Intervening in the mTOR pathway: a promising anti-ageing therapy for humans?

rijksuniversiteit groningen

Essay (5ECTS) for the Biomedical Sciences master
Dorieke Dijkstra
Student number: 1864432
Supervision: Dr. C.F. Calkhoven
Examination: E.A.A. Nollen

Abstract

Life expectancy at birth is increasing every year, resulting in more humans reaching a high age. However, ageing comes with a risk of developing age-related diseases, including cancer and metabolic diseases. Targeting the mTOR pathway might be a way to promote healthy ageing and increase life span. mTOR is the catalytic subunit of two distinct multiprotein complexes: mTORC1 and mTORC2. Little is known about mTORC2 signaling. mTORC1 is activated by amino acids, insulin and insulin-like growth factor and high energy levels, while it is repressed by cellular stress, including energy deprivation, hypoxia and DNA damage. When activated, mTORC1 promotes the transcription of SREBP1, PPARγ, STAT3, HIF1α, YY1 and PCG1α, which result in cellular survival, growth, and proliferation. Furthermore, mTORC1 promotes transcription and represses autophagy. Overactivation of mTORC1 can lead to age-related diseases, including cancer, autoimmune disorders, diabetes and obesity. Furthermore, mTORC1 accelerates ageing on cell and tissue level. On cell level, mTORC1 activation decreases autophagy and increases the amount of reactive oxygen species, which leads to increased levels of damaged and aggregated proteins and organelles in the cell, a hallmark of ageing. On tissue level, mTORC1 stimulates proliferation, which can lead to exhaustion or senescence of stem cells and thereby to an increasing amount of aged cells. Low mTORC1 or TOR1 activity, achieved by caloric restriction, genetic manipulation or drugs, has been shown to decline ageing in yeast, fruit flies, nematodes and mice. Caloric restriction seems to decline ageing in non-human primates as well and this is possibly mediated by decreased mTOR signaling. Since high levels of caloric restriction would be hard to achieve for humans, mTOR-inhibiting drugs are of great interest. The drug rapamycin inhibits mTORC1 directly, which results in declined ageing in several animal models, but comes with severe side effects in humans, including immunosuppression and insulin resistance. Metformin simulates a state of energy deprivation. It decreases mTORC1 activity by activating AMPK, but it seems to have no effect on aged mice. Since directly inhibiting mTORC1 leads to severe side effects, new anti-ageing drug should either mimic an mTORC1-repressive status or target downstream effectors of mTORC1.

mTOR signaling

Because of improved hygiene, medical care and life style, humans are getting older and older. Since the middle of the 19th century, life expectancy at birth has raised by 2,5 years per decade. However, increasing age is a risk factor for many diseases, including cancer and metabolic, cardiovascular and neurodegenerative diseases. Nowadays, these age-related diseases are major causes of death (Partridge 2014). A current subject of medical research is how ageing can be declined in order to prevent the developing of these age-related diseases. In this essay, the role of mammalian (or mechanistic) Target of Rapamycin (mTOR) in ageing will be addressed and it will be discussed whether the mTOR pathway is a useful target to delay the onset and progression of ageing and age-related diseases in humans.

In order to survive, an organism has to react to environmental cues. Anabolic and catabolic processes need to be adapted to the amount of nutrients and energy available. One of the pathways involved in this metabolic regulation is the intracellular mTOR signaling pathway, which promotes anabolic processes (Andre and Cota 2012). mTOR, a serine-threonine kinase, is the catalytic subunit of two distinct multiprotein complexes, mTOR complex (mTORC) 1 and mTORC2, which differ in their upstream and downstream signaling. Next to the mTOR subunit, both mTORC1 and mTORC2 contain mLST8 (mammalian lethal with sec-13 protein 8), TTI1/TEL2 and Deptor (DEP domain containing mTOR-interacting protein). Specific for mTORC1 are PRAS40 (proline-rich Akt substrate 40 kDa) and raptor (regulatory-associated protein of mTOR). Specific for mTORC2 are mSIN1 (mammalian stress-activated map kinase-interacting protein 1, protor (protein observed with rictor 1 and 2) and rictor (rapamycin-insensitive companion of mTOR) (Figure 1) (Perluigi, Di Domenico, Butterfield 2015). The main characteristic component of mTORC1 is raptor. Raptor is involved in amino acid sensing, which regulates the intracellular localization and thereby activation of mTORC1 (Sancak et al. 2008). mTORC2 signaling is less well understood than mTORC1 signaling. For that reason, this essay will focus on the role of mTORC1.

