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mTOR as Regulator of Lifespan, Aging, and Cellular Senescence: A Mini-Review

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Abstract
The mechanistic target of rapamycin (mTOR) network is an evolutionary conserved signaling hub that senses and integrates environmental and intracellular nutrient and growth factor signals to coordinate basic cellular and organismal responses such as cell growth, proliferation, apoptosis, and inflammation depending on the individual cell and tissue. A growing list of evidence suggests that mTOR signaling influences longevity and aging. Inhibition of the mTOR complex 1 (mTORC1) with rapamycin is currently the only known pharmacological treatment that increases lifespan in all model organisms studied. This review discusses the potential mechanisms how mTOR signaling controls lifespan and influences aging-related processes such as cellular senescence, metabolism, and stem cell function. Understanding these processes might provide novel therapeutic approaches to influence longevity and aging-related diseases.

Introduction

The mechanistic (formerly mammalian) target of rapamycin (mTOR) is an evolutionary conserved serine-threonine kinase that senses and integrates a diverse set of environmental and intracellular signals, such as growth factors and nutrients to direct cellular and organismal responses [1]. The name TOR (target of rapamycin) is derived from its inhibitor rapamycin, which was initially isolated in the 1970s from a soil bacterium on Rapa Nui (Easter Island). Rapamycin, also known as sirolimus, forms a complex with FK506-binding protein 12 (FKBP12) and in this form inhibits the activity of mTOR. Rapamycin was first described as an antifungal drug and used to inhibit the growth of yeast, but was later found to potently decrease proliferation of T lymphocytes [1]. We now know that the role of mTOR goes far beyond proliferation and coordinates a cell-tailored metabolic program to control cell growth and many biological processes including aging, cellular senescence, and lifespan. Rapamycin is currently the only known pharmacological substance to prolong lifespan in all studied model organisms and the only one in mammals. This review focuses on the role of the mTOR network in aging-related processes and discusses underlying molecular mechanisms that are controlled by mTOR. We discuss recent studies that may allow us to better understand the clinical consequences of mTOR inhibition in rapamycin-treated individuals.

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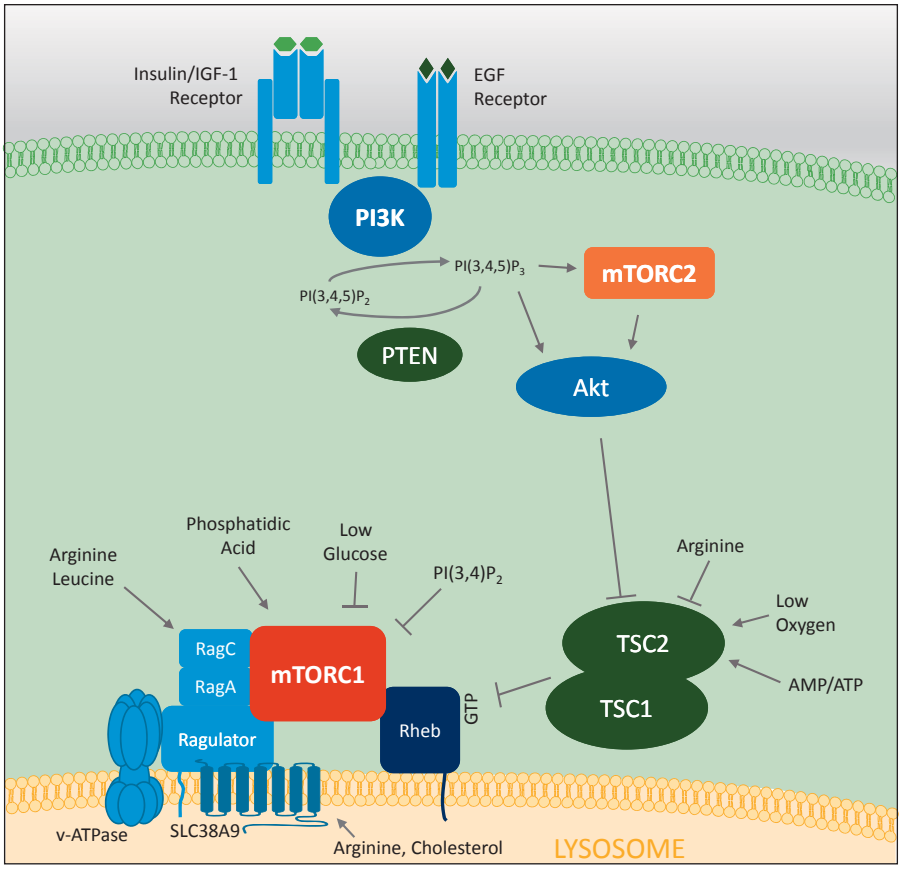

