2. mTOR, aging and the potential for intervention

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We review current knowledge indicating that mTOR plays a central role in limiting longevity by potentiating, aging and age-associated diseases. Experiments in genetically heterogeneous as well as inbred strains of mice provide proof-of-concept evidence that rapamycin, or similar inhibitors, deserve clinical testing as potential prophylactics against aging related dysfunction and diseases. We also review evidence against the widely held view that chronic use of rapamycin (and other mTORC1 inhibitors) is immunosuppressive in terms of infectious disease. To tackle the increasing toll in human suffering and economic burden of the aging population and their associated diseases, we argue for more research on therapeutic approaches, exemplified by rapamycin treatment, which target the processes that underlie the exponential increase with advancing age in risk for virtually all diseases and disability.

1 mTOR, growth regulation and aging

The mechanistic [or mammalian (see Hall [11])] target of rapamycin and its ancillary signaling inputs and outputs is one of the most important cell regulatory nodes in biology—and maybe one of the most important nodes modulating aging. While mTOR is critical for normal development, its recently discovered linkage to aging and associated diseases has quickly elevated it to one of the most studied systems in biology. Using mTOR as the search term, NCBI returns about 40–50 papers per week. The challenge to understanding its actions in terms of their impact on aging is the complexity of its cell-autonomous regulatory circuits compounded by its equally multifaceted non-cell autonomous functions. Although we know that mTOR is central to modulating aging, understanding the web of effects from the pathways it effects to the outcomes that modulate aging remains a major challenge—indeed a challenge not unique to mTOR. For example, one of the most reliable anti-aging interventions (and experimental tools), diet restriction (DR), has been intensely studied for thirty years, with many hypotheses but little proof or clarity to the detailed mechanisms and pathways modulated by DR that in turn modulating aging [12].

[Keywords]
mTOR, mTORC1, ITP, rapamycin

mTOR, aging and the potential for intervention
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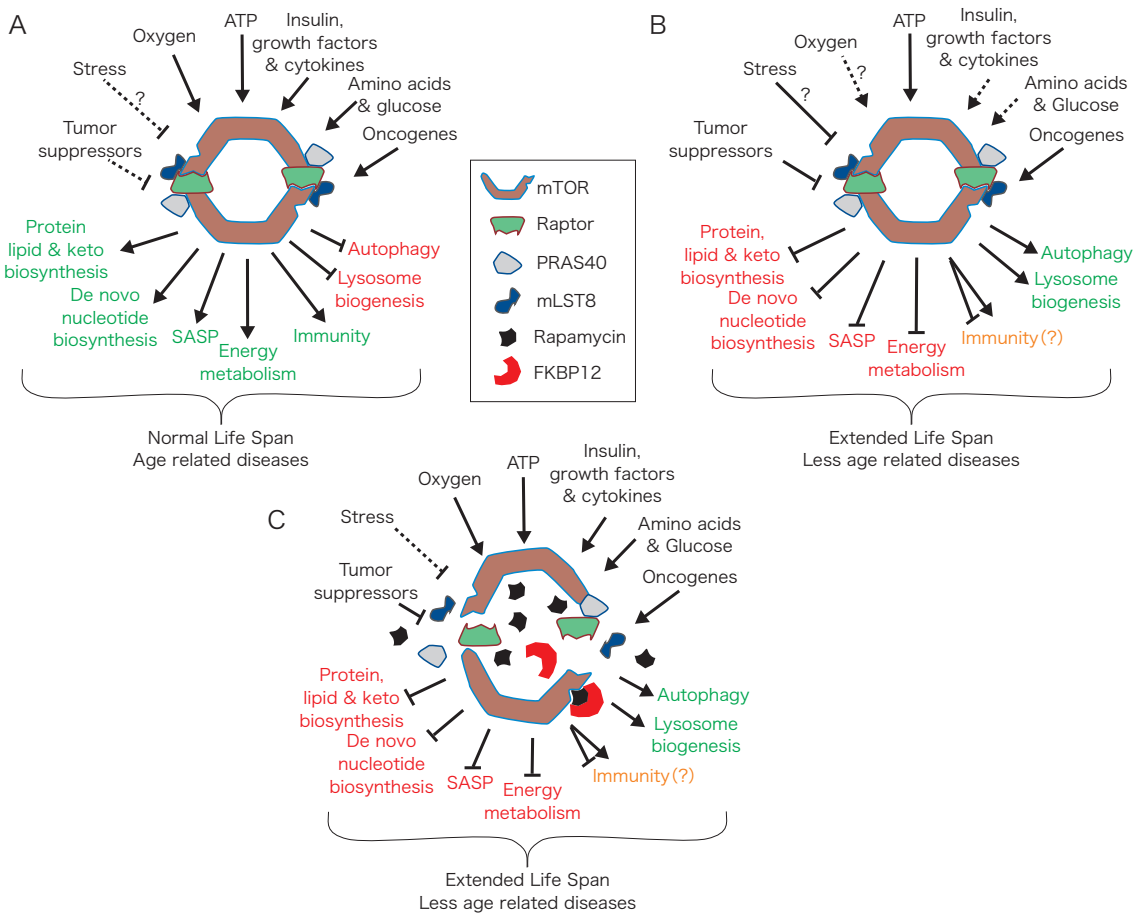

