Chapter 5

Where and How in the mTOR Pathway Inhibitors Fight Aging: Rapamycin, Resveratrol, and Metformin

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Additional information is available at the end of the chapter

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Abstract

The molecular mechanisms underlying the quality and quantity of life extension appear to sometimes be orthogonal. For example, while resveratrol has continued to prove beneficial in reducing obesity, it has had less efficacy in extending lifespan. On the other hand, rapamycin and the chemically similar rapalogs extend lifespan across genera of life from yeast, to nematodes, to mice. Caloric restriction (CR) and bioavailable small molecules, which mimic a fasted state, upregulate autophagy, catabolism of fats over anabolism of carbohydrates, and decrease oxidative stress and inflammation. CR mimics are currently being investigated to elucidate the best dosage, route of administration, timing in life, where best to inhibit in the mTOR pathway, and effects of long-term use on mTORC1 verse mTORC2 complexes. Comparisons between rapamycin, resveratrol, and metformin targets, downstream pathway effects, dosage, and clinical trials will be discussed.

Keywords: rapamycin, rapalogs, resveratrol, metformin, mTOR, senescence, aging, longevity, autophagy, inflammation

1. Introduction

It has been shown across the animal kingdom that caloric restriction (CR) extends lifespan. It is logistically harder to test this in longer living animals due to the length of studies needed, but there are studies in non-human primates [1] and ongoing human test groups who show fewer signs of cardiovascular aging [2]. Two trials calorically constricting macaques began in the 1980s and initially had conflicting results. A study out of University of Wisconsin found a drastic 30% increased survival in the CR group compared to control [3], while a latter study

by the National Institute on aging (NIA) did not find a statistically relevant effect [4]. It was later found that in the NIA study, control monkeys consumed fewer calories than expected, and some in the CR groups began consuming reduced calories as juveniles, which is known to reduce lifespan. A reanalysis of all data by both groups agreed caloric restriction appears to increase macaque longevity by almost 10% (3 years in macaques which would translate to 9 years in humans) [1].

In general results have seemed positive, extending life, but to a lesser degree than in small animal models, such as mice which have seen up to a 50% increase in lifespan from CR [5]. The search for pharmaceuticals to mimic CR life extension will need to continue the long and expensive process of large human studies due to humans' unique interaction with calories in our post-industrial world. Study designs for larger caloric restriction studies are often questioned. Particularly concerning humans, the ability to accurately track caloric consumption in people living outside a clinical setting has often relied on caloric approximations such as food diaries, pictures of food eaten [6–8], or dietary consumption habits at a national level when comparing between countries. The larger percentage life extension effects from CR has been seen across many simpler organisms with budding yeast, fruit flies and worms having their lifespan increased 2–3 fold. However, no mammals have had such large effects. Indeed no one has suggested humans could achieve such great gains with CR which would extend our current upper lifespan from ~100 years to over 200 years. However, mammals such as rats and mice have shown a 20–50% reduction in calories can result in a lifespan increase of up to 50% [9, 10].

Even with these qualifiers in mind, it seems likely that a 30–60% reduction in calories could extend human life 10–20%. This gain of ~1% of lifetime for every ~3% reduction in calories translates to a likely ~10–20 years of extra life for humans, which is similar to the 9-year human equivalent life extension seen in the recent reanalysis of primates undergoing CR [1]. This 1:3 ratio of %lifetimeExtension:%caloricRestriction (LE:CR) may end up being 1:4 or 1:2 in humans, but in either scenario it is most likely caloric restriction will show a statistically significant life extension in humans. However, it is not likely to be a panacea that would give us 50 extra years bringing us past 150 years-of-age, despite that relative effect in mice.

The current dilemma has been elucidating the root cause(s) responsible for life extension which are being targeted as pharmaceutical targets. The "inputs" that one might measure which lead to an increased lifespan in humans (e.g. obesity, cholesterol, cancer, bone density) are numerous and often orthogonal in nature. For example at pharmacological concentrations resveratrol does inhibit obesity but did not inhibit cellular senescence like rapamycin does [11]. While resveratrol and rapamycin were at times thought to act similarly, their mechanistic and pharmacological issues are diverging. While resveratrol had been found to extend life in studies there have been negative results with some labs failing to find life extension in all strains of yeast [12], worms, and flies [13]. Indeed rapamycin but not resveratrol has been shown to extend lifespan in mice [14]. Resveratrol may increase our quality of life while rapamycin (and rapalogs) could increase our quantity of life. In addition, one of resveratrol's main issues is its bioavailability (it's good we just want more), whereas rapamycin may shut down people's immune system too much leading to cancer (it's good but too much is bad). The mechanism of action for rapamycin, resveratrol, and metformin, as well as animal and human studies will be discussed.

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The ideal "biological scale" at which aging can be targeted is also still in question (a single gene, pathway, salvaging a cell, or killing unrecoverable cells) (**Figure 1**). Single genes continue to be investigated with inhibition by siRNA, conditional knockouts, or reducing posttranslational modifications such as lipid anchoring [15–21], while activation could be investigated via upregulation of transcription factors or viral therapy such as CRISPR. However, due to overlapping inputs the field often addresses how entire pathways are being affected (such as increased mitochondria biogenesis by caloric restriction). In addition while in vitro studies have often looked at modifying a cells genetic profile to have more of a centenarian profile (i.e. to rescue human cells via an intervention), it has recently been shown many cells become senescent and causing those to undergo apoptosis can save other cells thereby resulting in organism longevity [22–28]. The easiest abnormal aging targets may be the overactive cancerous cells we have become use to targeting via single genetic markers (e.g. targeting estrogen receptor sensitivity in breast cancer). Pathways can be targeted via some important individual targets, for example rescuing p53 deficiency or inhibiting mdm2 over activity to cause apoptosis. However rescuing cells from becoming senescent is the hardest and most distant task, required to truly push human longevity beyond a ~125 year limit (**Figure 1**). An important comparison is the case of the hydra which has been pointed to in the last couple of decades as an immortal multicellular organism [29, 30]. The hydra however, has a structure in which stem cells continually differentiate and move the periphery where they fluff off. There is not a large repository of persistent differentiated cells that can never become senescent for their hydra to continue living. In this regard the hydra can be thought of amputating any problem cells which it can replace [31–33]. Many of humanity's growing diseases involve multi-organ