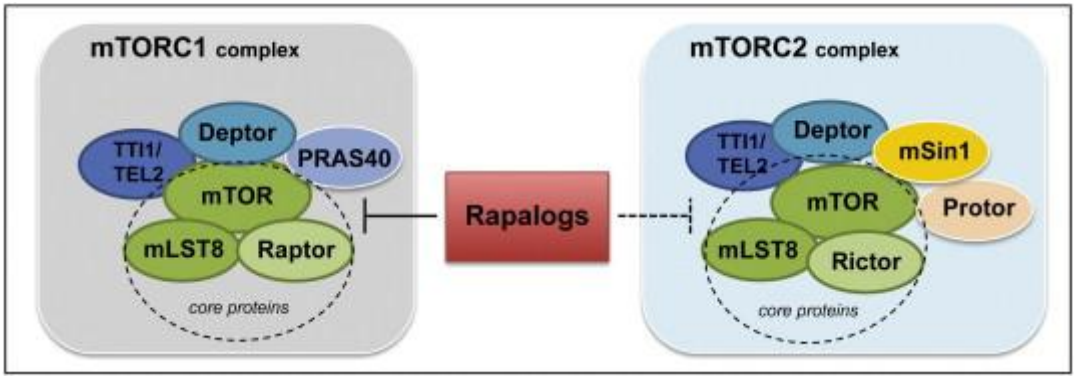

Figure 1 The composition of mammalian Target of Rapamycin Complex (mTORC) 1 and 2 (Perluigi et al., 2015).

Regulation of mTORC1 by upstream signals

Nutrients, growth factors, energy and stress determine mTORC1 activity. mTORC1 activity is regulated primarily via two mechanisms: direct modification of one of the components of the complex or regulation of Ras homologue enriched in brain (Rheb). Rheb is a GTPase which activates mTORC1 when loaded with guanosine triphosphate (GTP) (Zoncu, Efeyan, Sabatini 2011). Rheb-GTP is located on late endosomes and lysosomes and the amount of amino acids determines if mTORC1 is

translocated to those organelles. Amino acids activate RAG GTPase heterodimers which interact with raptor, resulting in localization of mTORC1 to the late endosomes or lysosomes and activation by Rheb-GTP. Tuberosclerosis complex (TSC) 2, a GTPase activating protein (GAP), can inhibit mTORC1 activity by promoting Rheb-GTP to Rheb-GDP conversion (Duran and Hall 2012).

The circulating growth factors insulin and insulin-like growth factor-1 (IGF-1) indicate the organism is in a fed state and are therefore promoting anabolic processes via mTORC1. Binding of insulin or IGF-1 to their receptors recruits insulin receptor substrate 1 (IRS1), which activates the phosphoinositide 3-kinase (PI3K) pathway, which leads to phosphorylation of protein kinase B (Akt). This leads to the phosphorylation of the heterodimer TSC1-TSC2 and thereby to inactivation of TSC2 and thus to less Rheb-GTP to Rheb-GDP conversion and increased activation of mTORC1 (Sengupta, Peterson, Sabatini 2010). Thus, the growth factors insulin and IGF-1 stimulate mTORC1 activation via PI3K, Akt, TSC2 and Rheb. TSC-independent mechanisms for activation of mTORC1 by growth factors also exist.

Cells can experience different kinds of stress, including energy deficiency, hypoxia and DNA damage, that require inhibited mTORC1 activation and cell cycle arrest. Glucose levels are reflected by the adenosine triphosphate (ATP) levels. Low ATP levels lead to a high adenosine monophosphate (AMP)/ATP ratio, which activates 5' AMP-activated protein kinase (AMPK). Via phosphorylation of IRS1, AMPK activates TSC2, resulting in less Rheb-GTP and repression of mTORC1 activation. AMPK is also able to directly inactivate raptor by phosphorylating it (Sengupta, Peterson, Sabatini 2010). Next to stress caused by energy shortage, low oxygen levels result in inhibited mTORC1 activation and cell cycle arrest as well. This happens via decrease cellular ATP by impaired mitochondrial respiration and via the hypoxia-inducible mTOR inhibitor Redd1 (Brugarolas et al. 2004). Another form of stress experienced by cells is DNA damage. DNA damage leads to reduced mTORC1 activity by expression of the mTORC1 inhibitors REDD1 and DEPTOR and by AMPK activation (Desantis et al. 2015; Budanov and Karin 2008). An overview of the upstream regulators of the mTOR pathway is shown in Figure 2.

Downstream effects of mTORC1

mTORC1 promotes cellular survival, growth, proliferation and translation. Substrates of mTORC1 involved in mRNA translation are eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP) 1 and the S6 kinases (S6K) 1 and 2. Phosphorylation of 4E-BP1 by mTOR inhibits the binding of 4E-BP1 to eIF4E on the 5' cap structure of mRNA, allowing eIF4E to initiate cap-dependent translation. Phosphorylation of S6K1 and 2 activates these proteins, which makes them promote mRNA biogenesis and cap-dependent translation and elongation. This increases ribosome biogenesis and thereby mRNA translation capacity (Catania, Binder, Cota 2011; Laplante and Sabatini 2009; Ma and Blenis 2009).

