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An anti-aging drug today: from senescence-promoting genes to anti-aging pill

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Numerous mutations increase lifespan in diverse organisms from worms to mammals. Most genes that affect longevity encode components of the target of rapamycin (TOR) pathway, thus revealing potential targets for pharmacological intervention. I propose that one target, TOR itself, stands out, simply because its inhibitor (rapamycin) is a non-toxic, well-tolerated drug that is suitable for everyday oral administration. Preclinical and clinical data indicate that rapamycin is a promising drug for age-related diseases and seems to have anti-tumor, bone-sparing and calorie-restriction-mimicking 'side-effects'. I also discuss other potential anti-aging agents (calorie restriction, metformin, resveratrol and sirtuins) and their targets, interference with the TOR pathway and combination with antioxidants.

Introduction

The lifespan of multicellular organisms can be extended genetically. From worms to mammals, mutations that inactivate specific signaling pathways can increase lifespan up to 2–3-fold [1–3]. If genetic inhibition (knockout of genes) extends lifespan, then it follows that pharmacological inhibition of the same genes should also prolong life. It is, however, a long way from genes to anti-aging drugs.

First, although numerous genetic mutations that prolong lifespan have been identified, none of them abrogates senescence completely [4]. As suggested by Kirkwood [4], the key 'senescence-promoting' gene either has not been identified or does not exist. Here I discuss the idea that, in fact, the key senescence-promoting gene seems to exist and is well known. According to the trade-off theory, the key senescence-promoting gene must be antagonistically pleiotropic: in other words, damaging in late life but essential early in life during organism growth and development. Complete genetic knockout of such a gene would cause lethality during development. The list of genes known to regulate longevity might therefore not include the key senescence-promoting gene itself but only those genes that affect it or function downstream of it. An ideal anti-aging drug, however, must inhibit the key target.

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Second, not only does a key target need to be identified but also a drug must be created. Most current anti-aging approaches are related to potential agents that would activate Sir2 (also known as SIRT1 or sirtuin 1), FOXO transcription factors and heat shock proteins to increase stress resistance. Potential drugs should not be toxic to healthy individuals. Third, clinical trials of anti-aging drugs might require almost a lifetime to determine effects on human aging. Thus, there is a long path from identifying a gene involved in aging to developing a clinically useful drug.

Fortunately, there are ways to circumvent these challenges. If we can identify an existing drug that inhibits the key senescence-promoting pathway, then such a drug could be used today. Thus, the problem has two unknowns: the key target and an existing drug that targets this target.

Vast amounts of data in the literature (from cell cycle regulation to clinical ophthalmology including gerontology itself) provide an opportunity for analytical research. To reveal the anti-aging drug, here I bring together three independent fields: (i) cell senescence; (ii) genetic control of longevity (from yeast to mammals); and (iii) human age-related diseases. If their juxtaposition helps to reveal the key target, then it should be possible to determine whether a currently available drug will affect this target. If so, the effects that these drugs have on longevity (if available) and age-related diseases (such as cardiovascular and neurodegenerative diseases and cancer) can be analyzed. Lastly, I also examine the side-effects of drugs