Figure 1 mTOR complex 1 (mTORC1) signaling in aging

A) Stimuli integrated by mTORC1 in the performance of its cell autonomous functions. In a pro-growth environment (including active growth factor/cytokine upstream stimulation), mTORC1 executes a pro-anabolic (growth in mass preceding cell division) program as indicated in its key outputs (red = down regulated state; green = up regulated). In adult non-proliferating tissues, mTORC1 activity is posited to contribute to the senescence associated secretory phenotype (SASP). Under these conditions, a normal life span includes age-associated diseases like Alzheimer's and cancer. B) Prolongevity interventions (reductions in growth factors and/or nutrients) lead to reduction of mTORC1 activity and decrease in downstream processes. This hypothetical shift in the state of mTORC1 and the related down regulation of its key outputs is posited to result in extended longevity, including the prevention, delay and/or reduction in severity of age-related diseases. Hypoxia also inhibits mTORC1 [1)–4)], but effects on life span are not known. C) Rapamycin-FKBP12 destabilizes mTORC1 [5)], which is hypothesized to mimic diet and/or growth factor restriction in effects on downstream effectors and longevity extension. Protein subunits of mTORC1 are indicated. Solid lines in arrows and blocks in mTORC1 stimuli indicate increased conditions, and dotted lines signify reduced conditions.

2 The Yin and Yang of mTOR

1) The Yin

mTOR, a member of the phosphoinositide 3-kinase (PI3K)-related protein kinases (PIKK) family, co-ordinates cell responses to various stimuli and environmental conditions summarized in **Figure 1**. The two complexes formed by mTOR (mTORC1 and mTORC2) each have diverse cell autonomous and non-cell autonomous functions. There is general agreement that mTORC1 plays a key role in modulating aging and age-associated diseases (Reviewed comprehensively by

Johnson, Rabinovitch and Kaeberlein [13]). When nutrients are abundant, mTORC1 promotes anabolic pathways for cell growth (mass accumulation). When nutrients are scarce, mTOR ramps down its anabolic stimuli, and becomes permissive for catabolic activities such as autophagy (**Figure 1**), important for cell survival. In addition and importantly, any stress experienced by cells (or organisms) represses mTORC1 and thus its downstream signaling effectors.

Metazoan mTORC1 has numerous cell autonomous and non-cell autonomous functions. Tissue- and organ- specific functions range from the regulation of organismal growth, appetite (energy balance), adipogenesis, muscle mass, glucose homeostasis, liver ketogenesis and adipogenesis, β -cell mass in the pancreas [14], and iron homeostasis [15]. In a recent example of non-cell autonomous function, Yilmaz et al. [16] showed that DR- and rapamycin (which together with it receptor, FKBP12 inhibits mTOR) regulates Paneth cell signaling for Lgr5+ stem cell renewal in intestinal crypts. DR appears to increase ISC self-renewal via an increase in extracellular signaling (cADPR) by Paneth cells in response to a reduction of mTORC1 signaling. Lgr5+ stem cells are the origin of intestinal tumors in the *Apc*$^{Min/+}$ model [17]. It is interesting that DR reduced tumor formation in *Apc*$^{Min/+}$ mice [18], illustrating how chronic mTORC1 inhibition can prevent disease or reduces their severity.

mTOR suppression plays an important role in learning and memory [19], raising the possibility that mTOR inhibitors may have therapeutic potential for the treatment of cognitive deficiencies [19], improved cognition [20-24], and neurodegenerative diseases [25]. Recently Cao and colleagues showed the mTORC1/4E-BP1 axis regulates central (suprachiasmatic) regulation of circadian rhythms [26].

The diverse functions modulated by mTOR signaling are consistent with its role in modulating longevity and aging. At the same time, they provide a formidable challenge to efforts to fully elucidate the role of mTOR in longevity regulation and the development of age-related diseases. In summary, mTORC1 appears to play a major role in regulating numerous aspects of cell autonomous and non-cell autonomous physiology and, for our discussion, lifespan in invertebrates and vertebrates.