Next to translation, mTORC1 controls activation of certain genes. Downstream of mTORC1, SREBP1 (sterol regulatory element-binding protein 1), PPARγ (peroxisome proliferator-activated receptor-γ), STAT3 (signal transducer and activator of transcription 3), HIF1α (hypoxia-inducible factor 1α), YY1 (yin-yang 1) and PCG1α (PPARγ coactivator-1) are activated (Laplante and Sabatini 2013). mTORC1 activates those proteins in different manners, including phosphorylation and promotion of transcription. All these proteins are promoting anabolic processes when active. STAT3 promotes cell growth, survival and proliferation (Laplante and Sabatini 2013), SREBP1 promotes lipogenesis (Bakan and Laplante 2012) and PPARγ promotes adipogenesis (Kim and Chen 2004). HIF1α activation promotes glycolysis and angiogenesis (Hudson et al. 2002). Moreover, mOTRC1 promotes a physical

interaction between PCG1α and YY1, which results in promotion of mitochondrial biogenesis (Cunningham et al. 2007).

Furthermore, mTORC1 is a major repressor of macroautophagy. During macroautophagy, a catabolic process, proteins and organelles are absorbed by autophagosomes and degraded by lysosomes. This is in order to maintain cellular nutrient levels, to help regulate intracellular organelle homeostasis and to clear up damaged or aggregated proteins and organelles. mTORC1 represses the initiation of autophagy, which is phagophore formation, by phosphorylating the Ulk1 complex, which contains several autophagy-related gene (ATG) proteins that are necessary for autophagy (Perluigi, Di Domenico, Butterfield 2015). Furthermore, phosphorylation of Transcription Factor EB (TFEB) by mTORC1 inhibits TFEB activity, leading to inhibiton of autophagosome formation and of the fusion with lysosomes (Settembre and Ballabio 2011; Settembre et al. 2012). Thus, low nutrient, growth hormone and energy levels and elevated cellular stress lead to an increase in autophagy via inhibition of mTORC1 (Mizushima et al. 2008; Perluigi, Di Domenico, Butterfield 2015). An overview of the downstream effectors of mTORC1 is given in figure 2.

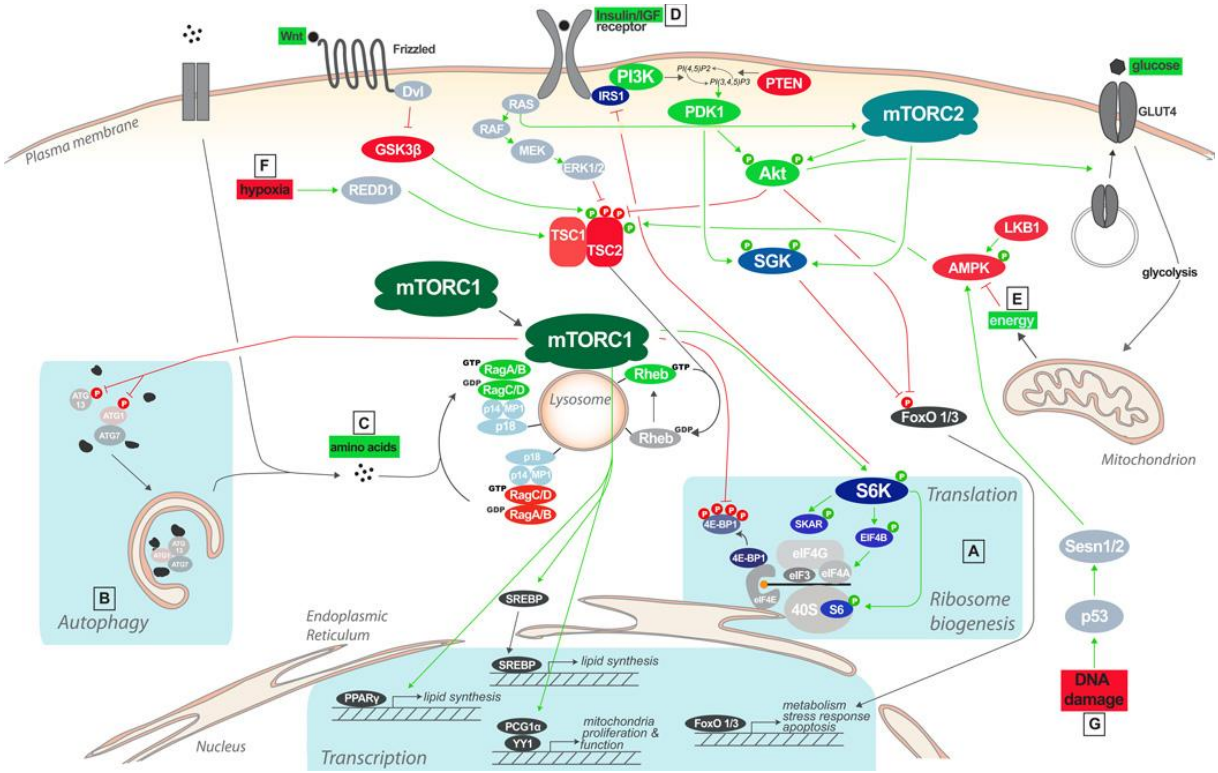

Figure 2 An overview of mTOR signaling. mTORC1 promotes translation **(A)** and inhibits autophagy **(B)** by integrating nutrient signals from amino acids **(C)**, growth factor signals from insulin and insulin-like growth factors **(D)**, energy signals from AMPK **(E)** and the stress signals hypoxia **(F)** and DNA damage **(G)**. mTORC1 regulates transcription of PPARγ, SREBP, PCG1α and YY1, ribosome biogenesis and autophagy (adapted from Zoncu et al., 2011).