Fig. 1. The mTOR signaling network. For details, see text.

mTOR Signaling

mTOR belongs to the phosphatidylinositol-3 kinases (PI3K)-related kinase (PIKK) family that is present as catalytic subunit in at least two protein complexes: mTOR complex 1 (mTORC1) and mTORC2 [1]. mTORC1 is composed of three core components: mTOR, Raptor (regulatory protein associated with mTOR), and mLST8 (mammalian lethal with Sec13 protein 8). In addition, mTORC1 comprises of DEPTOR (DEP domain containing mTOR interacting protein) and PRAS40 (proline-rich Akt substrate of 40 kDa), which are inhibitory subunits [1]. Numerous growth factor receptors such as the insulin receptor or the epidermal growth factor receptor activate tyrosine kinase adaptor molecules at the cell membrane leading to the recruitment of the class I family of PI3K to the receptor complex (Fig. 1). Following receptor engagement, PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate ($PI[3,4,5]P_2$) to generate phosphatidylinositol-3,4,5-trisphosphate ($PI[3,4,5]P_3$) that recruits and activates the serine-threonine kinase Akt (also known as protein kinase B) via phosphorylation on threonine 308 by phosphoinositide-dependent protein kinase 1 (PDPK1) [2, 3]. mTORC2 is also activated by PI3K through $PI(3,4,5)P_3$ [4] and phosphorylates Akt on serine 473, which is important for full activation and substrate specificity of Akt [3]. A main target of Akt is tuberous sclerosis 2 (TSC2). TSC2 forms a heterodimeric complex with TSC1 and inhibits mTORC1. Phosphorylation of TSC2 at threonine 1462 (Thr1462) by Akt blocks its GTPase-activating protein (GAP) activity for the small GTPase RAS homologue enriched in brain (Rheb), which therefore remains in a GTP-bound state and activates mTORC1 [1, 3]. Thus, class I PI3K activation finally leads to mTORC1 activation through the inhibition of TSC2. Interestingly, the class II PI3K β (PI3KC2β) synthesizes phosphatidylinositol 3,4-bisphosphate ($PI[3,4]P_2$) under growth factor-deprived conditions to inhibit mTORC1 on the lysosome [5].

mTORC1 activation occurs on cellular organelles such as peroxisomes or lysosomes [6]. The activation of mTOR on the lysosome is best understood: mTORC1

binds to a complex consisting of the v-ATPase, Ragulator, and SLC38A9 [1] (Fig. 1). Our current understanding of full mTORC1 activation requires the presence of main nutrient and energy sources: amino acids, glucose, lipids, oxygen, and a high ATP/AMP ratio [7]. mTORC1 senses amino acid sufficiency, especially leucine and arginine on the lysosome via Ras-related GTPases (Rag) and arginine by SLC38A9. In addition, arginine, independently of Rag family members, inhibits lysosomal localization of TSC2 to stimulate mTORC1 activity [8]. Low levels of glucose-6-phosphate decrease mTORC1 activity by stimulating its binding to hexokinase 2 (HK2). A direct interaction of the lipid molecule phosphatidic acid with mTORC1 is also assumed as precondition for mTORC1 activation. Oxygen deprivation stimulates expression of DDIT4 (DNA damage-inducible transcript 4; also known as REDD1 or RTP801), which inhibits mTORC1 via TSC2 [2]. Thus, the presence of these nutrients and energy is supposed to be required for full mTORC1 activation. However, this concept has been established currently only in heavily transformed cancer cell lines such as HeLa cells and remains to be shown in primary cell types. Thus, it is possible that distinct cells require only specific signals to allow activation of mTORC1. Moreover, during homeostasis under nonproliferating conditions in vivo, most cells do not show active mTORC1 or mTORC2 signaling. This is in stark contrast to in vitro cell culture, where these pathways are always active under normal growth-promoting conditions. While rapamycin through FKBP12 directly inhibits mTORC1, mTORC2 is insensitive to acute rapamycin treatment [9]. Like mTORC1, mTORC2 also contains mTOR and mLST8, but contains Rictor (rapamycin-insensitive companion of mTOR), DEPTOR, as well as the regulatory subunits mSin1 and Protor1/2 [1]. Prolonged rapamycin treatment can abrogate mTORC2 signaling in some cells and in mice in vivo [9, 10]; nevertheless the growth-promoting functions of mTOR inhibitors are thought to be mediated by mTORC1.