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.01.004

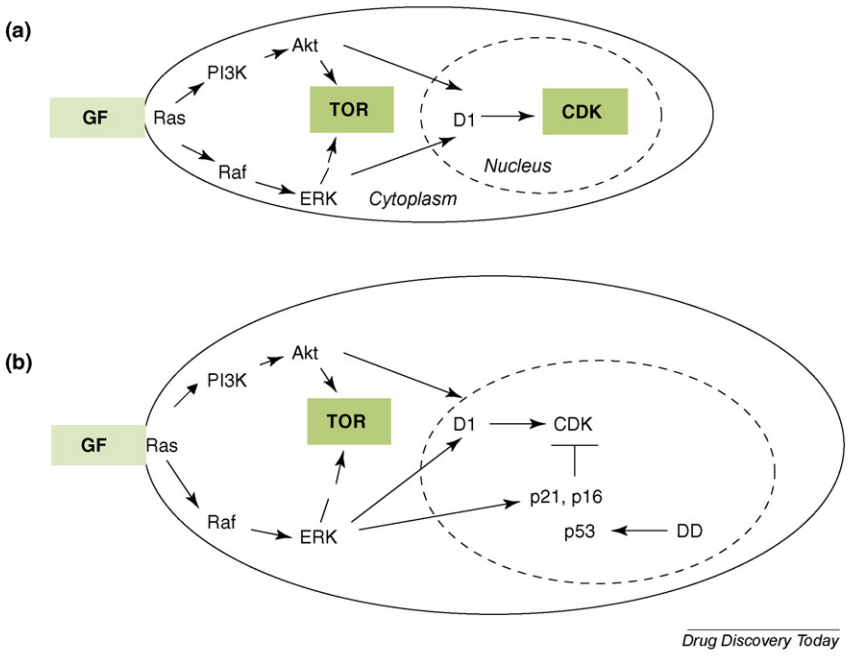

FIGURE 1

Mitogen-activated signaling, cell growth and cell cycle pathways. **(a)** Cell growth and cell cycle progression. Mitogens and growth factors (GF) activate the Ras/PI3K/Akt and Raf-1/MEK/ERK signaling pathways. Both pathways activate TOR through Tsc1. (The effects of MEK/ERK are indirect: MEK and ERK activate the pathway upstream and downstream of TOR). In turn, TOR stimulates protein synthesis and cell size growth. In addition, the Raf-1/MEK/ERK and PI3K/Akt pathways induce cyclin D1 (D1) expression, thereby initiating cell cycle progression. Thus, mitogens and growth factors stimulate both cell growth through TOR, and cell cycle progression through cyclin D and cyclin-dependent kinase (CDK). CDK drives the cell cycle and cell proliferation. Thus, cell growth is balanced by cell division. **(b)** Cell cycle arrest without growth arrest. Strong activation of Ras/Raf-1/MEK/ERK and other mitogen-stimulated pathways can induce p53, p21 and p16, inhibiting CDK and blocking the cell cycle. In addition, DNA damage (DD) and other inducers of p53, p21 and p16 block the cell cycle. When the cell cycle is blocked, the activated TOR pathway causes cell hypertrophy (size growth without cell division). Note that growth factor withdrawal causes cell quiescence when neither the mitogenic pathways nor TOR or CDK is activated (not shown).

because some side-effects might be a manifestation of anti-aging effects.

Cell senescence

First, I will analyze cellular senescence. The most noticeable feature of cell senescence is cell cycle arrest, thereby highlighting telomeres, p53, p16 and p21. But quiescence (arrest in G0) is also cell cycle arrest; thus, the question is what, beyond cell cycle arrest, is the difference between cell quiescence and cell senescence?

Much evidence and reasoning support the hypothesis that the activation of growth-promoting pathways, when the cell cycle is blocked, is a hallmark of cell senescence. Normally, mitogens (growth factors) activate the Raf-1/MEK/ERK and phosphatidylinositol 3-kinase (PI3K)/Akt kinase signaling pathways (Figure 1). In turn, these pathways activate TOR, stimulating protein synthesis and cell growth (both mass and size) [5,6]. In addition, the Raf-1/MEK/ERK and PI3K/Akt pathways stimulate expression of cyclin D1, thereby initiating cell cycle progression [7]. Thus, cell growth is balanced by cell division. Quiescence (G0 arrest) due to mitogen withdrawal is associated with lack of activation of growth-promoting pathways and with cell cycle arrest [8]. This is a perfect balance: no mass growth, no cell divisions. In cell senescence, by contrast, the cell cycle is blocked but growth-promoting pathways are not (see Ref. [8]). For example, oncogenic Ras, Raf-1, B-Raf, MEK, Akt and PI3K activate the TOR pathway, but (by inducing p21, p16 and p53) can cause cycle arrest leading to cell senescence [8,9]. DNA damage, which causes accelerated cell senescence, arrests the cell cycle but does not prevent cell growth [8].