During fasting, mTORC1 activates a range of processes that contribute to energy release. Low mTORC1 levels promote the production of ketone bodies in the liver via PPARα (Sengupta et al. 2010) and lysosome biogenesis via bHLH leucine zipper transcription factor EB (TFEB) to help cells cope with the stress of energy deprivation (Roczniak-Ferguson et al. 2012). Ketone bodies can be used as source of energy, while lysosomes break down biomolecules to release energy. Moreover,

translation, glycogen synthesis and adipogenesis are repressed, while autophagy and gluconeogenesis are promoted by the transcription of genes mentioned above.

mTORC1 overactivation could lead to insulin resistance and many other disease states. mTORC1 promotes deposition of fat in white adipose tissue, which contributes to obesity and insulin resistance. mTORC1-activated S6K1 can phosphorylate IRS1, which also contributes to insulin resistance. Insulin resistance results in decreased Akt activity and thereby in an increased gluconeogenesis and a worsening of hyperglycemia and hyperinsulinemia (Jia et al. 2014; Zoncu, Efeyan, Sabatini 2011). Activation of mTORC1 leads to increased oxidative stress via increased mitochondrial activity and this is involved in the pathogenesis of autoimmune disorders (Perl 2015). Dysregulation of mTOR signaling and its downstream effectors are involved in many disease states, including cancer, autoimmune disorders, diabetes and obesity. Cell proliferation, growth and survival, which is stimulated by mTORC1, are main hallmarks of cancer (Guertin and Sabatini 2007; Hanahan and Weinberg 2011) and mutations in the mTOR pathway are abundant in human cancers (Zoncu, Efeyan, Sabatini 2011).

mTORC1 in ageing

Increased mTOR signaling has not only be associated with these, partly age-related, diseases, but also with ageing in general (Zoncu, Efeyan, Sabatini 2011).

On cell level, increased mTORC1 signaling accelerates ageing by decreasing autophagy and increasing the amount of reactive oxygen species (ROS). Autophagy is necessary to clear up damaged and aggregated proteins and organelles. It is known that both chaperone-mediated autophagy and macroautophagy decline during ageing (Cuervo et al. 2005; Del Roso et al. 2003). This causes accumulation of damaged intracellular particles and thereby ageing of cells and an even further decline in lysosomal functioning (Cuervo et al. 2005; Mizushima et al. 2008). Indeed, preservation of chaperone-mediated autophagic function during ageing in a double transgenic mouse model resulted in less intracellular accumulation of damaged proteins, better handling of protein damage and improved organ function (Zhang and Cuervo 2008). Since mTORC1 inhibits macroautophagy, low mTOR levels promote autophagy and decline ageing. High mTORC1 levels can lead to accumulation of damaged proteins and organelles and thereby to cellular ageing and senescence. In addition to impaired autophagy, elevated protein synthesis by mTORC1 may result in even more accumulation of unfolded proteins by high demands of the protein folding system and endoplasmatic reticulum stress (Zoncu, Efeyan, Sabatini 2011). Since the copy number of mitochondria and the expression of genes encoding for proteins involved in oxidative metabolism are upregulated by mTORC1, ATP production is positively correlated with mTORC1 levels (Cunningham et al. 2007; Schieke et al. 2006). This ATP production comes with ROS as byproduct, while the maximal oxidative capacity of cell declines with ageing. This is causing increasing damage to DNA, proteins and membranes which is a hallmark of aged cells (Zoncu, Efeyan, Sabatini 2011). Furthermore, the mtDNA is easily damaged by ROS, causing impaired mitochondrial function, which is a hallmark of ageing and causes an accelerated ageing phenotype (Gonzalez-Freire et al. 2015; Lopez-Otin et al. 2013).

On tissue level, stem cells are responsible for the replacement of aged or damaged cells. Since proliferation requires a sufficient amount of nutrients and energy, mTORC1 signaling stimulates this. However, overstimulation of proliferation can lead to exhaustion or senescence of stem cells and

thereby to an increasing amount of aged cells. A summary of the involvement of mTORC1 in ageing is shown in Figure 3.

