Cheating Death: Does the New Biology of Aging Show Us the Way?

Perry E. Bickel, M.D.
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The Seventh Seal, Ingmar Bergman

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Purpose & Overview:
To review recent advancements in the molecular mechanisms of aging and in the development of potential anti-aging therapeutics.

Objectives:

1. To identify the hallmarks of aging conserved between humans and other species.
2. To understand the major nutrient sensing pathways that have been implicated in aging.
3. To learn how these pathways are being targeted for the development of anti-aging therapeutics.

Biosketch:

Dr. Perry E. Bickel is an Associate Professor of Medicine and Chief of Endocrinology at UT Southwestern Medical Center. He holds the J. D. & Maggie E. Wilson Distinguished Chair in Biomedical Research and is Director of the Jean D. Wilson Center for Biomedical Research. He received his B.A. in Philosophy from Yale University and his M.D. from the University of Texas Southwestern Medical School at Dallas. He completed his internal medicine residency and endocrinology fellowship at Massachusetts General Hospital. He completed a postdoctoral fellowship at the Whitehead Institute in Cambridge, MA with Harvey Lodish before moving to Washington University School of Medicine for his first faculty position, where he developed a research interest in lipid droplet proteins and their role in the regulation of lipid storage and utilization within cells. In 2007 Dr. Bickel moved to the Brown Foundation Institute of Molecular Medicine at the University of Texas Health Science Center at Houston to lead the development of a new research center in metabolic diseases. Two years ago Dr. Bickel joined the Internal Medicine faculty at UT Southwestern Medical Center, where his lab is focused on the role of the lipid droplet protein perilipin 5/OXPAT in the control of metabolic gene expression. Dr. Bickel's lab has discovered a surprising function of perilipin 5 as an activator of SIRT1 deacetylase activity. SIRT1 features prominently in one of the aging pathways to be discussed in this Grand Rounds.

Spring and Fall: to a Young Child

Margaret, are you grieving
Over Goldengrove unleaving?
Leaves, like the things of man, you
With your fresh thoughts care for, can you?
Ah! as the heart grows older
It will come to such sights colder
By and by, nor spare a sigh
Though worlds of wanwood leafmeal lie;
And yet you *will* weep and know why.
Now no matter, child, the name:
Sorrow's springs are the same.
Nor mouth had, no nor mind, expressed
What héart héard of, ghóst guéssed:
It is the blight man was born for,
It is Margaret you mourn for.

Gerard Manley Hopkins (1844-1889)

I am not afraid of death, I just don't want to be there when it happens.

Woody Allen (1935-)

Introduction

The inevitability of aging and death has preoccupied poets like Gerald Manley Hopkins and comedians like Woody Allen. Aging may be defined as the "time-dependent functional decline that affects most living organisms" (Lopez-Otin, 2013). Everyone alive today has a stake in the questions that surround longevity and aging. Can we live longer lives? Can we eliminate or at least delay the diseases and disabilities associated with aging? Can we increase our productive years so we can live independently longer? To answer these questions, to the extent they are answerable, we must achieve a mechanistic understanding of how we age at the molecular, cellular, and organismal levels. No one approach or methodology will suffice. Rather, the effort will require the work of multiple fields, including genetics, physiology, neurobiology, endocrinology, stem cell biology, biochemistry, pharmacology, ethics, economics, political science, and many others. Over the past 30 years, research on model organisms and in human genetics has revealed a new understanding of the biology of aging that points to potential therapeutic targets for the prevention of aging-related diseases and perhaps to the extension of human lifespan. This manuscript will review a selection of the major discoveries in the field that are starting to feed into the

pipelines of drug development. I will emphasize the therapeutic target SIRT1, a member of the sirtuin family of NAD^+-dependent histone deacetylases, because there has arisen much confusion and controversy about its validity as a therapeutic target for aging, and because resveratrol, a natural product activator of SIRT1, is already in wide use by the public as a dietary supplement for its purported anti-aging effects.

Historical Background and Definitions

Life expectancy is the age that 50% of a population achieves. By this definition life expectancy has increased in developed countries over the past 100 years from ~45 years to ~75 and ~80 years for men and women, respectively (Fig.1). This remarkable increase is due primarily to reduced infant mortality, better prevention and treatment of infectious diseases, better health care in general, and improved public health measures.