Because it is TOR that stimulates cell growth, it is possible that TOR activity is necessary for acquiring a senescent phenotype. Indeed, lack of TOR activity prevents p21-induced and DNA-damage-induced cell senescence (M.V.B. *et al.*, unpublished data). In addition, eIF-4E, a downstream effector of TOR, induces cell senescence [10]. The effects of TOR and the senescent cell phenotype look intriguingly similar. TOR stimulates peroxisome proliferator-activated receptor-γ (PPAR-γ), rDNA transcription, ribosome biogenesis, secretion of mitogens and vascular endothelial growth factor (VEGF), changes in cell morphology, protein synthesis and cell growth [6]. The senescent cell has a large cell morphology and secretes proteases, matrix, mitogens, VEGF and other biologically active molecules [11,12]. Senescent cells are hypertrophic [8,12]. Cellular hypertrophy can in theory be connected to hallmarks of aging (e.g. skin wrinkles and prostate enlargement in men) and diseases of aging (e.g. atherosclerotic plaques).

Although the link between TOR and cell senescence needs further investigation, it is sufficiently evident for our purpose: namely, to spot TOR among numerous genes that affect lifespan. With this in mind, what is the evidence that TOR controls animal longevity?

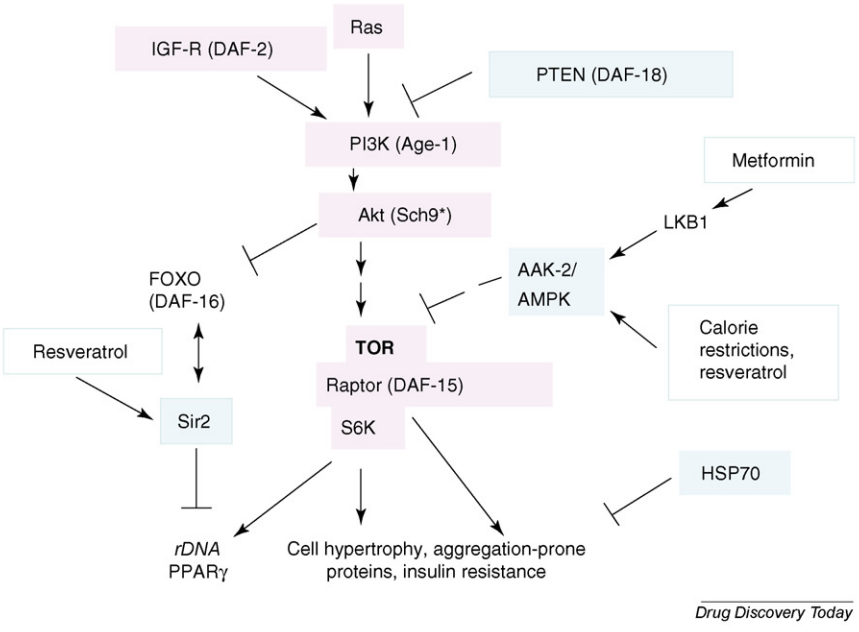

FIGURE 2

Pathways that affect longevity merge on TOR. Genes that increase lifespan when inactivated (genes for aging) are shown in pink, whereas those that decrease lifespan when inactivated (genes for longevity) are shown in blue. *C. elegans* orthologs of mammalian genes are shown in parentheses (but note that Sch9 is a yeast ortholog). Calorific restriction and metformin affect TOR through multistep pathways. Sir2 and Hsp70 antagonize downstream effects of TOR: namely, Hsp70 prevents toxicities of aggregation-prone proteins that are instrumental in neurodegenerative diseases; Sir2 antagonizes rDNA transcription, thereby antagonizing downstream effects of TOR, and its mammalian ortholog SIRT1 antagonizes PPAR-γ. Numerous members of the FOXO family have different and perhaps opposing roles in different species. For simplicity, only one link between FOXO/Sir2 and the TOR pathway is shown. TORC2 phosphorylates Akt and regulates FOXO in mammals [61] (not shown).

Is TOR among genes whose mutations prolong lifespan?