Identification of the mTOR Network as Lifespan Regulator

The first indications that mTOR is a regulator of lifespan stem from experiments with the nematode *Caenorhabditis elegans* [11] and the fruit fly *Drosophila melanogaster* [12]. Afterwards, mTOR could also be linked to have a role in controlling lifespan in the yeast strain *Saccharomyces cerevisiae* [13]. In this simple organism, inhibition of mTOR with rapamycin doubled the lifespan (defined as survival time of nondividing cells). The interest in the mTOR network as regulator of aging and lifespan was strengthened by the finding that rapamycin extents the lifespan of genetically heterogeneous mice at three independent test locations by about 10–18% depending on sex [14]. Interestingly, treatment was only started late when the mice were 600 days of age equivalent to roughly 60 years of age in a human person. This proposes that inhibition of mTOR in the elderly might be enough to prolong life. The findings were confirmed and extended in mice, in which rapamycin treatment started earlier [15]. However, they failed to substantially observe larger effects on longevity. The maximal lifespan extension seems to be dose-dependent [16]. A lot of other reports confirmed mTOR as lifespan regulator in mice [14, 17–21]. Deleting the mTORC1 substrate S6K1 similarly increases lifespan, but only in female and not male mice [22]. These findings in total suggest an evolutionary conserved role of mTOR as longevity regulator. The lifespan-enhancing effects of mTOR inhibitors have been linked to mTORC1 inhibition, whereas inhibition of mTORC2 might even be detrimental, because mTORC2 controls insulin-mediated suppression of hepatic gluconeogenesis [10, 23].

mTOR, Lifespan, Aging, and the Mechanisms

It is now accepted that mTOR inhibition increases lifespan; yet, the mechanism through which this occurs is still uncertain. mTORC1 inhibition may not delay aging itself, but may delay age-related diseases [24–27]. However, many researchers directly link the longevity effects of mTOR inhibitors to a decrease in aging. Aging is generally characterized by a progressive loss of physiological integrity, which leads to impaired functions, and therefore increases vulnerability to death thus limiting lifespan [28]. Conserved hallmarks of aging have recently been proposed and include telomere attrition, epigenetic alterations, genomic instability, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [28, 29]. The mTOR network is known to regulate some of these aging hallmarks as described below. Ultimately, the prominence of mTORC1 signaling in aging likely reflects its exceptional capacity to regulate such a wide variety of key cellular functions (Fig. 2).

mRNA Translation

mTORC1 regulates cap-dependent and cap-independent translation of mRNAs by phosphorylating the translation inhibitors eIF4E-binding protein 1 (4E-BP1) and 4E-BP2, which then release eukaryotic translation initiation factor 4E (eIF4E). mTORC1 is particularly potent in promoting translation initiation of mRNAs containing a 5′ terminal oligopyrimidine tract (5′ TOP) or a pyrimidine-rich translational element (PRTE); many of those encode translation- and ribosomal-related proteins but also metabolism-related genes [30]. In addition, ribosomal protein S6 kinase 1 (S6K1) and S6K2 are two other well-characterized targets of mTORC1-mediated phosphorylation, which subsequently phosphorylate and activate the ribosomal protein S6 to stimulate protein translation [1]. Several studies suggest that a general decrease in mRNA translation caused by mTORC1 inhibition slows aging. Mechanistically, this is explained by reducing the accumulation of proteotoxic and oxidative stress [1, 22]. Deletion of the translational regulator S6K1 prolongs lifespan in mammals, although a direct link to altered global translation has not been shown so far [22]. Hence, although counterintuitive at first thought, reducing mRNA translation might permit easier endogenous protein degradation or repair to preserve homeostasis when facing oxidative damage as well as protein aggregation during ageing [31].

