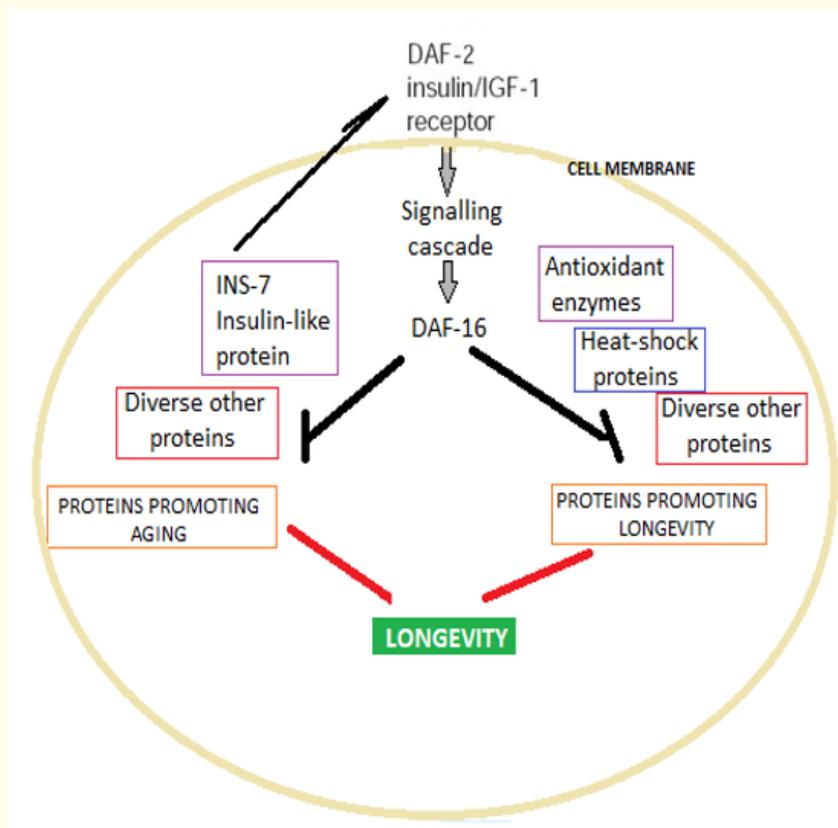

Figure 3: *The IGF and insulin signalling pathway and DAF-2 and DAF-16 transcription factors in C. elegans influencing aging and longevity.*