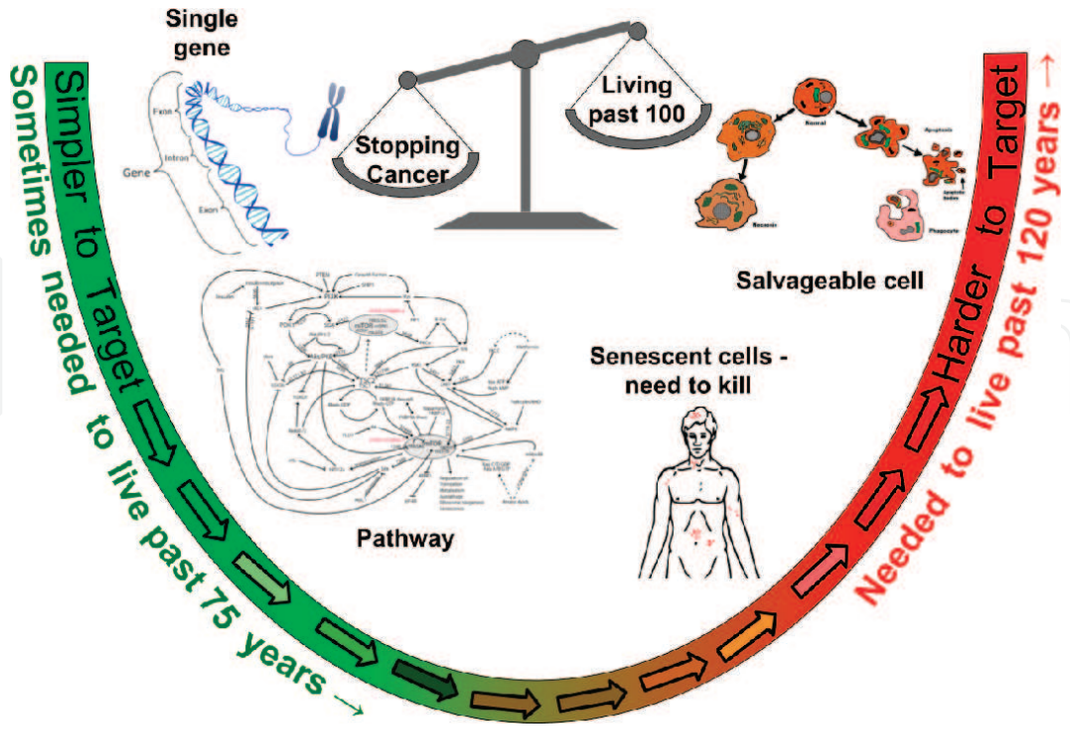

Figure 1. Therapeutics for "aging" will likely having differing levels of complexity in their targets depending on if they are targeting a single gene (easiest), a pathway, cause death of senescent cells, or trying to salvage a cell from becoming senescent (most difficult). Overactive cancerous cells can be targeted simply to kill based on one receptor or gene, while salvaging neurons from death (e.g. various dementias) is a harder therapeutic task.

systems with terminally differentiated cells which cannot be easily replaced. For example, neurodegenerative diseases such as Alzheimer disease (AD) have phenotypic effects when neurons start dying in large numbers. While CR and CR mimics may increase autophagy and delay cell death, as discussed below, there is not evidence that inhibition of the mTOR pathway can perpetually shift humans as an organism to a hydra like state of immortality. Since 1932 the correlation between mass and metabolic rate for mammals has been investigated as a foundation for humans' upper lifespan limit [34, 35]. It could be that the lower molecular activity from CR will shift humans to a longer lifespan following the three-quarters power law (or Kleiber's Law), although more recent studies seem to be elucidating cellular and molecular minutia in a more fine-grained manner than Kleiber's course mass does [36–38].

2. mTOR pathways: rapamycin, resveratrol, and metformin

A wealth of studies has confirmed that rapamycin and rapalogs directly inhibit mTOR, whereas resveratrol's targets are more numerous. Initially resveratrol was thought to act primarily through activation of sirtuins, with sirtuin-1 (SIRT1) known to help reduce obesity [39]. It is now known resveratrol also activates adenylyl cyclase and AMP-activated protein kinase (AMPK), while inhibiting a slew of proteins including lipoxygenase, protein kinase C (PKC), p53, mitogen-activated protein kinase 3 (MAPK3), proto-oncogene tyrosine-protein kinase (Src), signal transducer and activator of transcription 3 (STAT3), and IκB alpha kinase (IKK) [40]. One of the main targets is now AMPK activation which itself activates SIRT1 leading to mTOR inhibition.

2.1. Anabolic vs. catabolic energy production

AMPK is one of the primary metabolic detectors conserved across genera being activated by conditions that cause a low ATP:ADP ratio such as hypoglycemia and hypoxia. Phosphorylation of likely over 1000 targets by AMPK [41] shuts off anabolic pathways (energy-using) and turns on catabolic pathways (energy-generating). One of AMPKs targets for phosphorylation is peroxisome proliferators-activated receptor gamma coactivator-1 alpha (PGC-1α) which becomes active resulting in increased mitochondria biogenesis, membrane potential, and fatty acid oxidation [42], a recurring feature found during caloric restriction [43, 44]. AMPK also activates forkhead transcription factors of the O class (FOXO) which leads to increased autophagy and antioxidants, both leading to increased oxidative metabolism, like PGC-1α does [44].