Autophagy and Mitochondria

The starvation-induced degradation of cytosolic components known as autophagy is crucial in providing substrates for energy production under catabolic conditions of limited nutrient supply and removal of damaged organelles [32]. mTORC1 controls the Ser/Thr kinase ULK1, which regulates autophagosomal formation and autophagic flux [32]. mTORC1 actively suppresses autophagy by phosphorylating ULK1 and, accordingly, inhibition of mTORC1 induces autophagy. It has been suggested that autophagy might decline with age resulting in the accumulation of damaged proteins and organelles such as mitochondria; however, the underlying reason remains unclear. mTORC1 inhibition could slow aging by stimulating autophagy, which clears old and dysfunctional mitochondria (mitophagy), the accumulation of which is linked to aging and aging-related diseases [1]. Additionally, mTORC1 regulates mitochondrial functions [33]. On the one hand, mTORC1 controls the cellular energy metabolism by increasing glycolytic flux and simultaneously limiting oxidative phosphorylation (a process called aerobic glycolysis or Warburg effect in cancer cells). On the other hand, mTORC1 increases mitochondrial functions and stimulates mitochondrial biogenesis through PGC-1α and the transcription factor YY-1 in some cells [1]. It is important to preserve the function and number of mitochondria during aging, but the cumulative role of mTORC1 in these processes is complex and might depend on the individual tissue.

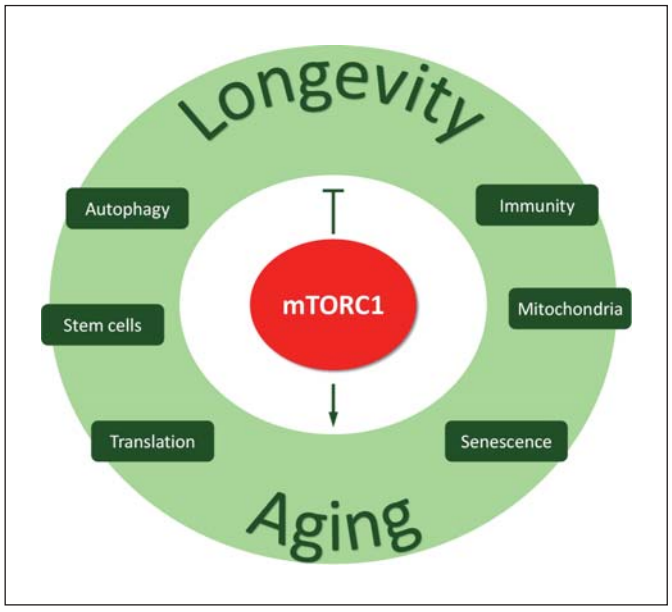

Fig. 2. The role of mTORC1 in longevity and aging. The mechanisms of how mTORC1 regulates longevity and aging.

Stem Cell and Immune Function

A decline in stem cell number and function might be a critical cause in age-related dysfunction of tissue homeostasis [34]. mTORC1 inhibition may preserve adult stem cell function in various tissues [31]. For example, treatment of old mice with rapamycin enhances intestinal stem cell function indirectly by reducing mTORC1 signaling in Paneth cells, which creates a better intestinal niche for stem cells [35]. In contrast, during calorie restriction, which enhances lifespan (see below), mTORC1 is induced in intestinal stem cells to allow their expansion [36]. Similarly, mTORC1 controls the adaptive transition of quiescent muscle stem cells from G0 arrest to an alert

state that is important to respond to injury-induced systemic signals [37].