Further, the *daf-2* and *daf-16* genes appear to represent a form of genetic regulation that allows *C. elegans* to mould its morphology and physiology according to the its habitat and suspend the development to the dauer state. In animal models, knocking out the activity of *daf-2* leads the developing nematodes into the dauer state, irrespective of the availability of nutrients [48]. This evolutionary conserved insulin signaling pathway has been shown to be critically involved in aging and longevity in mammals including humans [49]. These genes act by influencing groups of other genes which coordinate the survival system within organisms (Figure 4). The mutations related to the insulin/IGF-1 pathway and receptors have been associated with the dysregulation that characterizes the increasing risk of both diabetes and cancer with age [50].
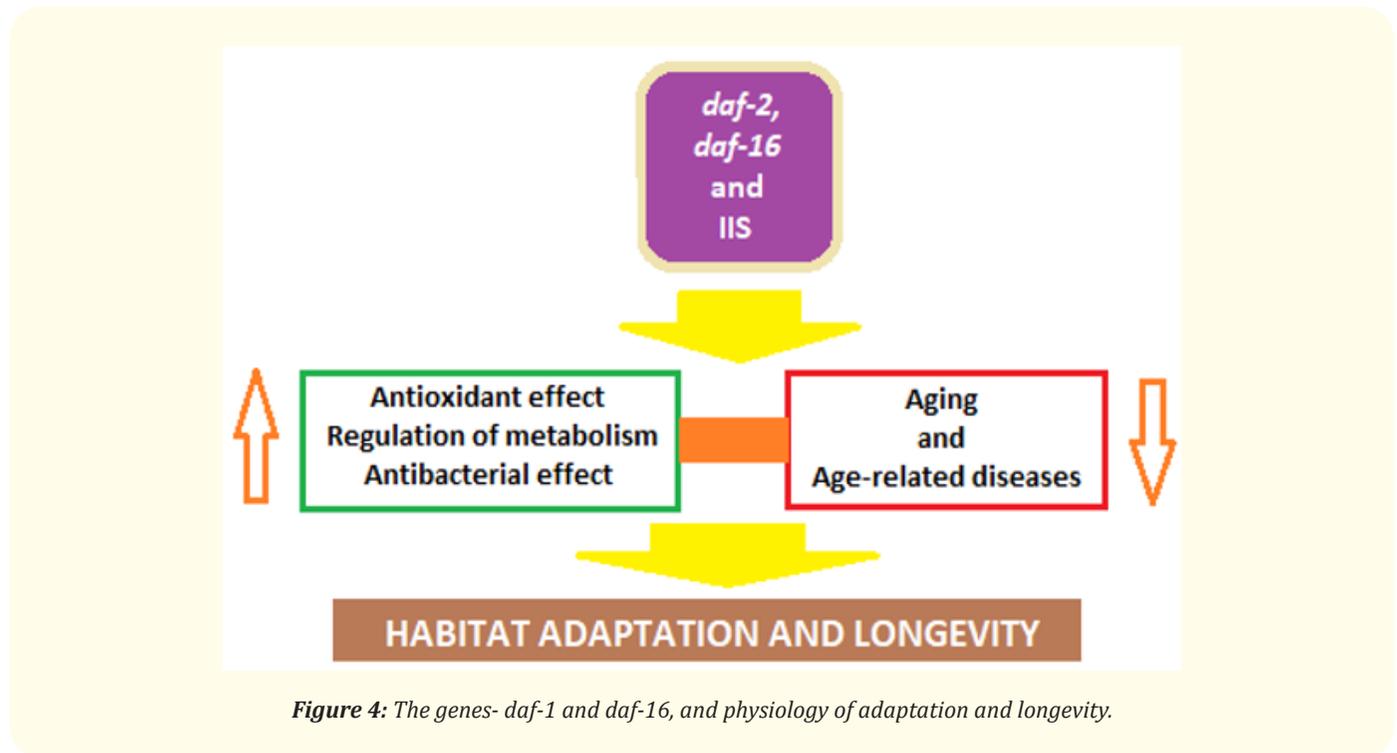

Figure 4: The genes- daf-1 and daf-16, and physiology of adaptation and longevity.

Interventions for taming pro-aging pathways

Physical exercise

The simplest and most efficient way to attenuate aging is to increase physical activity. The exercise improves physiological parameters like maximum oxygen consumption and reduces serum levels of cholesterol and triglycerides. It improves physical and psychical debility associated with aging, as well. Apart from the improved physiological parameters, the exercise has protective influence on the cardiovascular system which is higher than can be explained by the improvement in the physiological parameters. In addition, there is a documented positive effect on the brain and cognitive functions. With the increased physical activity, there occurs stimulation of neuronal growth in the hippocampus, an area critically important for memory functioning. The physical exercise has been documented to increase hippocampal volume and improve functional connectivity between the default mode network and the prefrontal cortex [51]. Based on various clinical studies, it can be inferred that increased physical activity leads to a significant improvement in memory functions in Alzheimer's disease [52].

Caloric restriction/dietary restriction

Reducing the amount of food/caloric intake appears to extend the lifespan. The experimental animals on caloric restriction not only living longer but also show less age-related deficits. In all, the reduced intake of calories is less important than the number of proteins. Thus,

the term caloric restriction should be substituted by dietary restriction. Apart from the amount of food consumed, the frequency of food intake is also important [53]. It has been documented that the experimental animals taking the food evenly distributed throughout the day do not show a definite positive effect as compared to animals being fed once a day. Another observation added to this, is that fasting every second day significantly improves the lifespan [54]. Experimentally, food intake with a ratio of 1:10 Proteins and Carbohydrates results in the longest lifespan in mice. The intermittent fasting (2 days per week or alternate days) has been documented to decrease insulin levels and inflammation, improve mitochondrial health, DNA repair and autophagy, and promote resistance to cardiac and neuronal stress [55].