2) The Yang

After mTOR performs its vital role in development, accumulating evidence suggests that the continued activity of mTOR into later adulthood at levels present in development is harmful in adult somatic tissues/organs. Support for this rested initially upon studies showing that reductions in mTOR activity in adulthood are associated with extended lifespan in *Saccharomyces cerevisiae* [27, 28], the adult round worm, *Caenorhabditis elegans* [27, 28], and the fruit fly, *Drosophila melanogaster* [29]. Proposed mechanisms for these dramatic effects include reduced ribosomal DNA recombination, lowered mRNA translation, less acetic acid production, improved oxidative stress resistance, enhanced mitochondrial function and better removal of damaged proteins through autophagy (reviewed in Ref. 30).

Inhibition of mTORC1-regulated protein synthesis (or Cap-dependent translation) and biosynthesis of lipid and other bio-macromolecules (needed for cell growth) appears to be fundamental to improved life span by way of mTORC1. **Figure 2** summarizes these functions. mTORC1 regulates eukaryotic initiation factor 4E (eIF4E)-binding proteins (4E-BPs), which represses its mRNA Cap-binding translation function [31]. The other key factor regulated by mTORC1, ribosome subunit 6 kinase 1 (S6K1), promotes protein synthesis via ribosome biogenesis by one of its substrates, ribosomal protein subunit 6 (rpS6) [6]. Of relevance to aging, overexpression of the 4E-BP (altered Cap-dependent translation) increased longevity of *D. melanogaster* [32]. Consistent with this line of thought, removal of IFE-2, a somatic isoform of eIF4E in *C. elegans*, lowers global protein production and oxidative stress—resulting in an extended life span [33]. In addition, decreased translation initiation complex components in worms (e.g., ifg-1, a homolog of mammalian eIF4G [31]) and loss of rsk-1, S6 kinase in mammals extend their life span [34]. Using a RNAi screen in *C. elegans*, Hamilton et al. [35] showed that inactivation of a homolog of the translation initiation factor eIF5A extended lifespan. These data indicate that decreased translation in the worm and a fly is a mechanism for extension of life span. Is there evidence in vertebrates?

In pituitary dwarf mice, which in the laboratory live much longer than wild-type, normal sized littermates, mTORC1 in liver and muscle is down-regulated [36, 37]. Deletion of S6K1, a substrate of mTORC1 (**Figure 2**), increased female mice life span and decreased age-related pathologies [38]. Small mice carrying two hypomorphic mTOR alleles [39] lived 20% longer than wild