In yeast, two TOR proteins are expressed: TOR1 and TOR2. Deletion of TOR1 extends lifespan, deletion of both TOR genes or only TOR2 is lethal [13]. Higher eukaryotes have only one TOR gene, which is essential for developmental growth. Disruption of the mouse TOR gene leads to early postimplantation lethality [14,15]. This outcome is exactly what might be expected for a key gene that promotes aging: that is, complete loss of the gene is lethal early in life. Nevertheless, genes that affect lifespan encode components of the TOR pathway (conserved from yeast to mice) and can be arranged into the TOR pathway [13,16–18]. They modulate the TOR pathway either upstream (insulin, PTEN, Akt) or downstream (S6K, Sir2, Hsp70) of TOR itself (Figure 2). In the same way that Mendeleev's periodic table predicted new chemical elements, the aging pathway might predict 'missed mutations' and their effects on longevity, depending on whether they activate or antagonize TOR. In this section I discuss the aging-promoting pathway in different species because, unlike TOR, other regulators (FOXO factors and Sir2) show considerable evolutionary differences.

In *Saccharomyces cerevisiae* (yeast), inhibition of TOR1 signaling slows down senescence and increases organism longevity [18]. Mutations in members of the TOR pathway (e.g. Ras, PI3K and Sch9) increase replicative lifespan [13]. Sir2, which inhibits rDNA transcription (thereby antagonizing TOR downstream), increases lifespan [19]. Lastly, rapamycin, a pharmacological inhibitor of TOR, increases lifespan [18].

In *Caenorhabditis elegans*, mutations in the genes encoding TOR and DAF-15 (also known as raptor) extend lifespan. DAF-15 forms a functional complex with TOR known as TOR complex 1 (TORC1) [6]. Expression of DAF-15 is transcriptionally regulated by DAF-16, a FOXO transcription factor that in turn is regulated by DAF-2 – also known as insulin/insulin growth factor-1 (IGF-1) receptor [20]. DAF-2 stimulates TOR (Figure 2). Mutations in DAF-2 and Age-1 (ortholog of mammalian PI3K) extend the lifespan of *C. elegans* [21]. Mutation in DAF-18 (a homolog of the PTEN tumor suppressor) can suppress the phenotype caused by inactivation of DAF-2 or Age-1 (PI3K). Thus, genetic defects that inhibit the TOR pathway (either upstream or downstream of TOR) increase lifespan in *C. elegans* [20,22,23]. Because Sir2 is a cofactor for DAF-16 [24], Sir2 and DAF-16 together might antagonize effects of the downstream TOR pathway (Figure 2).

In *Drosophila*, genetic inhibition of the TOR pathway, either by dominant-negative forms of TOR or by mutations in components upstream (Tsc1 and Tsc2) or downstream (S6K) of TOR, extends lifespan [16]. In *Drosophila*, activation of TOR causes neurodegeneration. Rapamycin protects against neurodegeneration in a fly model of Huntington disease [25]. Reduction in TOR function protects against age-dependent decline in heart function and increases longevity [26].

In genetic mouse models, decreased signaling by IGF-1 (upstream of TOR) increases lifespan [3]. Owing to decreased levels of IGF-1, TOR activity is reduced in long-lived mice [17]. In agreement, mice deficient in S6K1 (an effector of TOR) are

hypersensitive to insulin and are protected against age- and diet-induced obesity [27].

In humans, reduced insulin/IGF-1 signaling and increased sensitivity to insulin are associated with extreme longevity [28,29]. Insulin sensitivity is a marker of genetically reduced TOR activity, because TOR causes insulin resistance by signaling through S6K [27].

Calorie restriction

The diet known as 'calorie restriction' increases insulin sensitivity. Calorie restriction increases lifespan in all animals tested including mammals [1,2,19]. There are at least three mechanisms for the life-prolonging effect of calorie restriction.