In the case of life extending interventions dosing becomes very important. Too much of a good thing, can definitely be bad (i.e. cancer), and the molecular mechanism effecting longevity are being elucidated. For example, in *Caenorhabditis elegans*, metformin is found to delay development under well-fed conditions and even reduces life span during starvation [45–47]. The improved mitochondrial function, decreases oxygen consumption needed, which causes a beneficial decrease in reactive oxygen species (ROS) [48, 49]. Mitochondrial biogenesis is controlled differently depending on tissue and disease state. For example, mTOR signaling has been found to increase expression of mitochondrial genes involved in oxidative metabolism,

through PGC-1α and Ying-Yang 1 (YY1). This increased mitochondrial biogenesis in the muscle of healthy individuals, but not in obese individuals perhaps due to decreased insulin sensitivity [50]. Not only is mTORC1 activity cell specific, but it is also concentration dependent being induced and inhibited by low and high levels of ROS respectively [51]. This concentration sensitivity of mTOR is beneficial since it acts as a hub for interdependent pathways, such as mTORs ability to modify both mitochondrial biogenesis and increase autophagy (which helps degrade damaged mitochondria and other organelles). Two models of aging have been established in yeast (*Saccharomyces cerevisiae*): replicative lifespan (RLS) and the chronological lifespan (CLS). RLS measures the number of asymmetric mitotic divisions a cell can undergo before cell cycle arrest and is a valuable model for fibroblasts, lymphocytes, or stem cells in humans [4, 52–54]. CLS in contrast measures how long stationary (G_o) cultures remain viable and is a model for postmitotic cells like neurons or muscle cells [52, 54, 55]. Organ specific analysis of human in vivo studies, while difficult, would help elucidate CR mimics at and upstream of mTOR.

Metformin is a third life extending compound worth contrasting to rapamycin and resveratrol because it inhibits the mitochondrial respiratory chain complex I, leading to decreased ATP:ADP, which activates AMPK [56]. In addition metformin has lots of human data since it is a common oral antidiabetic drug used for overweight people with type 2 diabetes mellitus (T2DM). Metformin inhibits hepatic glucose production, reduces insulin resistance, and has recently been investigated as anti-aging therapeutic. Metformin is currently being investigated for use in various cancers [57, 58]; however, metformin has also been linked to the development of some solid tumors in humans, namely colorectal, breast, and pancreas cancer [59]. Mitochondrial complex I is clearly inhibited by metformin leading to the AMPK dependent activation of TSC2 which inhibits mTOR. AMPK can also directly inactivate mTORC1 complex via phosphorylation of its subunit Raptor. However, it has also been shown that metformin can act in an AMPK independent manner, though that mechanism is less clear but could involve nuclear pore complex (NPC) or late endosome interactions which have been documented [46]. The NPC interaction was found when *C. elegans* ortholog of acyl-CoA dehydrogenase family member 10 (CeACAD10) knockdown was found to have a 3-fold resistance to metformin. CeACAD10 expression was more than doubled by 50 mM metformin, and an unbiased, forward genetic screen found the nuclear pore complex is required for metformin to induce CeACAD10 [47]. That molecular pathway is currently unique to metformin, compared to resveratrol or rapamycin, and while multiple targets have been found for metformin, some pathways overlapping between these three molecules allow a more robust understanding of caloric restrictions possible of life extension mechanisms. Not only mTORC1, but even upstream AMPK, has been shown to be required for the positive effects of all three molecules rapamycin [60], resveratrol [61, 62], and metformin [63, 64]. Metformin's molecular pathway has also been elucidated upstream of AMPK. Metformin interacts with organelle Na1/H1 exchangers (eNHE) and the V-type-ATPase (V-ATPase) which supports the idea of the late endosome/lysosome, which is required by both the AMPK and mTOR pathway, acting as a signaling hub for metabolism [45].

While gross metrics such as weight are often reported in studies and useful to follow, they are not sufficient to investigate the aging phenomenon. For example, mice administered

resveratrol have been found to not lose weight [65, 66]. The degree to which resveratrol mimics caloric restriction (CR) has been shown at a molecular level in mice with changes in gene expression overlapping in the adipose tissue, skeletal muscle, heart, liver, and neocortex. Interestingly, both resveratrol and CR slowed age-related decline in organ function, showing the benefit from resveratrol was not dependent on weight loss [65, 66]. The other side of the caloric coin which is frequently investigated independently of CR is exercise induced caloric deficit. In general CR has more robust life extension properties than an exercise induced caloric deficit. For modern humans it is clear it is extremely difficult to exercise one's way into the same caloric deficit that can be attained through CR. In short, it is harder to run off a fast food meal than to not have the meal in the first place. It has been shown in rodents that increased activity to achieve a 30% relative energy deficit did not extend maximal lifespan but did increase average lifespan [9, 67]. The ability of resveratrol to increase lifespan has varied significantly between studies, but been roughly 40% for yeast, 15% for worms, 30% for fish, and 10% for mice [68].