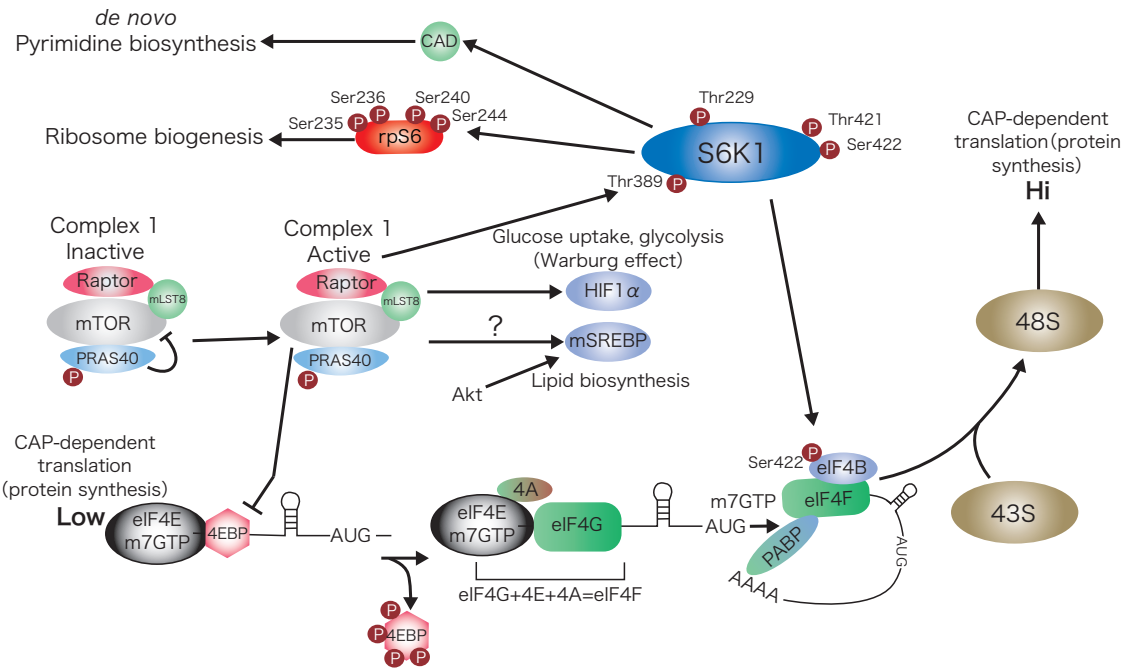

Figure 2 mTORC1 regulation of macromolecular biosynthesis

When environmental conditions warrant, mTORC1 becomes active toward substrates. Shown are two of the best studied substrates, S6 kinase 1 and some of its downstream substrates and the translation repressor, eukaryotic initiation factor 4E (eIF4E) binding protein (4E-BP1). Activated mTORC1 phosphorylates S6K1, which then phosphorylates ribosomal protein subunit 6 (rpS6) important for anabolic ribosome biogenesis via a transcriptional program [6]. As part of anabolic program, S6K1 also phosphorylates Ser1859 on CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase) to promote *de novo* pyrimidine biosynthesis. S6K1 also help promote protein synthesis by phosphorylation of eIF4B. Lipid biosynthesis for anabolism is by activation of the transcription factor SREBP-1 via complex molecular connection involving Akt [7]. S6 kinase has numerous other substrates not shown including Grb10 [8,9] important in insulin signaling. mTORC1 signaling can also stimulate glucose uptake, glycolysis, and NADPH synthesis to support anabolism. Transcription factor activation is largely responsible but post-translational modifications are also involved. Increased translation of hypoxia inducible factor 1 α (HIF1 α) promotes expression of glucose transporters and glycolytic enzymes and induces a switch from mitochondrial oxidative metabolism to glycolysis, Warburg effect in cancer cells (reviewed in Ref. 10). Cap-dependent translation is de-repressed by activated mTORC1 through phosphorylation of 4E-BP1 leading to formation of the eIF4F complex and subsequent assembly of the 48S complex which together with the 60S subunit forms ribosomes.

type controls [40]. Importantly, these mice showed reductions in a number of aging tissue biomarkers and functional preservation of some, but not all, organ systems. Thus, reductions of mTORC1 in adults appear to result not only in extended life span but also in markers associated with extended healthspan—indicating that normal activity of mTORC1 in adulthood limits lifespan and the duration of at least some aspects of healthy life. What specifically are the deleterious actions of mTORC1 in adulthood are discussed below.

3 The need for a new clinical approach to aging

The United Nations Population Division [141] reports that the number of people 60 years or older in 2012 is 809,743,000 (one out of nine). In 2050, that number balloons to an astonishing 2,031,337,000 (one out of five). For Japan, the population over 60 was 39,967,000 in 2012, which increases to 45,005,000 in 2050. If nothing is done, how will these numbers impact health and care for the elderly? The funda-

mental weakness of our current approach can be exemplified using cancer, a disease of aging [41], as an example. In 2011, Siegel et al. [42] projected the diagnosis of 1,596,670 new cancer cases associated with 571,950 deaths. Edwards et al. [43] examined the effect of these demographics on cancer and ominously reported a) that the number of cancer patients will double between 2000 and 2050; b) a dramatic increase in the proportion of the elderly will increase (e.g., 389,000 or 30% in 2000 to 1,102,000 or 42% in 2050); c) a four fold increase in cancer patients aged 85; d) a doubling of the absolute number of cancers in people 65 and older. The risk of developing cancer and dying from it becomes highly significant as the population ages since people over 65 have an age-adjusted cancer mortality rate 15 times greater than young people. If we could miraculously prevent and/or cure all cancers, would this in fact address the aging problem? The argument is compelling that curing all cancer without mitigating the effects of aging would be an imprudent health and economic policy. Eliminating all adult cancer is estimated to add 4 years to life but would raise healthcare costs 8.3% [44] due to the cost of treating other age-related diseases (e.g., dementias, cardiovasculardiseases, sarcopenia, frailty and diseases associated with immune senescence). Eliminating cardiovascular disease would increase longevity 5.3 years and health costs 5.2% [44]. Clearly, the single-disease approach is flawed in the context of aging. Aging is, by far, the most significant risk factor for a large number of diseases [45], including cancer [46] and the ideal strategy is to discover interventions that target "aging" , which would ideally reduce the incidence or ameliorate the impact of multiple diseases simultaneously.