First, the nutrient-sensing TOR pathway is an obvious candidate because nutrients (amino acids and glucose) activate the TOR pathway [13,16,20]. In *Drosophila*, it has been suggested that dietary restrictions extend lifespan by inhibiting the TOR pathway [16]. In mammals, calorie restriction or 'fasting' is associated with fat mobilization from adipose tissue (lipolysis), leading to weight loss. Lipolysis is caused by increased activity of adipose-tissue lipase, driving lipids from the fat tissue into the blood, and by decreased activity of lipoprotein lipase, preventing accumulation of lipids in fat tissue. As discussed below, pharmacological inhibition of TOR in humans causes lipolysis by a similar mechanism.

Second, it has been suggested that calorie restriction extends lifespan by inducing Sir2 [19]. Third, calorie restriction might affect the production of free radicals or reactive oxygen species (ROS). In the last case, it would be expected that antioxidants will also extend lifespan.

Antioxidants

Despite recent advances in genetic control of longevity, the free radical theory remains the mainstream theory of aging. It is generally assumed (albeit not scientifically proven) that all mutations that affect lifespan also affect ROS. However, lifespan can be extended markedly without, for example, reducing ROS production [30].

There are several theoretical and practical problems with the ROS theory that are beyond the scope of this review. Importantly, antioxidants have failed in clinical trials [31]. At least 15 clinical trials (testing tocopherol, β-carotine, vitamins C and E, retinol, folic acid) have been conducted on more than 500 000 humans [31]: none has shown a statistical decrease in age-related diseases and/or mortality, and some trials have been stopped early owing to an increased incidence of disease and mortality [31]. I can suggest at least three reasons for such failures. First, ROS might be irrelevant to aging and age-related diseases (i.e. the ROS theory is totally wrong). Second, current antioxidants are not capable of inhibiting ROS sufficiently and in addition exert independent side-effects. Third, free radicals do not have role in animal and human longevity because animals first die of other causes controlled by the genetic (TOR) pathway (Figure 2). If TOR-driven aging can be pharmacologically inhibited, then antioxidants might prolong lifespan further.

Metformin

By causing insulin resistance, TOR might have a role in the development of type II (age-related) diabetes [6]. Metformin is an anti-diabetic agent that increases insulin sensitivity. Notably, metformin and its analog phenformin extend lifespan in rodents [32,33]. Is there a connection between metformin and the TOR pathway? It has been recently shown that metformin activates the LKB1/AMPK kinase pathway, thereby inhibiting TOR (Figure 2) [34]. Inhibition of TOR restores insulin sensitivity, thus explaining the anti-diabetic effects of metformin. I speculate that the anti-aging effects of metformin also result from inhibition of TOR.

Resveratrol

Resveratrol, a polyphenol found in red wine, extends lifespan in yeast, fish and mice [35–37]. Although the exact target of resveratrol remains elusive, there are at least three possible mechanisms of action. First, resveratrol might be an antioxidant. Second, resveratrol activates SIRT1 [35,37]. Third, resveratrol suppresses mitogen-induced phosphorylation of S6K (a target of TOR) and subsequent cell hypertrophy [38], which is exactly what rapamycin does. Perhaps resveratrol is an indirect inhibitor of the TOR pathway. Indeed, similar to metformin, resveratrol activates the kinase AMPK [34,37,39]; and induces PPAR-γ coactivator 1α (PGC-1α) [37,40]. It has been suggested that resveratrol induces PGC-1α by activating SIRT1 [37]. Alternatively, AMPK is known to activate PGC-1α. In fact, both resveratrol and metformin activate AMPK and induce PGC-1α [40]. Thus, I suggest that resveratrol causes anti-aging affects by inhibiting the TOR pathway. This speculation warrants further investigations.

Sirtuin activators

It has been suggested that calorie restriction extends the replicative lifespan of yeast by increasing the activity of Sir2, a member of the conserved sirtuin family of NAD(+)-dependent protein deacetylases that includes SIRT1, a human deacetylase. Drug screening has revealed three classes of small molecules that activate sirtuins [35]. The effects of Sir2 on chronological lifespan are, however, are opposite to those on replicative lifespan and suggest that the relevant activities of Sir2-like deacetylases might be also complex in higher eukaryotes [41].