There are two mTOR multisubunit protein complexes which have been shown to be differentially regulated. mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) share the protein components DEP domain containing mTOR-interacting protein (DEPTOR), mammalian lethal with sec-13 protein 8 (mLST8, also known as GβL), telomere maintenance 2 (telO2), and telO2-Interacting Protein 1 (tti1) (shown as light blue in **Figure 2**). mTORC1 has three core components: mTOR, regulatory-associated protein of mTOR (Raptor), and mammalian lethal with sec-13 protein 8 (mLST8). Whereas mTOR complex 2 (mTORC2) core components share mTOR, mLST8, but also include rapamycin-insensitive companion of TOR (Rictor), and mammalian stress-activated map kinase-interacting protein 1 (mSIN1) (**Figure 2**) [69]. mTORC1 is activated by nutrients and growth factors while being inhibited in low energy cellular states. A known complexity with the mTOR pathway is the difference in response to inhibitors, not only by mTORC1 and mTORC2, but also by tissue. mTORC1 is universally inhibited by rapamycin, whereas mTORC2 needs long term exposure to be inhibited by rapamycin which continues to be investigated. While DEPTOR is known to partially inhibit mTORC1 it may not decrease lipogenesis or inflammation alone, however in conjunction with AKT Serine/Threonine Kinase 1 (AKT) inhibitors can result in both decreases in lipogenesis and inflammation [70]. Combination therapy may be necessary in targeting mTORC1 to attained desired effects.

While multiple targets upstream of mTOR continue to be investigated, well described downstream actions of mTOR help in analysis of in vivo, in vitro, and clinical studies. While the major downstream effect of mTOR activation is anabolic energy production (with inhibitors shifting to catabolic energy production from fat), another significant downstream effect of mTOR activation is increased inflammation. In general people living in the western world live in a state of excess inflammation. Time restricted feeding (TRF) was found to help immune response, reducing systemic low-grade inflammation and age-related chronic diseases linked to immunosenescence, without compromising muscle performance [71]. The reduced inflammation seen in calorically restricted individuals is partially due to an increase in autophagy from CR (see below). The mTOR pathway has been shown to trigger the development of T cells, B cells, and antigen-presenting cells (APC). Indeed resveratrol (found in plants such as

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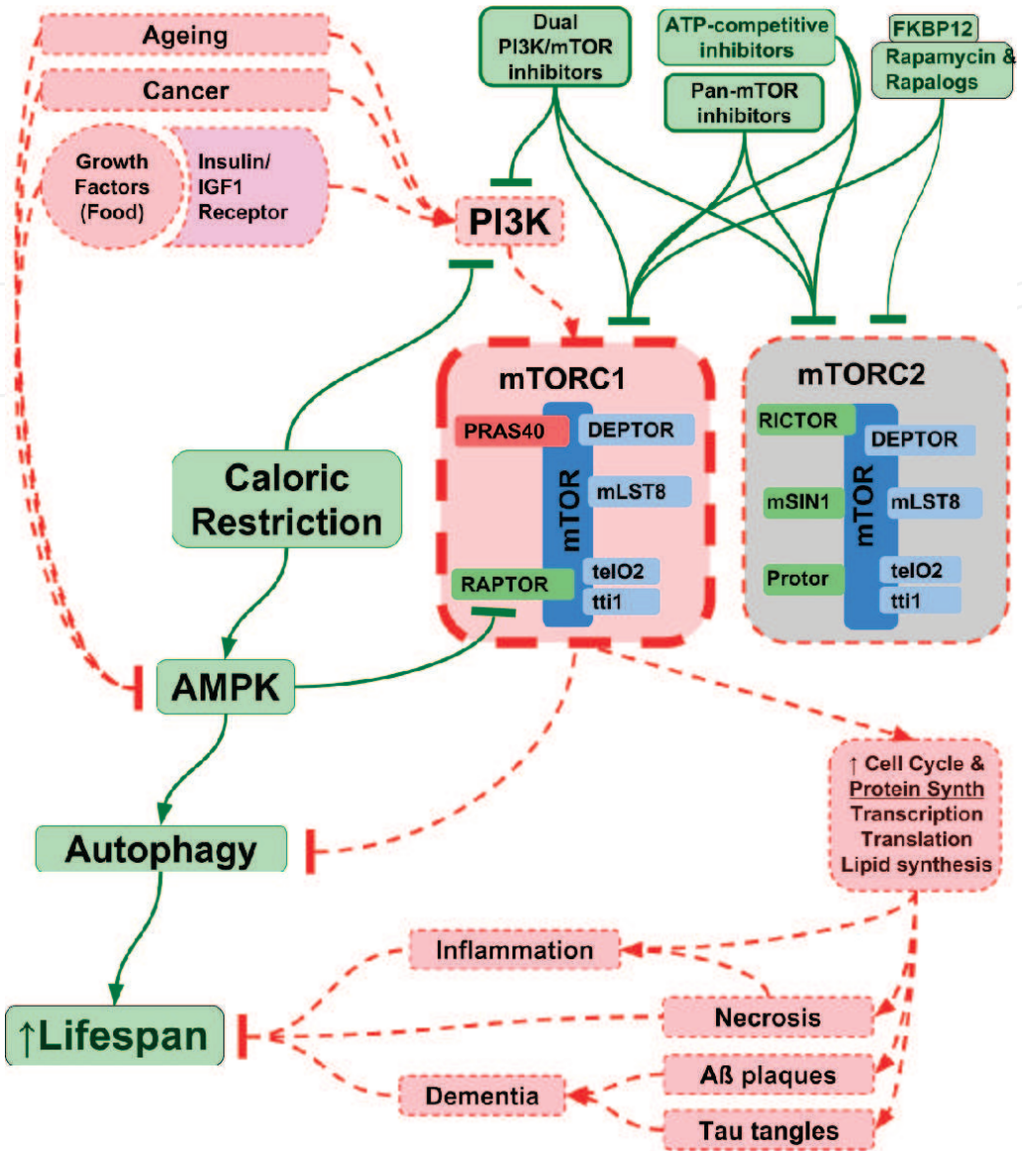