Biogerontological research has uncovered nutritional, genetic and pharmacologic interventions that provide "proof-of-principal" in animal models that aging can be targeted with clinical interventions. Research in aging has revealed interventions that have achieved both age extension and disease alleviation, referred to as the "longevity dividend" [47]. Consider the immense and still growing body of work showing that DR increases maximum life span [48], and improves most measures of health [49-52]. We remind the reader that an increase in maximum life span can only be achieved by reducing all competing causes of mortality [53]. Genetic mutations, such as those resulting in pituitary dwarfism also extend maximum life span (Reviewed by Richardson [54]), reduce cancer [55,56] and delay other age-sensitive traits [57].

Is there an underlying etiology shared by DR, dwarfism and other genetic interventions that can be targeted? One theory posits that, in somatic organs/tissues, there appears to be a slow, steady (in most cases) buildup of damaged or aggregate macromolecules that drives the decline seen in aging, and which likely plays a role in associated maladies. Arguing against this, Blagosklonny proposes a "quasi-programmed" process driven by continually active growth-promoting mTOR [58]. Velarde et al. [59] proposed that aging senescent cells acquire the unhealthy ability to alter their microenvironment by acquisition of a senescence associated secretory phenotype, or SASP. By way of autocrine signaling and paracrine (inflammasome) stimulation, SASP cells promote increased senescence and pro-tumorigenic conditions [60-62]. A role for mTOR in this process arises from evidence showing that mTORC1 inhibition suppresses senescence [63-68].

As a key regulator of numerous biological processes, mTOR is at least one candidate target with evidence linking it to aging and associated diseases. We argue that, targeting mTOR, using mTORC1 inhibitors such as rapamycin, opens up the prospect of being able to suppress both aging and its diseases at the same time.

4 A program to identify anti-aging drugs

Nearly a decade ago, the National Institute on Aging established a pioneering effort, the Interventions Testing Program (ITP [69,70]) to test candidate interventions in a model system, genetically heterogeneous mice (UM-HET3) for their ability to extend life span. Dr. Nancy Nadon oversees the ITP and an Access Committee and Steering Committee reviews proposals for feasibility. To date, the ITP website indicates they have tested or in the process of testing 23 different compounds, some at varied doses and in combination. Seven publications from the ITP have reported increases in life span from four compounds [69-75]. Importantly, the ITP also reports compounds that do not extend life span. This program, initially met with much skepticism, has provided proof-of-principle that drugs can delay aging processes and extend healthy life in mammals. The studies of the mTORC1 inhibitor, rapamycin that have been underwritten by the ITP have been a key catalyst in attracting scientific as

well as commercial resources to the problem of aging. How was rapamycin, FDA approved as a suppressor of immunity in transplant patients, chosen to be tested by the ITP?

In 2004, one of us (Sharp) proposed to the ITP that chronic treatment with rapamycin would mimic diet and/or growth factor restriction as a pro-longevity intervention. The rationale for this approach is shown in **Figure 1**. Although less was known at the time, we knew that mTOR sensed the nutrient state of the cell and responded to growth factor stimuli. The first results of the rapamycin studies, published in 2009 [71], showed that administration of an enteric formulation of rapamycin (termed eRapa) begun late in life significantly extended maximum life span of both sexes. In 2011, a follow-up paper showed that eRapa intervention started in mid-life also extended maximum life span in both sexes [72]. Recently, a dose-response study by Wilkinson et al. [75] reported positive and negative effects of eRapa has on health of genetically heterogeneous mice, and Zhang et al. [76] reported similar evidence for C57BL/6Nia mice. In addition, rapamycin also prolonged lifespan when given as subcutaneous injections to female mice carrying the tumorigenic HER-2/neu transgene [77], to female inbred 129/Sv mice [78], or as a 6-week treatment to old C57BL/6 mice [79]. Neff et al. [80], in a comprehensive study, showed that eRapa extended the life span of male C57BL/6 mice. As proofs of principle, these papers show the feasibility of pharmacologically extending not only life span, but also health span in mammalian species. Will other inhibitors of mTORC1 also do this?

5 Prolongevity potential of other drugs that target mTORC1

Identifying alternatives to rapamycin is an important pharmacological goal. We will briefly discuss a few examples. Metformin, originally viewed as an activator of adenosine monophosphate-activated protein kinase (AMPK), has no direct effect on it or its upstream kinase, LKB1 [81]. Through inhibition of mitochondrial function that increases AMP and/or ADP levels, metformin indirectly inhibits AMPK, although its mode of action as an anti-diabetic drug remains unclear. Metformin also indirectly inhibits mTORC1 by way of two pathways; first by suppressing the RagGTPase system [82], which functions in the amino acid sensing system associated with lysosomes [10) 14]; second by inhibiting mTORC1 through REDD1 and p53 [83].