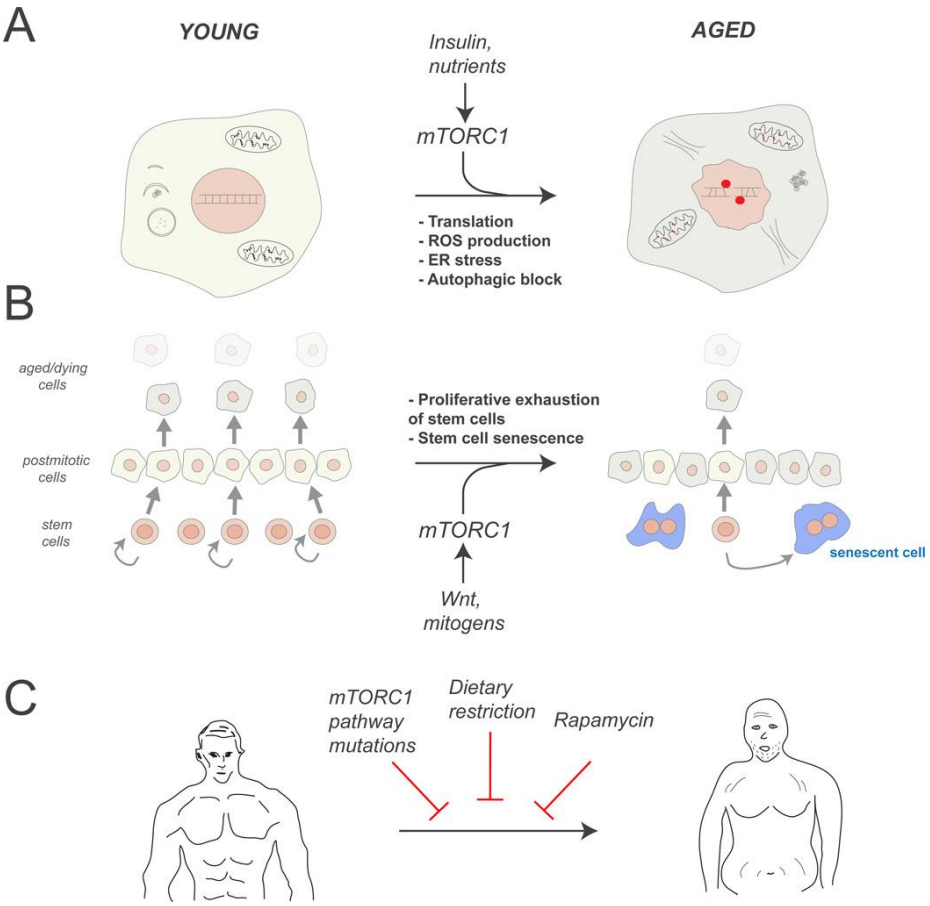

Figure 3 An overview of the role of mTORC1 in ageing. **A** On cellular level, mTORC1 stimulates translation, ROS production and endoplasmatic reticulum stress and blocks autophagy, resulting in accelerated ageing. **B** On tissue level, mTORC1 stimulates proliferation and thereby exhaustion of stem cells and stem cell senescence, resulting in accelerated ageing. **C.** Dietary restriction, mTORC1 pathway mutations and rapamycin can attenuate the ageing process (Zoncu et al., 2011).

TOR in animal models for ageing

Since low mTORC1 activity has many beneficial effects on both general ageing and age-related diseases, it is not surprising that reduced mTORC1 signaling by calorie restriction, genetic manipulation or drugs has been shown to have anti-ageing capacities in several animal models (Zoncu, Efeyan, Sabatini 2011).

Inhibition of TOR, the non-mammalian homolog of mTOR, increases life span in several animal models. In yeast (S. cerevisiae), caloric restriction extends replicative life span by up to 40%. This can be mimicked by knocking out TOR1 and caloric restriction in TOR1 knock out models fails to extend replicative life span further. Thus, in yeast, the extension of life span by caloric restriction is mediated by TOR signaling (Kaeberlein et al. 2005). In the nematode C. elegans, life span was more than doubled by inhibiting TOR signaling. This effect was not found when certain genes required for autophagy were inhibited, indicating that autophagy is mediating the effects of TOR signaling on life span (Hansen et al. 2008). In D. melanogaster, caloric restriction also leads to extended life span. In

this animal model, inhibiting TOR by TSC1/2 or a dominant negative form of TOR or inhibiting S6K mimics caloric restriction (Kapahi et al. 2010).

In rodents, caloric restriction (CR) increases life span and delays the onset of age-related diseases (Anderson, Shanmuganayagam, Weindruch 2009). The importance of the TOR pathway in ageing in rodents has been confirmed by genetic manipulation. In mice, deletion of S6K1, which is part of the mTOR signaling pathway, resulted in increased life span and protection against age-related diseases. The gene pattern observed was similar to that seen in calorie restricted mice, which indicates that the mTOR pathway is the mediator between CR and extended life span (Selman et al. 2009). Furthermore, Ma et al showed that mTOR and S6K1 activation in the brain is decreased after 44 weeks of caloric restriction. This is probably mediating the positive effects of CR on learning and memory (Ma et al. 2015). Furthermore, research in non-human primates indicates CR could postpone ageing in these primates as well (Colman et al. 2014). However, it is not proven that declined ageing by caloric restriction in mammals is mediated by reduced mTOR signaling.