Figure 2. mTORC components, signaling, and inhibitors. Food, old age, and cancer activate PI3K and inactivation of AMPK which cause an increase mTOR activity in both complexes mTORC1 and mTORC2 and decrease the level of cellular autophagy. Autophagy can be restored through mTOR inhibitors (rapalogs, ATP-competitive inhibitors, Pan-mTOR inhibitors, dual PI3K/mTOR inhibitors) or reduced caloric intake (growth signals)-all restore autophagy. Beneficial and deleterious interactions or macromolecules are shown in green and or dashed red respectively. Proteins found in both mTOR1 and mTOR2 are colored blue. *Abbreviations*: AMPK, AMP-activated protein kinase; DEPTOR, DEP domain containing mTOR-interacting protein; mLST8, mammalian lethal with sec-13 protein 8 (also known as GβL); mSin1, mammalian stress-activated map kinase-interacting protein 1; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; PRAS40, proline-rich Akt substrate 40 kDa; protor1/2, protein observed with rictor 1 and 2; RAPTOR, regulatory-associated protein of mammalian target of rapamycin; RICTOR, rapamycin-insensitive companion of mTOR; telO2, telomere maintenance 2; tti1, telO2-interacting protein 1.

grapes, red wine, mulberries, and peanuts) has been described as a broad spectrum of action anti-inflammatory which attenuates microglial cell overactivation through mTOR inhibition [72]. Resveratrol inhibition of NF-κB causes an increase in superoxide dismutase (SOD) and results in decreased proinflammatory cytokines IL-1β, IL-6, and TNF-α [73–76].

Genome wide analysis will likely be needed to elucidate the beneficial molecular level causes of caloric restriction. For example, Dato et al. recently analyzed pathway-based SNP-SNP interactions of 3 pathways: the insulin/insulin-like growth factor signaling (IIS), DNA repair, and pro/antioxidants. Synergistic effects on longevity were found in the combination of growth hormone secretagogue receptor (GHSR) and double strand break repair nuclease MRE11 homolog (MRE11A) genes which are involved in IIS signaling. TP53 also had synergistic effects with either ERCC Excision Repair 2 (ERCC2) or thioredoxin reductase 1 (TXNRD1). Those results highlighted the central role of TP53 in activating DNA repair and pro-antioxidant pathways [77].

2.2. Autophagy

One pathway difference between rapamycin and resveratrol is the large magnitude with which rapamycin increases autophagy over apoptosis, which helps in regards to life extension but could prove problematic in cancer use. Pharmacological levels of resveratrol on the other hand prevent upregulation of Akt activation and autophagy thereby causing apoptosis. Resveratrol does inhibit obesity at pharmacological concentrations, prevent heightened hyperinsulinemia, or inhibit mTOR in vitro and therefore did not inhibit cellular senescence like rapamycin does [11]. Large levels of resveratrol have recently been shown to induce autophagy when inhibiting mTOR directly through ATP competition [78]. Combination therapy of rapamycin and resveratrol has proven synergistic in treatment of breast cancer cells [79, 80].

Caloric restriction has been shown to increase autophagy through inhibition of mTOR and delay molecular events associated with dementia. The rise in neurodegenerative diseases, which are exacerbated by low autophagy levels, heightens interest in mTOR inhibitors. Caloric restriction achieves mTOR inhibition through two pathways: decreased PI3K activity and increased AMPK activity (**Figure 2**). Cells in low energy states (calorically restricted) have low PI3K activity, lowering Akt activity, which then lowers mTORC1 via inhibition by Tsc1/2 (**Figure 3**). Rapamycin directly inhibits mTOR but metformin and resveratrol inhibit mTOR through upstream pathways, inhibiting the mitochondrial complex I activity and increasing AMPK respectively. In the well fed state mTORC1 inhibits autophagy via inhibition of SIRT1, Unc-51 like autophagy activating kinase (ULK1), transcription factor EB/E3 (TFEB/TFE3). Active mTOR also stimulates eukaryotic translation though phosphorylation and inhibition of 4E-BP1 which in turn releases the bound cap-binding eukaryotic translation initiation factor 4E (eif-4E). When eif-4E is released it can participate in forming the eIF4F complex required for initiation of cap-dependent translation. Ribosomal proteins S6 and S6K are also stimulated by mTOR which leads to increased protein synthesis and lipogenesis. In the fasted state ULK1 starts autophagosome maturation and TFEB/TFE3 increases lysosomal biogenesis and autophagy. Ras-related GTPases (Rags) actually tether mTORC1 to the lysosomal surface and that connection is controlled through amino acid sensing of the vacuolar H^+-adenosine triphosphatase ATPase (v-ATPase) as well as the proton-assisted amino acid transporter 1 (PAT1) (**Figure 3**). SIRT1 is also activated in the fasted state, and by CR mimetics, which increases SOD, p53, and activates FOXO leading to increases in cellular autophagy and mitochondrial biogenesis (**Figure 2**).