Indeed, humans contain nine sirtuins. SIRT1 seems to antagonize the TOR pathway downstream of TOR. In fact, TOR activates the fat regulator PPAR-γ, which is essential for adipocyte differentiation and fat accumulation [42]. By contrast, SIRT1 represses genes controlled by PPAR-γ, thus antagonizing TOR downstream [43]. In addition, whereas TOR induces rDNA transcription, SIRT1 silences it. Thus, a model emerges in which SIRT1, metformin, calorie restriction and resveratrol all indirectly antagonize TOR signaling, thereby increasing lifespan. Could we design a direct inhibitor of TOR? Fortunately, we do not need to 'discover' the first inhibitor of TOR, because TOR itself was first identified as the target of rapamycin.

Rapamycin

Rapamycin is an antifungal antibiotic – a natural product that, in low nanomolar concentrations, selectively and effectively inhibits TOR. Because TOR is evolutionarily conserved, rapamycin inhibits TOR in both yeast and human cells. Moreover, TOR is antagonistically pleiotropic; thus, rapamycin inhibits yeast growth, while decelerating yeast senescence. Similarly, TOR is essential for the

developmental growth but might be dispensable later in life. If so, rapamycin must be well-tolerated in humans.

Indeed, rapamycin is used in combinations with other drugs to prevent organ rejection after renal transplantation [44]. Renal transplant patients who receive rapamycin daily for several years do not seem to have obligatory problems from sustained blockage of TOR [44]. Among the side-effects of rapamycin, there are no pronounced immunosuppressive problems (viral, bacterial and fungal infections, Kaposi's sarcoma or osteoporosis). Furthermore, rapamycin can be used to treat invasive fungal infections and HIV [45]. The antifungal antibiotic rapamycin is not a classic immunosuppressant; rather, it modulates the immune response. Ironically, it was assumed that rapamycin would predispose patients to osteoporosis and cancer, but instead it turns out that rapamycin has anti-cancer and bone-sparing activities [46,47].

Lessons of side-effects

Historically, renal transplant patients receiving immunosuppressants have often developed tumors (particularly Kaposi's sarcoma) and osteoporosis. In 1997, rapamycin (Sirolimus) was added to the list of immunosuppressive regimens. Unexpectedly, rapamycin was found to prevent tumors in renal transplant patients [47,48]. Rapamycin not only prevented new tumors but also led to regression of pre-existing tumors [49,50]. For example, three months after Sirolimus (rapamycin) therapy was begun, all cutaneous Kaposi's sarcoma lesions had disappeared in the 15 patients treated [51]. Moreover, rapamycin does not cause osteoporosis in patients [46] and inhibits bone resorption in animals [52]. Thus, prevention of both tumors and osteoporosis are side-effects of rapamycin. What are its other side-effects?

As its most common side-effect, rapamycin increases blood lipids such as triglycerides (hyperlipidemia and hypertriglyceridemia) [44,53]. Importantly, rapamycin-induced hyperlipidemia is manageable with the administration of lipid-lowering agents. It is worth looking at this side-effect in more detail. Increased blood triglycerides could be a manifestation of the calorie-restriction-mimetic effect. Lipolysis and consequently hyperlipidemia are exactly what happens under calorie restriction, when the organism consumes fat from the fat tissue. Rapamycin-induced lipolysis causes hypertriglyceridemia [54]. Rapamycin both increases adipose-tissue lipase activity, driving lipids from the fat tissue into the blood, and decreases lipoprotein lipase activity, preventing lipid accumulation in tissues including the vascular wall. These effects resemble those of calorie restriction. Indeed, a 'lipolytic side-effect' might antagonize age-associated increases in visceral fat and might slow down atherosclerosis.

In animal models, systemic administration of rapamycin reduces neointimal thickening and slows the progression of atherosclerosis in apoE-deficient mice, which have high levels of cholesterol [55]. In addition, rapamycin often causes mild anemia and leucopenia. Mild anemia results from a reduction in serum iron and transferrin saturation levels, which might be beneficial. Thus, rapamycin has several remarkable side-effects: (i) anti-cancer effects; (ii) bone-sparing effects; (iii) lipolysis that resembles the effects of calorie-restriction; (iv) mild anemia owing to decreased iron levels; and (v) mild leucopenia, which might counteract age-associated pro-inflammatory states.