Molecules slowing the aging process

Sirtuins

Sirtuins interact with IGF-1, mTOR and AMPK signaling pathways and regulate energy metabolism, DNA repair, inflammation, cell survival and tissue regeneration. The sirtuin-activating compounds (STACs) activate sirtuins and raise NAD^+ levels to promote general health during aging, protect against cardiovascular disease, T2DM, neurodegeneration and cancer [56]. Resveratrol, a polyphenol, activates SIRT1 and AMPK, to mediate the effects like caloric restriction, stimulates autophagy and has antioxidant, anti-inflammatory and neuroprotective properties.

mTOR

The mTOR is a serine/threonine kinase which regulates metabolism and aging [57]. The factors, such as insulin, IGF-1, amino acids and glucose have been shown to activate mTOR. The mTOR, in turn, inhibits autophagy and promotes growth. Rapamycin inhibits mTOR and has been shown to increase lifespan in various experimental organisms including mice.

AMP-activated protein kinase (AMPK)

The AMPK is a major constituent for energy-sensing pathway [58]. It modulates metabolic pathways and positively influences glucose uptake and fatty acid oxidation and inhibits protein and lipid synthesis [59]. It promotes autophagy and mitochondrial biogenesis and inhibits mTOR and the process of inflamm-aging and aging process [60]. With aging, there is documented decline in the AMPK signaling activity. Metformin and resveratrol influence the AMPK activity. Metformin has been shown to inhibit synthesis of glucose in the liver through the activation of AMPK, inhibit mTOR and complex I of the mitochondrial electron transfer chain resulting in a reduced production of ROS, stimulate autophagy, dampen inflammatory processes and senescence and increase the health-span and lifespan.

Spermidine is a polyamine which stimulates the synthesis of anti-inflammatory cytokines as well as autophagy in a SIRT1-independent manner. It has a positive influence on lipid metabolism, neuroprotective and cardioprotective effects, decreases aging process and has been demonstrated to increase the lifespan in mice.

Other factors

Vitamin D has an important metabolic regulatory function. It has been shown to prevent loss of muscle mass (sarcopenia) as well as the age-dependent deposition of fat in muscles. Simultaneously, it appears to positively modulate the cognitive function. The mice lacking the vitamin D receptor have been documented to age prematurely. Further, following vitamin D supplementation the mice model for Alzheimer's disease shows improvement in memory and various pathological markers for Alzheimer's disease.

Hormones/growth factors: A recent study in older mice has documented the Oxytocin have demonstrated a rejuvenating effect of oxytocin. Further, another important study has correlated successful aging with Chemerin, Fetuin-A and fibroblast growth factors, FGF19 and FGF21 [61]. The FGF21 has been shown to enhance autophagy and influence glucose and lipid metabolism as well as important for the maintenance of homeostasis during cellular stress [62].

Adiponectin, expressed and secreted from small adipocytes, increases insulin sensitivity, has anti-atherosclerotic effects and improves metabolism in skeletal muscle, liver and adipose tissue through AMPK and SIRT1 and acts like an exercise mimicking factor [63].

The acetylcholinesterase inhibitors increase of acetylcholine levels and improve cognitive function and have beneficial effects on the comorbidities linked to Alzheimer's and Parkinson's diseases.

Future prospects for taming aging process

Selective elimination of senescent cells

The removal of senescent cells in experimental mice by activating immune system appears to retard aging process and improve the health span. The elimination of senescent cells via drugs (senolysis) or to trigger apoptosis (synopsis) is another prospective therapeutic option in near future. There have been identified six senescent-cell anti-apoptotic pathways (SCAPs), which can be promising targets for senolytic drugs [64]. The senolytic drugs like quercetin, raise hope for the treatment of diseases like idiopathic pulmonary fibrosis (IPF) [65].