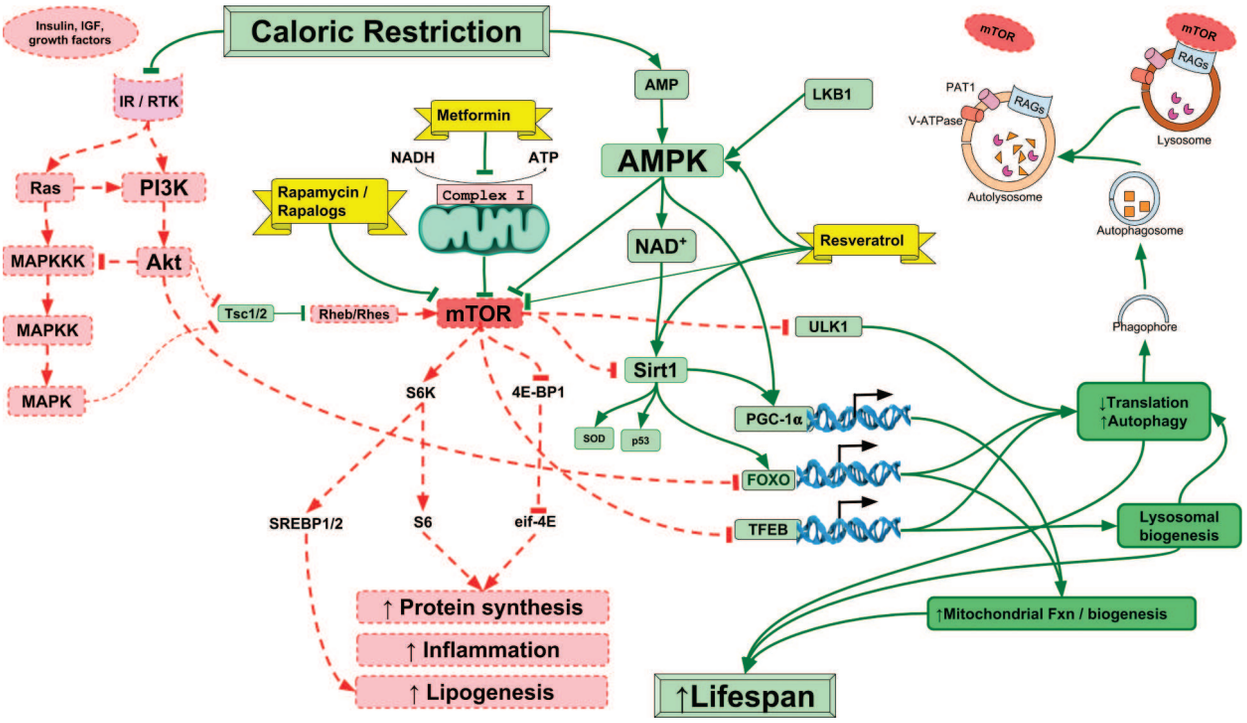

Figure 3. mTOR pathway activation, inhibitors, and downstream effects. The molecular pathways increased by caloric restriction that increase lifespan are also targeted by rapamycin, resveratrol, and metformin. Metformin and resveratrol inhibit mTOR through upstream pathways, inhibiting the mitochondrial complex I activity and increasing AMPK respectively. Rapamycin, and rapalogs, on the other hand inhibit mTOR directly. Beneficial downstream effects of mTOR inhibition include increase mitochondrial function and biogenesis, lysosomal biogenesis, autophagy, and decreased translation. Deleterious effects of an active mTOR pathway include increased lipogenesis and inflammation. Beneficial and deleterious interactions or macromolecules are shown in green and or dashed red respectively. *Abbreviations*: AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; SOD, superoxide dismutase; PGC-1**α**, peroxisome proliferators-activated receptor gamma coactivator-1 alpha; IR, insulin receptor; RTK, receptor tyrosine kinase; Ras; MAPKKK, mitogen-activated protein kinase kinase kinase; MAPKK, mitogen-activated protein kinase kinase; MAPK, mitogen-activated protein kinase; PI3K; Akt = AKT Serine/Threonine Kinase 1; S6, ribosomal protein S6; S6K, ribosomal protein S6 kinase beta-1; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; eif-4E, eukaryotic translation initiation factor 4E; SREBP1/2, sterol regulatory element-binding protein; Rheb/Rhes; Tsc1/2, tuberous sclerosis proteins; PAT1, proton-assisted amino acid transporter 1; V-ATPase, vacuolar H^+-adenosine triphosphatase ATPase; RAGs, Ras-related GTPases; ULK1, Unc-51 like autophagy activating kinase; TFEB/TFE3, transcription factor EB/E3. Ras, Ras superfamily (RAt Sarcoma); PI3K, Phosphoinositide 3-Kinase Gamma; Rheb, Ras homologue enriched in brain; Rhes, Ras homolog enriched in striatum.

Therapeutics to help extend human lifespan far past the ~100 year limit will likely need to increase autophagy to avoid dementias, a later life disease state. With various dementias (PD, ALS, HD, and AD) having mitochondrial dysfunction [81–84], and mTOR activation known to increase oxidative stress, antioxidant therapies are being investigated. It has been found conjugating a cation compound to the antioxidant increases uptake into the mitochondria 80-fold and potency up to 800-fold [85] due to its 165 mV negative potential [86]. Low levels of autophagy also result in necrosis instead of apoptosis, with the resulting ramped up immune system increasing inflammation. Intracellular stress acts through Bcl-2 to open the mitochondrial permeability transition pore (mPTP) leading to caspase dependent intrinsic apoptosis [69, 87, 88]. The mPTP is known to exist in 3 states: closed, transiently open in low conductance, and permanently open in high conductance [89–91], the latter resulting in mitochondrial depolarization, loss of ATP production, and caspase independent necrosis since the

Lifestyle	♀	♂
Physical activity (≥30 min/day)	8	7
Not smoking	9	12
Healthy diet	5	4
Low alcohol (15♀, 30♂ g/d = 2♀, 4♂ drinks/d)	3	2
BMI (18–25 kg/m^2)	4	5
Extra years if all 5	14	12

Five healthy lifestyles (exercise, healthy diet, ideal BMI, low alcohol, and not smoking) were found to add 12–14 years of life starting at age 50 when compared to people that did not follow any of the five lifestyles. The healthiest and worst habits within each lifestyle had very different life expectancies as well, with nonsmokers and excessive smokers having the largest lifespan gap (9–12 years). The second greatest gain in lifespan (7–8 years) came from getting more than 30 min of exercise a day compared to never exercising. Data from [94].