A drug for age-related diseases or for anti-aging

Potential clinical applications of rapamycin are emerging in different fields of medicine from cardiology to ophthalmology and oncology. In each disease, rapamycin is indicated on different and unrelated grounds such as its anti-inflammatory, anti-proliferative, anti-angiogenic, immunosuppressive, anti-tumor and anti-atherosclerotic effects. I suggest that applications of rapamycin to seemingly unrelated conditions are not coincidental. If rapamycin is an anti-aging drug, it will affect all age-related diseases. No one dies from healthy aging, and aging people develop age-related diseases. Age-related diseases are a manifestation of aging. In fact, TOR is involved in most age-related diseases [6,56]. Below, I consider some examples.

Osteoporosis

Despite the popular believe that a deficiency of dietary calcium and vitamin D causes age-related osteoporosis, this disease results from hyper-functioning osteoclasts – cells that resorb bones. Bone-resorbing osteoclasts depend on the TOR pathway for their formation, function and survival. Rapamycin and other inhibitors of TOR inhibit osteoclast formation and activity, and suppress bone resorption [52].

Fibrosis and cardiac hypertrophy

Organ fibrosis is a common condition associated with aging. TOR stimulates fibrosis and rapamycin can exert direct antifibrotic activities [57]. Rapamycin treatment regresses cardiac hypertrophy and cardiac fibrosis in mice. Cardiac hypertrophy results from overactivation of the PI3K/TOR pathway [58]. Hypertrophic stimuli, such as angiotensin II, activate the TOR pathway and protein synthesis; rapamycin abolishes this activation [59].

Cancer

As discussed above, rapamycin has undergone clinical trials for cancer treatment, because mutations of the PI3K/TOR pathway are most common in cancer. But, this is only part of the story. Senescent cells can also promote pre-malignant neighboring cells to proliferate and to form tumors [11,12]. In other words, malignant tumorigenesis requires a permissive tissue environment in which mutant cells can survive, proliferate and express their neoplastic phenotype. Thus, senescent stromal cells create angiogenic conditions and can promote pre-malignant cells to grow [11,12].

The incidence of occult microscopic cancers is much higher than the incidence of clinical cancers. Senescent cells provide a permissive environment and angiogenesis can transform occult cancer to a clinical disease. As a result, it might be predicted that rapamycin will be most useful in preventing but not curing cancer. Similarly, rapamycin might be most effective in preventing osteoporosis but not repairing broken bones, and in slowing atherosclerosis rather than treating its consequences such as stroke and infarction. In short, rapamycin might be administered for the prevention of various age-related diseases: that is how this anti-aging drug could be used today.

Conclusion

To identify an anti-aging drug that could be used today, here I have brought together cellular senescence, genetic control of longevity and age-related diseases. Not only does the TOR pathway seem to

be involved in cell senescence, animal aging and human diseases, but also its inhibitor (rapamycin) is clinically available. Rapamycin is administered daily to transplant patients for several years and is safe enough to be given to healthy volunteers [60]. Its use is considered in different diseases from atherosclerosis and obesity to cancer and macular degeneration. Forthcoming clinical applications of rapamycin for seemingly unrelated diseases will enable us to evaluate its anti-aging potential. Such applications will be 'accidental' clinical trials of a potential anti-aging drug.

Certainly, owing to feedback loops in the TOR pathway, indirect inhibitors of TOR will be also needed. Currently, metformin and resveratrol, which activate AMPK (Figure 2), are available for clinical use. Resveratrol might also antagonize some downstream effects of the TOR pathway through SIRT1. Development of activators of sirtuins might empower the anti-aging drug arsenal. These drugs, if non-toxic, could be added to drug combinations, although it might take decades to develop such drugs for clinical use. New targets will be also exploited and perhaps new pathways will be identified. Finally, antioxidants might find their place in drug combinations to prolong further the extended lifespan.

Acknowledgements

I would like to thank all the authors whose work was not cited here owing to page limitations.

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