Phenformin, metformin and other biguanide anti-diabetic drugs extend survival in animal models with spontaneous, genotoxic-induced, and genetically predisposed tumors—a first indication that they might function as pro-longevity drugs. In support, chronic treatment with metformin in the drinking water extended mean and maximum life span of outbred SHR female mice (prone to mammary carcinoma and leukemia), without any effect on the incidence of spontaneous malignant tumors [84]. Alone and in combination with rapamycin, metformin is currently under study by the ITP for pro-longevity effects in UM-HET3 mice. Because metformin is one of the most prescribed drugs in the world with a good safety record, this ITP study has significant implications if it is confirmed to extend longevity and delay age-related disease and dysfunction. A systematic review and meta-analysis revealed that metformin "was the only antidiabetic agent not associated with harm in patients with heart failure and diabetes" [85].

Another potential mTOR inhibiting candidate is resveratrol, an activator of SIRT1, one of seven mammalian sirtuins. Reviewed by Baur et al. [86], it has been extensively studied for its anti-cancer and anti-aging effects. Numerous studies have shown that resveratrol reduces mTORC1 [87-91], perhaps explaining its anti-cancer effects and lifespan extending effects. Valenzano et al. [92] showed that resveratrol extended the life span of the short-lived seasonal fish *Nothobranchius furzeri*. Importantly, Baur et al. reported that resveratrol extended life span in mice fed a high fat diet [86]. The ITP reported that two doses of resveratrol (300 ppm and 1200 ppm in standard diet) starting at 12 months of age had no effect on life span for UM-HET3 mice fed a normal diet [72]. Testing initiation of drug treatment at an earlier age (4 months of age), the ITP reported 300 ppm resveratrol again had no effect on life span [73]. The different outcomes of resveratrol on longevity in normal and high-fat fed environments underscore the importance of careful documentation of the environmental circumstances under which interventions are tested.

6 Limitations and negative effects of rapamycin therapy

No drug is without side-effects and no intervention that extends lifespan, whether nutritional, genetic or pharmacologic, has been found without limitations in

terms of the array of aging associated traits it effects or deleterious actions. Dietary restriction often reduces fertility [93] and renders animals more susceptible to cold stress [94]. Dwarf mice, in addition to size abnormality, require special housing to survive the neonatal period (Nelson and Diaz, unpublished observations). Rapamycin reversibly reduces markers of male reproductive function and leads to stomatitis in humans [95] [96]. In C57BL/6 mice, Neff et al. [80], showed that eRapa had two negative effects, testicular degeneration and nephrotoxicity, in addition to extending lifespan and improving several age-related conditions. Zhang et al. [76] studying C57BL/6 mice and Wilkinson et al. [75] analyzing HET-3 mice found testicular degeneration but no nephrotoxicity. It should also be noted that Neff et al. [80] argued that eRapa is not broadly anti-aging. In a comprehensive study of many traits showing age-related changes, they reported that only a small fraction of the changes were attenuated by chronic eRapa treatment. Moreover, they noted that for most of the traits whose age-related deficits were reduced by eRapa, a similar effect was observed after short-term treatment with eRapa in young mice. For example, eRapa enhanced measures of cognition in young and old mice similarly, leaving the age-related change unaffected. Whether these findings definitively indicate a lessened role for rapamycin in aging is open to debate and we refer to an accompanying commentary that offers an opposing argument (Richardson, 2013). The bottom line is rapamycin extends lifespan in multiple murine models ranging from inbred strains to genetically heterogeneous and mutant strains with shortened lifespan. Moreover, it enhances measures of activity, cognition and immune function in old mice. Indeed, the fact that relatively short term treatment with rapamycin in old mice can result in improvements in these measures of healthspan is consistent with a short-term enhancement of these functions in young mice and points to a potential ability to reverse these age-related changes—a potential deserving further study to identify the underlying mechanisms.