Optimal immune functions are evidently critical during aging for maintaining organismal fitness against pathogens, cancers, or other diseases. mTORC1 has many central and often divergent functions in the innate and adaptive immune system to enhance or limit inflammation or immune responses [2, 38–40]. Importantly, mTORC1 inhibition is used as immunosuppressive therapy to limit T cell activation and prevent transplant rejection after organ transplantation. In contrast, inhibition of mTORC1 augments CD8+ T cell memory responses that are critical for viral defense [41]. The role and activity of mTORC1 in immune cells during aging is, however, not well studied. One study reported that an age-related decline in hematopoietic stem cell (HSC) function, which may contribute to anemia, poor vaccination, or enhanced tumorigenesis, is associated with an increased mTORC1 activity in HSCs from old mice [42]. Hence, rapamycin restores self-renewal and hematopoiesis of HSCs, which enables effective vaccination of old mice against a lethal challenge with influenza virus [42]. In summary, although mTORC1 inhibition clearly enhances overall lifespan, it may exert positive and negative functions on stem and immune cells that may differentially impact aging.

Cellular Senescence

Cellular senescence is historically defined as an irreversible cell cycle exit, whilst preserving cell viability [34]. Cellular senescence has been suggested to function as a tumor suppressor mechanism and promotor of tissue remodeling after wounding [34, 43]. However, senescent cells may also directly contribute to aging [28, 34]. Senescent cells show marked changes in morphology including an enlarged size, irregular cell shape, prominent and sometimes multiple nuclei, accumulation of mitochondrial and lysosomal mass, increased granularity and highly prominent stress fibers that are accompanied by shifts in metabolism and a failure of autophagy. Interestingly, many of these phenotypes are regulated by mTORC1 in various cell types [44, 45]. Senescent cells secrete proinflammatory and pro-oxidant signals, which can cause inflammation, and due to a suppression of apoptosis, they occupy key cellular niches [28, 46]. Due to these mechanisms, senescent cells steadily accumulate with age and contribute to aging-related diseases and morbidity [34]. Hence, clearance of senescent cells improves aging-related disorders [47].

Senescence-Associated Secretory Phenotype Regulated by mTORC1

The secretion of proinflammatory mediators by senescent cells contributes to aging and has been termed senescence-associated secretory phenotype (SASP). Recent data identified a main role of mTORC1 to promote the SASP [48, 49]. Rapamycin blunts the proinflammatory phenotype of senescent cells by specifically suppressing translation of the membrane-bound cytokine IL1A [48]. This reduction of IL1A diminishes transcription of inflammatory genes regulated by the proinflammatory transcription factors NF-κB. In parallel, mTOR controls translation of MK2, which in turn phosphorylates the RNA-binding protein ZFP36L1 during senescence. This phosphorylation of ZFP36L1 inhibits its ability to degrade transcripts of numerous SASP components. Thus, rapamycin activates ZFP36L1 to induce SASP component degradation [49]. The reason for mTORC1 activation in senescent cells might result from defects in amino acid and growth factor sensing [50]. Senescent human fibroblasts induced by stress, replicative exhaustion, or oncogene activation, show constitutive mTORC1 activation, which is resistant to serum and amino acid starvation. This is mediated in part by a depolarization of the plasma membrane resulting in a failure to abrogate growth factor signaling. Moreover, increased autophagy provides high amino acid levels to support mTORC1 activation [50]. Additionally, two proteins important for the DNA damage repair, O-6-methylguanine-DNA methyltransferase (MGMT) and N-myc downstream-regulated gene 1 (NDRG1) are negatively regulated by mTORC1 in senescent mice and cells [51]. These results in toto indicate that mTORC1 inhibitors inhibit the SASP through various mutually nonexclusive mechanisms.