Transplantation of stem cells

Stem cells are important for regeneration and function integrity of various organs. Identifying the tissue defects and transplanting stem cells into target organs opens-up the possibility for repair and recovery of functional deficit. A related development is the discovery of transcription factors (Yamanka factors - OCT3/4, SOX2, KLF4 and MYC) for inducing reprogramming the stem cells pluripotency [66]. In various clinical trials, tissue specific cells differentiated from induced pluripotent stem cells (iPSCs) have shown therapeutic potential for spinal cord injuries, Alzheimer's and Parkinson's diseases, diabetes mellitus and heart failure [67,68]. The stem cell therapy based strategies for replacement of senescent cells and rejuvenation of damaged tissues and diseased organs through offers limitless possibilities [69].

Conclusion

Understanding biological basis of the aging process has led to insights that are able to potentially identify measures to slow down the aging process. The aging, thus, becomes a modifiable risk factor and there are expectant possibilities to extend the lifespan and improve health-span.

Through targeted lifestyle changes and potentially effective interventions through nutraceuticals and pharmaceuticals and stem cell therapy, it may be possible to reduce the age-related chronic and debilitating morbidities and improve the health-span.

Bibliography

1. Fabrizio P and Longo VD. "The chronological life span of *Saccharomyces cerevisiae*". *Methods in Molecular Biology* 371 (2007): 89-95.

2. Longo VD and Fabrizio P. "Chronological Aging in *Saccharomyces cerevisiae*". *Subcellular Biochemistry* 57 (2012): 101-121.

3. Kenyon C. "A conserved regulatory system for aging". *Cell* 105 (2001): 165-168.

4. Fabrizio P., *et al.* "Regulation of longevity and stress resistance by Sch9 in yeast". *Science* 292 (2001): 288-290.

5. Longo VD. "Ras: The Other Pro-Aging Pathway". *Science of Aging Knowledge Environment* 39 (2004): 36.

6. Pichova A., *et al.* "Mutants in the *Saccharomyces cerevisiae* RAS2 gene influence life span, cytoskeleton, and regulation of mitosis". *Canadian Journal of Microbiology* 43 (1997): 774-781.

7. Longo VD. "Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells". *Neurobiology of Aging* 20 (1999): 479-486.

8. Hertweck M., *et al.* "*C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span". *Developmental Cell* 6 (2004): 577-588.

9.   Lee AC., *et al*. "Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species". *Journal of Biological Chemistry* 274 (1999): 7936-7940.

10. Lin SJ., *et al*. "Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*". *Science* 289 (2000): 2126-2128.

11. Fabrizio P., *et al*. "Sir2 Blocks Extreme Life-Span Extension". *Cell* 123.4 (2005): 655-667.

12. Holzenberger M. "Igf-I signaling and effects on longevity". *Nestlé Nutrition Workshop Series Paediatric Programme* 68 (2011): 237-245.

13. Brown-Borg HM. "Hormonal regulation of aging and life span". *Trends in Endocrinology and Metabolism* 14.4 (2003): 151-153.

14. Pan H and Finkel T. "Key proteins and pathways that regulate lifespan". *Journal of Biological Chemistry* 292.16 (2017): 6452-6460.

15. Bonkowski MS and Sinclair DA. "Slowing ageing by design: the rise of NAD+ and sirtuin-activating compounds". *Nature Reviews Molecular Cell Biology* 17 (2016): 679-690.

16. Kaeberlein M., *et al*. "The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms". *Genes and Development* 13 (1999): 2570-2580.

17. Lin SJ., *et al*. "Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*". *Science* 289 (2000): 2126-2128.

18. Wątroba M and Szukiewicz D. "The role of sirtuins in aging and age-related diseases". *Advances in Medical Sciences* 61 (2016): 52-62.