Table 1. Five healthy lifestyles that extend lifespan more than 10 years.

controlled apoptotic pathway requires energy [92]. Multiple types of cancer show increased mTOR pathway signaling which is what the first mTOR inhibitors were FDA approved for: sirolimus, everolimus (Afinitor), temsirolimus (Torisel), and ridaforolimus, with sirolimus and everolimus also finding use as immunosuppressants after organ transplants [93].

While caloric mimics will the not be the panacea pushing human life past 200 years it should be pointed out the large effect it could have in humans compared to other currently measurable lifestyle interventions. The effect was recently quantified for the top five frequent lifestyle interventions: smoking cessation, physical activity, healthy diet, healthy BMI, and low alcohol consumption (**Table 1**) [94]. Starting at age 50 women and men were found to be able to add on average 14 and 12 years respectively, if all 5 healthy lifestyles were adopted. Never smoking was the strongest healthy habit of the five, with a close second being engaging in physical activity over 30 min a day (which included brisk walking or anything more strenuous). The healthy diet and BMI (18–25 kg/m^2) can both clearly be linked to a CR lifestyle. It will be interesting to compare the magnitude of CR life extension to the years gained by aspects of a "healthy diet" which is usually cataloged by many more variables than just caloric count (e.g. vitamin/antioxidants, omega-3 vs. omega-6 vs. saturated fat content). In summary, CR alone seems likely to have as big, or slightly larger, of an effect than the 5 healthy lifestyles in concert. If rapamycin, resveratrol, metformin, or a combination thereof, prove capable of reproducing even half the years of life extension that CR extension can, it would be a multi-billion dollar market (USD) and could be among the best therapeutics measured on the Quality Adjusted Life Years (QALY) scale.

3. Preclinical and clinical studies

While there are chemically similar caloric restriction (CR) mimetics such as rapalogs for rapamycin, the main compounds discussed: resveratrol, rapamycin, and metformin are

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chemically distinct. Both resveratrol and metformin are hydrogen-donor rich, having hydroxyls and amides, respectively. Rapamycin is a much larger macrocycle molecule (MW = 914) with both hydrogen donor and acceptor moieties compared to the smaller resveratrol (MW = 228) and rapamycin (MW = 129) (**Figure 4**). Future docking, crystallography, and NMR studies would be interesting to determine if other molecules could mimic ATP, directly binding to the ATP pocket on mTOR as it has been suggested resveratrol does [78]. A structural mimic of ATP acting as an antagonist can seem conceptually attractive and likely have broad effects on multiple energy sensing proteins, but would also likely have lower than desired specificity.

All three compounds resveratrol, rapamycin, and metformin have had numerous human clinical trials. Metformin is unique among the three in that it is currently an approved and recommended therapy for a massive population, specifically obese individuals with type II diabetes, and therefore has a much larger dataset of patients to pull safety and efficacy information from. Resveratrol and rapamycin are both natural compounds with a plethora of academic papers in animal models, but rapamycin studies have the added nuance/diversity of involving a host of rapalogs with modified activity. A search of clinical trials including the keywords resveratrol, metformin and rapamycin and grouped by topic is shown in **Table 2** (as of April 11th 2018). Resveratrol has the lowest number of ongoing clinical trials (137), metformin has over 2.5 fold as many (359), and rapamycin has almost fivefold ongoing clinical trials (646). Resveratrol and metformin have largely overlapping pathway targets in clinical trials with the most common being endocrine system diseases, diabetes mellitus, obesity, and insulin resistance. The main topics for rapamycin are neoplasms by histological type, vascular

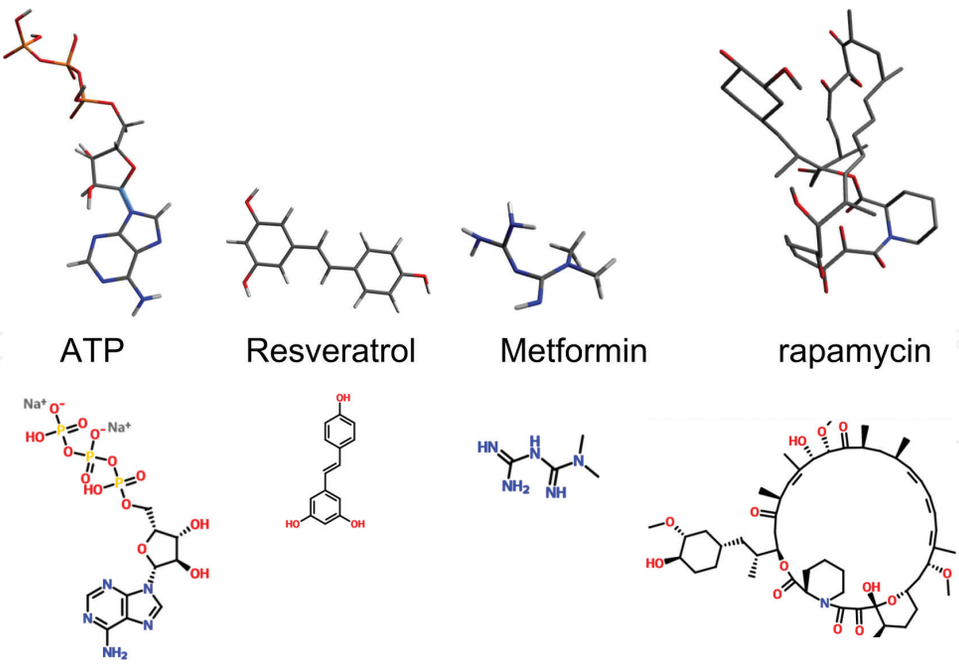

Figure 4. Structures of metabolism modifiers. The structures of compounds discussed: resveratrol, metformin, rapamycin, and ATP. Some have had suggested competitive binding pockets, such as resveratrol and ATP. However on a small molecule scale metformin, resveratrol, and rapamycin have very different complexity and differ by orders of magnitude in molecular weight. All compounds do have significant number of polar groups for hydrogen binding to protein surfaces and pockets.