Macronutrient content

In an extensive mice study, Solon-Biet et al found that macronutrient content is more important for healthy ageing than total caloric intake. An *ad libitum* low-protein, high-carbohydrate diet resulted in healthy ageing and an extended life span, while a calorie restricted high-protein, low carbohydrate diet did not result in healthy ageing. This suggest longevity can be achieved by a low-protein diet, rather than by caloric restriction. It was shown that hepatic mTOR signaling and mitochondrial function was reduced by a low-protein diet (Solon-Biet et al. 2014). It has not been established yet whether this applies to humans. A low-protein diet would be more feasible for humans than long-term caloric restriction.

Anti-ageing drugs

Even if a restriction in food intake would result in declined ageing in humans, it would be hard to achieve. A low-protein diet without caloric restriction would be more feasible but, to date, evidence for the anti-ageing affects of a low-protein diet is limited to one study. Furthermore, proteins are necessary for a healthy metabolism and the side effects of a low-protein diet are not known yet. Therefore, the search for anti-ageing drugs in ongoing and drugs intervening with the mTOR pathway are of great interest. The most investigated drug is Rapamycin, an mTOR inhibitor. Furthermore, AMPK activators have been examined as potential anti-ageing drugs.

Rapamycin

Rapamycin has been shown to extend life span in S. cerevisiae, C. elegans, D. melanogaster and mice (Kapahi et al. 2010). Rapamycin inhibits mTORC1 by binding to the intracellular protein FKBP12. This FKBP12-rapamycin complex binds the mTORC1 complex and inhibit its kinase activity (Sarbassov et al. 2006). Rapamycin mainly targets mTORC1 but can also inhibit mTORC2, when administered in high concentrations. Several mice studies have confirmed and anti-ageing role for rapamycin. Harrisson *et al* were the first to report extended life span due to rapamycin treatment late in life. Life span was extended in both males and females and the mice were genetically heterogeneous, which indicates the role for mTORC1 is not genotype-specific (Harrison et al. 2009). Zhang *et al* confirmed this and found that rapamycin treatment extends health span as well, while mORC1 levels were found lower in several tissues (Zhang et al. 2014). Moreover, Chang *et al* reported that rapamycin protects against high fat diet-induced obesity. After being fed a high fat diet for 16 weeks, mice were

injected weekly with rapamycin for 16 weeks while high fat diet consumption continued. The rapamycin-group had significantly lower body adiposity mass and insulin levels than the controls, even though they had a significantly higher food intake (Chang et al. 2009). In a primary culture of human preadiposites, it was shown that rapamycin inhibits adipocyte differentiation, which indicates it negatively regulates adipogenesis (Bell, Grunder, Sorisky 2000). Even though the molecular mechanism was not examined, this is in line with the finding that mTORC1 promotes adipogenesis via PPARγ. High adiposity promotes many age-related diseases, including diabetes and cancer. Thus, one of the ways by which rapamycin promotes healthy ageing is by lowering body adiposity, probably via lowering mTORC1 levels.

In humans, rapamycin is used to prevent graft rejection after transplantation because of its immunosuppressive function. Via mTORC1 inhibiton, rapamycin inhibits B-cell and natural killer cell proliferation, dendritic cell differentiation and maturation of effector T cells (Thomson, Turnquist, Raimondi 2009). When used as a therapy against ageing, immunosuppression would be a severe side effect. Furthermore, rapamycin has negative effects on metabolism. Even though rapamycin negatively regulates adipogenesis, prolonged use of this drug has been associated with insulin resistance, hyperinsulinemia and hyperglycemia in humans (Morrisett et al. 2002). Furthermore, mice show hyperlipidemia and glucose intolerance after prolonged use of rapamycin (Fang et al. 2013).

AMPK activators

Instead of directly targeting MondoA, factors upstream of MondoA could be inhibited or activated as well. In times of energy deprivation, AMPK levels are high and, as a result of that, mTORC1 is inhibited. This could be mimicked with AMPK activators. Some AMPK activators are currently on the market as therapy for diabetes type 2 patients, including metformin and thiazolidinediones (Coughlan et al. 2014). Metformin leads to improved insulin resistance, partly by activating AMPK, which represses mTORC1 activity. Metformin use has little side effects and the severe immunosuppressive effects of rapamycin have not been reported for metformin. Metformin-induced AMPK activation enhances memory T cell formation by enhancing fatty acid oxidation right after the peak of infection, resulting in an enhanced immune response (Pearce et al. 2009). The anti-ageing effect of metformin at the cellular level was proven in SHR mice (Arkad'eva et al. 2011). The usefulness of metformin as anti-ageing drug later in life is questioned, since it was reported that metformin has no effect on S6K1 expression and 4E-BP1 phosphorylation in skeletal muscle cells of aged mice (Dungan et al. 2015).