7 Possible mechanism(s) and novel interventional opportunities

We postulate that an important effect of chronic treatment with eRapa in mice is a delay or slower progression in age-related disease development, (e.g., by immune protection, see below), or an enhanced ability to tolerate or mitigate the deleterious effects of the disease process. What is the underlying mechanism? A comprehensive understanding of how rapamycin works *in vivo* to extend life span and repress diseases will be as complex and hard to understand as has been efforts to understand the mechanisms whereby DR acts. Our study of the effect of chronic rapamycin in a preclinical model of cancer promoted by loss of the tumor suppressor, pRb1, illustrates the difficulties in comparing/understanding how DR and chronic rapamycin works in cancer prevention. eRapa treatment of male and female $Rb1^{+/-}$ mice robustly extended their life span in part by preventing or delaying growth of $Rb1^{-/-}$ neuroendocrine tumors [97]. In contrast 50% DR in this mode had minimal, if any, effect on life span, tumor incidence or multiplicity [98]. Both DR and rapamycin inhibit mTOR, yet rapamycin works in $Rb1^{-/-}$ tumors and DR does not. How and why rapamycin works remains a mystery. One possibility is there is an off-target effect. A dependence of most cancer cells to (hyper) active mTORC1 is responsible for the up-regulated pro growth state (increased biomass accumulation for cell division) of most cancer cells [99]. It may be possible that aging is similarly dependent to increased mTORC1? If so, to what aspect of mTORC1 action are cancer cells (or cells from aged organisms) addicted?

Regardless of the conditions, responses to a cell's nutrient state by mTORC1 provide a key decision point between anabolic versus catabolic metabolism [10]. Laplante and Sabatini [14] provided an excellent review of the processes (e.g., ribosome biogenesis) that cells exploit, and in which mTORC1 has a regulatory role.

Translation, especially translation initiation, is replete with opportunities for the development of new drugs that target aging and age-associated diseases [100]. Transcription has been studied exhaustively in aging. How transcription and translation regulation are coordinated remained a mystery until recently. For maintenance of an anabolic state, Santagata et al. [101] performed a detailed study to determine the interplay of transcription and translation. In response to translation inhibition, they identified heat shock transcription factor 1 (HSF1) as a key coordinator. In a chemical screen for HSF1 inhibitors, these investigators identified rocaglamide, a natural product previously known to have potent anti-cancer activity [102]–[104]. Interestingly and in common with rapamycin, it has anti-inflammatory activity [105] and antifungal properties [106]. Rohinitib, a stronger derivative of rocaglamide,

strongly inhibits translation initation [101]. This study also emphasizes the vital role that translation initiation plays in the maintenance of the anabolic state, and the potential of translation initiation for the development of new drugs that target this and aging.

Ribosome elongation also presents an opportunity for development of anti-aging drugs. Ribosome profiling [107)-109)] compares the translation fingerprint of cells, and was used for the development of a unified "model for mTORC1-mediated regulation of mRNA translation" [110]. Using ribosome profiling to study translation elongation in response to proteotoxic stress uncovered an association with ribosome stalling due to reductions of the Hsc70/Hsp70 chaperones [111]. New polypeptide chains need Hsc70/Hsp70 to exit from ribosomes, and small molecule inhibitors of Hsc/Hsp70 are being investigated as anti-cancer agents [112], and might serve to promote increase longevity and improve health span.

mTORC1 also regulates other processes supporting the anabolic program [113]. One of these up regulated programs to support cell growth and proliferation is *de novo* fatty acid and lipid synthesis [7) 14) 114)]. mTORC1 relays anabolic signaling to pro-lipogenic transcription factor SREBP1 [115]. In addition to increasing uptake of glucose needed for anabolism, activated mTORC1 also regulates genes expression supporting the pentose phosphate pathway (PPP) for its oxidative, NADPH-producing branch coordinated through SREBP (reviewed in Ref. 10). Nucleic acid biosynthesis relies on ribose production by the PPP acutely regulated in parallel with the metabolic flux through the *de novo* pyrimidine synthetic pathway regulated by S6K1 (a down-stream substrate of mTORC1)-mediated phosphorylation of enzyme CAD (carbamoyl phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase) [116) 117)]. Translation of hypoxia-inducible factor-1 α (HIF-1 α) represents another branch of regulation by mTORC1 that up-regulates glucose transporters, enzymes for glycolysis, and, promotes a change to aerobic glycolysis (Warburg effect) seen in most growing cancer cells [118]. Evolutionarily conserved notch signaling, which is important in neurodevelopment [119], appears to regulate both glucose and lipid biosynthesis via mTORC1 in liver. All of these points of regulation represent opportunities for new drug targets to positively impact aging and reduce effects of age-related diseases. How chronic inhibition by rapamycin affects these processes is not known. Short-term inhibition by rapamycin or active site (kinase) inhibitors have been studied in some detail.

Ribosome profiling studies [120] revealed that rapamycin or active-site mTOR inhibitors PP242 and clinical grade INK128 have interesting transcript-specific control mediated by anabolic mTORC1 signaling. The question of cell specificity of this response is unknown, with ribosome biogenesis linked to mTOR activation likely involved. These studies also revealed that active site inhibitors of the mTOR kinase are more efficient in generating this response than rapamycin/FKBP12, an allosteric inhibitor. The new generation of ATP-competitive inhibitors, which target the mTOR catalytic site directly, show promise as more effective cancer therapeutic agents [121]. How effective these will be at both cancer prevention and anti-aging remains to be tested.