19. Jing H and Lin HN. "Sirtuins in epigenetic regulation". *Chemical Reviews* 115.6 (2015): 2350-2375.

20. Imai S and Guarente L. "It takes two to tango: NAD(+) and sirtuins in aging/longevity control". *NPJ Aging and Mechanisms of Disease* 2 (2016): 16017.

21. Someya S., *et al*. "Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction". *Cell* 143 (2010): 802-812.

22. Gambini J., *et al*. "Properties of resveratrol: *In vitro* and *In vivo* studies about metabolism, bioavailability, and biological effects in animal models and humans". *Oxidative Medicine and Cellular Longevity* (2015): 837042.

23. Mitchell SJ., *et al*. "Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans". *Cell Metabolism* 14 (2014): 612-622.

24. Mattison JA., *et al*. "Resveratrol prevents high fat/sucrose diet-induced central arterial wall inflammation and stiffening in nonhuman primates". *Cell Metabolism* 20 (2014): 183-190.

25. Milne JC., *et al*. "Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes". *Nature* 450 (2007): 712-716.

26. Mitchell SJ., *et al*. "The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet". *Cell Reports* 6 (2014): 836-843.

27. Mercken EM., *et al*. "SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass". *Aging Cell* 13 (2014): 787-796.

28. Dorman JB., *et al*. "The age-1 and daf-2 genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*". *Genetics* 141 (1995): 1399-1406.

29. Altintas O., *et al*. "The role of insulin/IGF-1 signaling in the longevity of model invertebrates, *C. elegans* and *D. melanogaster*". *BMB Reports* 49 (2016): 81-92.

30. Lee SS., *et al*. "DAF-16 target genes that control *C. elegans* lifespan and metabolism". *Science* 300 (2003): 644-647.

31. Albert V and Hall MN. "mTOR signaling in cellular and organismal energetics". *Current Opinion in Cell Biology* 33 (2015): 55-66.

32. Lee IH., *et al*. "A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy". *Proceedings of the National Academy of Sciences of the United States of America* 105 (2008): 3374-3379.

33. Gelino S and Hansen M. "Autophagy: an emerging anti-aging mechanism". *International Journal of Clinical and Experimental Pathology* 4 (2012): 006.

34. Sun N., *et al*. "The mitochondrial basis of aging*". Molecular cell* 61 (2016): 654-666.

35. Gladyshev VN. "Aging: Progressive decline in fitness due to the rising deleteriome adjusted by genetic, environmental, and stochastic processes". *Aging Cell* 15.4 (2016): 594-602.

36. Horvath S. "DNA methylation age of human tissues and cell types". *Genome Biology* 14 (2013): 10 R115.

37. Zampieri M., *et al*. "Reconfiguration of DNA methylation in aging". *Mechanisms of Ageing and Development* 151 (2015): 60-70.

38. Klaips CL., *et al*. "Pathways of cellular proteostasis in aging and disease". *The Journal of Cell Biology* 217 (2017): 1 51-163.

39. Labbadia J and Morimoto RI. "The biology of Proteostasis in aging and disease". *Annual Review of Biochemistry* 84 (2015): 435-464.

40. Bonkowski MS and Sinclair DA. "Slowing ageing by design: The rise of NAD(+) and sirtuin-activating compounds". *Nature Reviews Molecular Cell Biology* 17.11 (2016): 679-690.

41. Sun N., *et al*. "The mitochondrial basis of aging". *Molecular Cell* 61.5 (2016): 654-666.

42. Wang Y and Hekimi S. "Mitochondrial dysfunction and longevity in animals: Untangling the knot". *Science* 350.6265 (2015): 1204-1207.

43. Criscione SW., *et al*. "The chromatin landscape of cellular senescence". *Trends in Genetics* 32.11 (2016): 751-761.

44. Urbanelli L., *et al*. "Extracellular vesicles as new players in cellular senescence". *International Journal of Molecular Sciences* 17.9 (2016): 1408.