The fact that rapamycin treatment comes with severe side effects while metformin treatment does not, suggests that the side effects of rapamycin are not a cause of mTORC1 inhibition but of rapamycin directly. It could be that rapamycin targets some proteins directly. It could also be that activating AMPK, which is upstream of mTORC1, leads to a more natural situation, since AMPK activation stimulates a range of other metabolic effects next to mTORC1 activation (Towler and Hardie 2007). It could thus be that simulating a natural condition, in this case mimicking energy deprivation, leads to better effects than direct mTOR inhibiton. In a breast cancer cell line, it was shown that metformin and rapamycin have distinct effects on the Akt pathway and on proliferation. Akt pathway phosphorylation results in its activation, which activates mTORC1. While both rapamycin and metformin lead to inhibiton of mTORC1 and S6K1, in this study it was found that rapamycin increases Akt activation while metformin decreases Akt activation. This is probably

because high AMPK levels phosphorylate IRS1 which leads to Akt repression, while direct inhibition of mTORC1 has no effect on IRS1 phosphorylation, giving feedback signals the chance to activate Akt (Zakikhani et al. 2010). Next to activating mTORC1, Akt is also known to repress FoxO proteins and to promote glycolysis. Thus, because a low nutritional state not only represses mTORC1 activation, but has a whole range of actions, it might be better to mimic a low nutritional state than to inhibit mTORC1 directly.

Discussion

mTOR signaling in relation to human ageing

To date, the use of mTORC1 inhibiting drugs to decline ageing has not been examined in humans. However, TOR signaling is a highly conserved mechanism. It is found in a wide range of species, and seems to function the same in mammals as in invertebrates, making it likely that humans posses the same signaling cascade. So, based on current knowledge, it would be likely that mTORC1 inhibition results in declined ageing in humans.

Caloric restriction would be an obvious way to reach mTORC1 inhibition. However, little is known about the effect of calorie restriction on human ageing. A few relatively short-term studies toward caloric restriction in humans have been performed. Six months of 25% CR resulted in a decrease in fasting insulin and core body temperature, which are both biomarkers of longevity. Furthermore, weight loss, a decrease in energy expenditure and a reduction in DNA damage were observed (Heilbronn et al. 2006). The CALERIE study reported that one year of CR resulted in weight loss and fat mass loss. Unfortunately, measurements of biomarkers for ageing did not took place in phase 1 of this study and the results of phase 2 are not yet published (Racette et al. 2006). However, this study did show that a high level of CR is hardly feasible. While the participants were prescribed 20% CR, they reached an average of 11,5% CR. Caloric restriction was higher in the first 6 months than in the last 6 months of the study. Fontana *et al* measured the concentrations of biomarkers involved in atherosclerosis in humans that voluntarily follow a calorie restricted diet for a longer term. They reported that the CR group had a lower body weight and fat mass percentage than their age-matched controls. Furthermore, the CR group had lower levels of, i.a., triglycerides, fasting glucose and fasting insulin (Fontana et al. 2004). Although the involvement of mTOR signaling in these effects of CR has not been examined, mTORC1 could be mediating these effects by lowering the levels of SREBP1, PPARγ and HIF1α. In that case, it is likely that autophagy is promoted and translation and mitochondrial functioning are repressed. It would be interesting to know how blood levels of mTOR relate to ageing in humans.

Mimicking mTORC1-repressive statuses

It is probably better to mimic an mTORC1-respressive status than to inhibit mTORC1 directly, as explained before. AMPK activation is a way to mimic energy deprivation and other upstream regulators of mTORC1 might be suitable targets as well. Insulin and IFG1 function as mTORC1 activators by promoting IRS1/Akt signaling, so inhibiting insulin and IGF1 signaling would be comparable to activating AMPK. Low glucose levels are reflected by high AMPK levels, so this would be comparable to AMPK activation as well. Hypoxia inhibits mTORC1 activation. Even if it was possible, stimulating hypoxia in order to decrease mTORC1 activation would not be a way to promote healthy ageing, since it has severe side effects, including cerebral and myocardial ischemia (Michiels

2004). Hypoxia induces REDD1 expression, which leads to dephosphorylation of Akt by phosphatase 2A (PP2A) and subsequently to dephosphorylation of TSC2, resulting in repressed mTORC1 signaling (Dennis et al. 2014). REDD1 suppresses mitochondrial ROS formation, which is associated with a decline in ageing. Furthermore, a loss of REDD1 induced HIF1α stabilization, indicating that REDD1 destabilizes HIF1α and thereby suppresses its function. This was independent of mTORC1 activity (Horak et al. 2010). Thus, activation of REDD1 could lead to a decline in ROS levels, to a decline in glycolysis and to mTORC1 activation. Therefore, REDD1 activation might be a new target in anti-ageing research. The DNA damage response phosphorylates and thereby activates Che-1, a RNA polymerase II binding protein, which promotes the transcription of two mTOR inhibitors: REDD1 and DEPTOR (Desantis et al. 2015). Both REDD1 and DEPTOR inhibit mTORC1 and the additional functions of REDD1 are described above. Resveratrol, which is found in red wine, promotes the interaction between DEPTOR and mTORC1 and is therefore an mTORC1 inhibitor (Liu and Liu 2011). Che-1 could also be a target of anti-ageing therapy, since it induces the expression of both REDD1 and DEPTOR. However, since REDD1 and DEPTOR directly inhibit mTORC1, chances are that they would have the same negative side effects as rapamycin. DNA damage also induces p53 expression. p53 promotes translation of sestrin 1 and 2, which can inhibit mTOR by activating AMPK and enhancing TSC2 phosphorylation (Budanov and Karin 2008).