In summary, numerous nodes of control in the ancillary mTORC1 pathways are viable targets of opportunity for the development of safe and effective drug to intervene in aging and associated diseases. Of course it remains to be determine whether active site inhibitors will be any safer or more effective than the founding drug—rapamycin.

8 Immunosuppression: the 800 lb Gorilla in the Geriatrician's Office

Rapamycin for prophylaxis against age-related diseases requires it to have minimal toxicity in healthy adults. Marketed to prevent organ allograft rejection, rapamycin carries an FDA black box warning for immunosuppression. Clinicians often use rapamycin in combination with other more potent calcineurin inhibitor-based immunosuppressants, meaning that its individual effects in this setting are not well known. We are unaware of published studies in healthy adults showing rapamycin is immune suppressive.

Our studies rigorously documented that rapamycin increases maximum life span of genetically heterogeneous mouse of both sexes in two independent studies conducted in three geographically separate laboratories. It must be recognized, however, that these studies were conducted in specific pathogen free animal colonies, where immunosuppression would be expected to have minimal effect in terms of infectious disease. However, preclinical studies raise the possibility that rapamycin may be functionally less immunosuppressive in terms of infectious agents than would be expected. In a specific examination of the effects

of rapamycin on immunity, Araki et al. [122] found that rapamycin boosts immunity to infections. In a study to address this paradox, Ferrer et al. [123] compared $CD8^+$ T cell responses to a pathogen or to a skin transplant with and without rapamycin. Using a transgenic model in which an identical monoclonal cell population would respond to the same epitope in either an infection or transplant setting, they found that rapamycin had disparate effects depending on the setting. Rapamycin boosted antigen-specific T cell responses to a bacterium, but not to a transplant. In their discussion, the authors to stated "many facets to the mTOR signaling pathway in immune cells that are still poorly understood" [123]. Jagannath et al. [124] showed that rapamycin pretreatment enhances immune function in tuberculosis. Pre-treatment also improved immune function in antitumor vaccine responses in mice [125], influenza [79], and vaccinia vaccine responses in non-human primates [122]. In old mice, pretreatment with eRapa also enhanced resistance to pneumococcal pneumonia through reduced cell senescence [65]. Clinical trials using rapamycin and rapalogs as an FDA approved cancer treatment (reviewed in Ref. 126) is also not consistent with serious immunosuppression, which would increase cancer. It is not likely that rapamycin is immunosuppressive in these studies, in fact reports suggest otherwise [127].

The age-related decline in the immune system negatively effects elderly populations [128] and rapamycin would be contraindicated if it exacerbated this decline. Naïve T cells show age-associated reduction in function through acquisition of functional defects including reduced ability to proliferate, alterations in cytokine secretion and deficits in ability to undergo effector T cell differentiation [129)-131]. Immune surveillance of cancer [132] may be negatively impacted. However, specific interventions can reverse age-associated decline in immunity [133) 134], improving efficacy of immunotherapy [135]. Since mTOR modulates the immune system [122) 136)-140] and aging, could the longevity and cancer prevention effects of chronic eRapa treatment be, in part at least, through immune system modulation? Remarkably, there is little known about the role on immune effects by mTOR inhibition in longevity extension and disease prevention.

Available data, in summary, do not support the expectation that single agent rapamycin in healthy, normal subjects is immunosuppressive. Preclinical data support the seemingly paradoxical concept that it may be an enhancer and/or modulator of immune system [13].

Summary

Based on the evidence that mTOR inhibition enhances important functions, including physical activity, cognition and cardiac function, whose declines reduces quality of life in the elderly, and has minimal negative effects, we believe it is time to investigate the use of mTORC1 inhibitors as prevention agents for aging and its debilitating diseases, especially for people at risk. Evidence suggest that even a small effect on age-related disability would have enormous economic impact and improvement in quality of life – the potential for mTOR intervention as one of the first efforts to target aging at a nodal point of control is strong and thus deserves greater attention.

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Potential Financial Conflict of Interest

ZDS and RS are unpaid consultants to Rapamycin Holdings, Inc.

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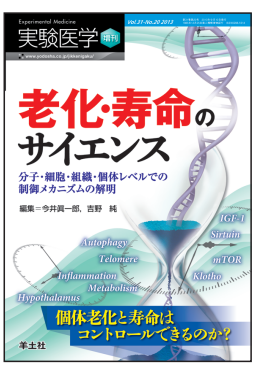

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