Metabolic Reprogramming in Senescence

Despite maintaining a nondividing state, senescent cells display a high metabolic rate [52]. Metabolic changes characteristic of replicative senescence often show a shift to glycolytic metabolism away from oxidative phosphorylation (which is also observed in proliferative cells), despite a marked increase in mitochondrial mass and markers of mitochondrial activity. This might stem from a rise in lysosomal pH as a consequence of proton pump failure, which leads to an inability to get rid of damaged organelles such as mitochondria caused by a failure of autophagy. Dysfunctional mitochondria not cleared by autophagy in senescent cells produce reactive oxygen spe-

cies, which cause cellular damage including DNA damage. mTORC1 has been postulated as main driver of these metabolic changes [1], which are overlapping with the functions described above in the section Autophagy and Mitochondria. Hence, rapamycin treatment prevents metabolic stress and delays cellular senescence.

Calorie Restriction

There is one other intervention besides mTOR inhibitors that extends lifespan in a lot of organisms: caloric restriction (CR). CR is defined as a reduction in nutrient intake without malnutrition. Because mTORC1 has an important role in sensing energy, nutrients, and insulin intracellularly and on the organismal level [1, 53], this fueled the speculation that the lifespan-enhancing effects of CR are at least partly mediated by decreased mTORC1 signaling. Indeed, CR-like regimens do not additionally lengthen the lifespan of yeast or worms when mTOR is inhibited, indicating overlapping mechanisms [12, 13, 54]. In contrast, mTORC1 inhibition and CR show additive effects on lifespan in flies [55]. In rhesus monkeys, CR has universal health benefits and but no clear-cut effects on survival [56]. Whether CR prolongs lifespan in human is currently unknown. In some animals including distinct strains of mice, CR does not always correlate with lifespan extension, although it consistently improves health [57]. Most CR studies (as well as mouse experiments in general) are designed in an ad libitum fashion; the animals can eat as much as they want. Hence, these studies may actually analyze more the effects of overfeeding, which is known to promote obesity-associated pathologies in our society [58].

Clinical Use of mTOR Inhibitors

The finding that inhibition of mTOR prolongs lifespan and postpones onset of age-associated diseases in mammals spurred the interest to develop mTOR inhibitors as drugs to augment human longevity. However, the side effect profiles of mTOR inhibitors are a major cause of concern. Because pharmacological inhibition of mTOR is an FDA-approved clinical principle, there is a wealth of information about the known side effects of mTOR inhibitors (rapalogs) in humans [59, 60]. Importantly, the introduction of mTOR inhibitors as a therapeutic regimen generated a new spectrum of side effects that resulted in 20–40% dropout rates in phase III clinical trials [61]. The most common side effects are immunosuppression, hyperglycemia, and dyslipidemia as well as interstitial pneumonitis [59]. Most of these side effects are dosage-dependent and may regress with lower dosage. Because mTOR inhibitors are mostly given in combination with other drugs such as steroids or proliferation inhibitors in already severely diseased patients, preventive mTOR inhibitor therapy in the general healthy population might be more tolerable. Moreover, the immunosuppressive properties of mTOR inhibitors for T cell responses might be particularly strong for graft-reactive alloantigens, but not for pathogen-specific responses such as infections [62]. Indeed, mTOR inhibitors might be future longevity drugs, because a clinical trial in elderly healthy humans receiving the mTORC1 inhibitor everolimus at a very low dose showed safety and even improved immune function [24]. Moreover, alternate dosing regimens may still promote longevity but reduce side effects [63, 64].

Outlook

mTOR inhibitors are currently the only known pharmacological intervention that increases lifespan in all experimental animal models tested. This raises the prospect that someday it might be possible to therapeutically increase lifespan, slow aging, or decrease aging-related diseases in humans. Nevertheless, we are currently only at the beginning of that journey. Further knowledge of the actual lifespan-enhancing effects of mTOR inhibition might generate more precise ways to promote longevity. For example, differential isoforms and splice variants exist for the individual mTOR pathway members; their role in normal cellular functions and for aging is currently poorly defined. Because RNA splicing is required for longevity in *C. elegans* downstream of the TOR pathway, this gap of knowledge should be closed [65]. Moreover, distinct mTORC1 and mTORC2 complexes may exist inside the cell at different locations that perform different functions that may differentially affect longevity [66].

In conclusion, defining the exact mechanisms and tissues in which mTOR inhibition promotes longevity and reduces age-related diseases is of scientific and general but also socioeconomic importance.

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