mTORC1 inhibition leads to a loss of the feedback signal of S6K and 4E-BP to the PI3K/Akt pathway, leading to activation of this pathway (Carracedo and Pandolfi 2008). This activation would lead to activation of mTORC1 and mTORC2 in the normal situation, but when mTORC1 is inhibited, only mTORC2 is activated. It was reported that overexpression of DEPTOR could induce this asymmetrical activation of mTOR, but it could also be the case for other mTORC1-specific inhibitors, including rapamycin (Peterson et al. 2009). mTORC2 activates SREBP1c and glucokinase in the liver, resulting in activation of lipogenesis and glycolysis (Hagiwara et al. 2012). Expression of mTORC2 wothout expression of mTORC1 could lead to an imbalance in metabolism. When examining mTOR inhibitors, it should also be considered that mTOR signaling is necessary for protein synthesis. Therefore, inhibition of mTORC1 could suppress processes requiring protein synthesis, including growth, tissue repair and regeneration (Zhang et al. 2014). Thus, mTORC1 should not be repressed below levels that are physiologically necessary.

Targeting downstream effectors of mTORC1

Directly inhibiting the 'master regulator' mTORC1 comes with severe side effects and might not be optimal in order to achieve healthy ageing. Mimicking a status of energy deprivation by activating AMPK might be a better way to inhibit mTORC1, since this mimics a natural situation. Other targets for healthy ageing might be factors that are downstream of mTORC1. In this manner, it could be possible to inhibit the factors that attenuate ageing but without impacting other functions. However, little is known about how inhibited mTORC1 activation leads to improved metabolic health.

Recently, Zidek et al found that repressing the formation of C/EBPβ-LIP, a transcription factor downstream of mTORC1, improves metabolic health in mice (Zidek et al. 2015). CCAAT/enhancer binding protein beta (C/EBPβ) is translated into the *cis*-isoform C/EBPβ-LIP when mTORC1 is activated. mTORC1 inhibition represses this translation. Mice lacking the cis-regulatory upstream open reading frame (uORF) in the C/EBPb-mRNA cannot form C/EBPβ-LIP, which is comparable to the effect of mTORC1 inhibition. This results in improved metabolic health, characterized by decreased fat mass and increased energy expenditure, insulin sensitivity, glucose tolerance and physical activity.

Since the metabolic characteristics are similar to those of mice on caloric restriction and since this is a factor downstream of mTORC1, C/EBPβ-LIP repression will probably increase life span. Thus, C/EBPβ-LIP formation could be a key process in the positive effects of mTORC1 inhibition on ageing. Therefore, suppressing C/EBPβ-LIP formation with drugs could lead to healthy ageing without the negative side effects of direct mTORC1 inhibition.

C/EBPβ could be mediating the effects of mTORC1 on several genes involved in glucose and lipid metabolism. Other positive effects of mTORC1 inhibition on ageing were caused by increased autophagy and decreased translation and oxidative metabolism. Therefore, these processes could be targets for anti-ageing therapy as well. Preventing TFEB phosphorylation would result in increased autophagy. Ballabio et al patented a TFEB phosphorylation inhibitor and the use thereof (Ballabio, Medina Sanabria, Settembre 2012). The fact that such an inhibitor exists is promising for anti-ageing research, especially because autophagy is declined during ageing. However, when promoting autophagy, chances are that protein formation will increase to restore protein homeostasis. High demands of the protein folding system could lead to misfolded proteins, having a counterproductive effect on ageing. Translation could be repressed by activation of 4E-BP or inhibition of eIF4E. Currently, several eIF4E inhibitors are tested as anti-cancer therapy (Bhat et al. 2015). Whether or not this would be useful for whole body anti-ageing therapy has not been examined yet. Oxidative metabolism can be harmful by ROS production. To lower the levels of ROS, the human body naturally has antioxidant defense system. Research towards mitochondrial-targeted antioxidants is ongoing. These antioxidants are a promising therapeutic target, since they eliminate ROS directly at their source (Oyewole and Birch-Machin 2015).

Conclusion

The first aim of this essay was to address the role of mTOR signaling is ageing. Reduced TOR or mTOR signaling has proven to decline ageing in many model organisms, including fruit flies, nematodes and mice. Since TOR signaling is highly conserved between organisms, it is likely that reduced mTOR signaling would lead to a delay in the onset of ageing and age-related diseases in humans as well. The second aim of this essay was to discuss whether the mTOR pathway is a useful target to delay the onset and progression of ageing and age-related diseases in humans. Targeting mTORC1 directly is not the best way to go, since this probably leads to severe side effects, as seen with rapamycin treatment. It would be better to either mimic a natural situation, for instance energy deprivation by activating AMPK, or to target one of mTORC1's downstream effectors involved in glucose and lipid metabolism, autophagy, translation or oxidative metabolism.

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