45. Adams JU. "Genetic Control of Aging and Life Span". *Nature Education* 1.1 (2008): 130.

46. Kenyon C., *et al*. "A *C. elegans* mutant that lives twice as long as wild type". *Nature* 366.6454 (1993): 461-464.

47. Braeckman BP and Vanfleteren JR. "Genetic control of longevity in *C. elegans*". *Experimental Gerontology* 42.1-2 (2007): 90-98.

48. Gami MS and Wolkow CA. "Studies of Caenorhabditis elegans DAF-2/insulin signaling reveal targets for pharmacological manipulation of life span". *Aging Cell* 5 (2006): 31-37.

49. Barbieri M., *et al*. "Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans". *The American Journal of Physiology: Endocrinology and Metabolism* 285.5 (2003): E1064-71.

50. Gems D and McElwee J. "Ageing: Micro-arraying mortality". *Nature* 424 (2003): 260.

51. Chen WW., *et al*. "Role of physical exercise in Alzheimer's disease". *Biomedical Reports* 4.4 (2016): 403-407.

52. Radak Z., *et al*. "Exercise plays a preventive role against Alzheimer's disease". *Journal of Alzheimer's Disease* 20.3 (2010): 777-783.

53. Fontana L and Partridge L. "Promoting health and longevity through diet: From model organisms to humans". *Cell* 161 (2015): 106-118.

54. Simpson SJ., *et al*. "Dietary protein, aging and nutritional geometry". *Ageing Research Reviews* 39 (2017): 78-86.

55. Mattson MP., *et al*. "Impact of intermittent fasting on health and disease processes". *Ageing Research Reviews* 39 (2017): 46-58.

56. Rinnerthaler M and Richter K. "The Basics of Biogerontology" (2017).

57. Kennedy BK and Lamming DW. "The mechanistic target of Rapamycin: The grand ConducTOR of metabolism and aging". *Cell Metabolism* 23.6 (2016): 990-1003.

58. Hardie DG., *et al*. "AMPK: An energy-sensing pathway with multiple inputs and outputs". *Trends in Cell Biology* 26.3 (2016): 190-201.

59. Garcia D and Shaw RJ. "AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance". *Molecular Cell* 66.6 (2017): 789-800.

60. Cordero MD., *et al*. "AMP-activated protein kinase regulation of the NLRP3 inflammasome during aging". *Trends in Endocrinology and Metabolism* 29.1 (2018): 8-17.

61. Sanchis-Gomar F., *et al*. "A preliminary candidate approach identifies the combination of chemerin, fetuin-a, and fibroblast growth factors 19 and 21 as a potential biomarker panel of successful aging". *Age* 37 (2015): 3-42.

62. Salminen A., *et al*. "Integrated stress response stimulates FGF21 expression: Systemic enhancer of longevity". *Cellular Signalling* 40 (2017): 10-21.

63. Iwabu M., *et al*. "Adiponectin/adiponectin receptor in disease and aging". *NPJ Aging and Mechanisms of Disease* 1 (2015): 15013.

64. Zhu Y., *et al*. "The Achilles' heel of senescent cells: From transcriptome to senolytic drugs". *Aging Cell* 14.4 (2015): 644-658.

65. McHugh D and Gil J. "Senescence and aging: Causes, consequences, and therapeutic avenues". *The Journal of Cell Biology* 217.1 (2018): 65-77.

66. Liu X., *et al*. "Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells". *Cell Research* 18.12 (2008): 1177-1189.

67. Martin U. "New muscle for old hearts: engineering tissue from pluripotent stem cells". *Human Gene Therapy* 26.5 (2015): 305-311.

68. Talkhabi M., *et al*. "Human cardiomyocyte generation from pluripotent stem cells: A state-of-art". *Life Sciences* 145 (2016): 98-113.

69. Martin U. "Therapeutic Application of Pluripotent Stem Cells: Challenges and Risks". *Frontiers in Medicine (Lausanne)* 4 (2017): 229.