Compound term search at clinicaltrials.gov	# CT ongoing	Insulin resistance	Diabetes mellitus	Endocrine system diseases	Obesity	Neoplasms by histologic type	Vascular diseases	Myocardial ischemia
Resveratrol	137	28	24	23	19	5	6	1
Metformin	359	34	178	195	34	45	14	5
Rapamycin	646	1	12	42	2	218	172	143

Clinical trials that are ongoing, as of April 11th 2018, were searched for the keywords resveratrol, metformin, and rapamycin. Resveratrol and metformin had overlapping metabolic clinical targets listed, while rapamycin had more numerous trials, which were focused on vascular diseases and cancer.

Table 2. Ongoing clinical trials for resveratrol, rapamycin, or metformin (April 11th 2018 search of clinicaltrials.gov).

disease, and myocardial ischemia. Metformin and rapamycin have some overlap, e.g. metformin has 45 current trials listed under neoplasms by histological type, and rapamycin has 42 trials listed under endocrine system diseases.

4. Conclusions

The use of therapeutics that mimic caloric restriction (CR) is likely to increase and add incremental quality-adjusted life years (QALYs). Natural CR compounds, and analogs based off of them, are fairly cheap with low side effects. Controlled animal studies will likely continue to be the avenue which exposes the degree to which molecular pathways are responsible for the increased quality and quantity of life. Resveratrol and metformin seem robust at increasing molecular pathways linked to quality of health and are useful to combat obesity and type II diabetes; while their ability to increase maximum lifespan remains in question. Data suggests rapamycin and the follow on rapalogs could add years to a human lifespan, although the magnitude of the effect could be enhanced or completely ablated based on accompanying lifestyle choices (diet, exercise, sleep).

Research shedding light on the optimum dosing of caloric mimics should be interesting to follow. Caloric restriction studies in humans fall into three categories: continual modest decrease in calories consumed (~1500 kcal/day), temporary drastic reduction in energy intake (~500 kcal/day), or intermittent fasting (0 kcal/day) in which only water is consumed for 1–3 days. Intermittent fasting has actually slightly outperformed all other methods of dieting methods (atkins, zone, weight watchers, ornish/vegan) in reducing weight in humans, which is partially due to increased compliance [95, 96]. The degree to which molecular pathway changes from intermittent fasting are responsible for reduced weight, such as increased autophagy, remains to be determined. The number of calories that can be consumed above complete fasting, while still increasing autophagy and decreasing inflammation, needs further investigation [1, 10, 44, 97–108].

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5. Recommendations

It would be very useful for clinicians and patients to have a curve in which the x-axis showed calories consumed per day and the y-axis showed %change in these important life extension pathways (e.g. autophagy, inflammation, lipogenesis, lysosomal biogenesis, and protein synthesis). These studies/curves would ideally be done separately for various groups (e.g. males, females, diabetics, elderly predementia, and elderly with early dementia). The interaction of therapeutics that mimic CR in combination with a changing intake of calories from fluctuating diet will require significant large studies and clear simplifications for clinicians and patients to utilize that information and make actionable in daily life. The actionable timeline for CR mimics is still being investigated, but if studies from intermittent fasting apply then administration for months could be useful but lifetime use will be needed to maximize benefits.

Abbreviations

4E-BP1	eukaryotic translation initiation factor 4E-binding protein 1
AD	Alzheimer disease
ALS	amyotrophic lateral sclerosis
AKT	AKT Serine/Threonine Kinase 1
AMPK	AMP-activated protein kinase
APC	antigen-presenting cells
CeACAD10	*C. elegans* ortholog of acyl-CoA dehydrogenase family member 10
CLS	chronological life span
CR	caloric restriction
DEPTOR	DEP domain containing mTOR-interacting protein
eif-4E	eukaryotic translation initiation factor 4E
eNHE	Na1/H1 exchangers
ERCC2	ERCC Excision Repair 2 (ERCC2)
FOXO	forkhead transcription factors of the O class
GHSR	growth hormone secretagogue receptor
HD	Huntington disease
IIS	insulin/insulin-like growth factor signaling

IKK	IκB alpha kinase
MAPK3	mitogen-activated protein kinase 3
mLST8	mammalian lethal with sec-13 protein 8
mPTP	mitochondrial permeability transition pore
MRE11A	double strand break repair nuclease MRE11
mSIN1	mammalian stress-activated map kinase-interacting protein 1
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
NIA	National Institute on aging
NPC	nuclear pore complex
PAT1	proton-assisted amino acid transporter 1
PD	Parkinson disease
PGC-1α	proliferators-activated receptor gamma coactivator-1 alpha
PKC	protein kinase C
QALY	Quality Adjusted Life Years
Rags	Ras-related GTPases
Raptor	regulatory-associated protein of mTOR
Rictor	rapamycin-insensitive companion of TOR
RLS	replicative lifespan
ROS	reactive oxygen species
SIRT1	sirtuin-1
SOD	superoxide dismutase
Src	proto-oncogene tyrosine-protein kinase
STAT3	signal transducer and activator of transcription 3
T2DM	type 2 diabetes mellitus
telO2	telomere maintenance 2
TFEB/TFE3	transcription factor EB/E3
tti1	telO2-interacting protein 1
TXNRD1	thioredoxin reductase

ULK1	Unc-51 like autophagy activating kinase
v-ATPase	vacuolar H^+-adenosine triphosphatase ATPase
V-ATPase	vacuolar (H+)-ATPase
YY1	Ying-Yang 1

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