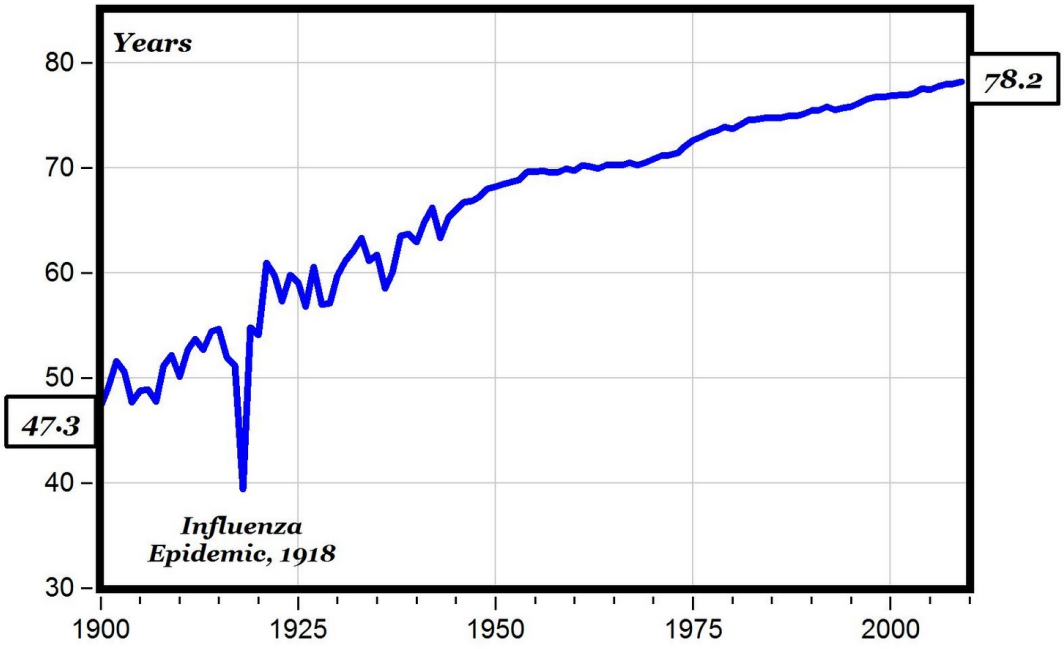

Figure 1. Life expectancy in the United States from 1900 to 2010.

For research purposes, maximum lifespan is defined as the average age achieved by the longest-lived decile of a particular group. The oldest well-documented age achieved was 122 years by Jeanne Louise Calment, who died in 1997 (reviewed in Fontana and Klein, 2007). As no one else has come close to reaching this age recently, 122 years is known as the "Calment Limit" (Coles and Young, 2012). The current oldest living person is Misao Okawa, who celebrated her 116th birthday on March 5, 2014. The website for the Gerontology Research Group (GRG) (www.grg.org) lists 75 known living supercentenarians (110 years or older) in the world, 71 female and 4 male. The GRG estimates that supercentenarians number between 300-400 currently living worldwide. Thus, we

have the potential to live to ~120 years old, but vanishingly few of us do. Our life expectancy has not surpassed that noted in Psalms.

> "The days of our years are threescore and ten; and if by reason of strength they be forescore years, yet is their strength labour and sorrow; for it is soon cut off and we fly away." (Psalm 90:10)

The "labour and sorrow" mentioned by the Psalmist reflect the burden of aging-associated diseases, including cardiovascular disease, type 2 diabetes, hypertension, cancer, osteoporosis, degenerative joint disease, sarcopenia, frailty, cataracts, and neurodegenerative diseases. The goals of aging research should not be simply to prolong the "labour and sorrow" but rather to prolong what has been termed "healthspan," which is the duration of one's life spent in optimal health. To be avoided are longevity strategies that produce the "Tithonus effect" (Richardson, 2013). Tithonus was a prince of Troy beloved by the goddess Eos. She kidnapped him and begged Zeus to grant Tithonus immortality, which he did. Eos forgot to also request for Tithonus eternal youth, so he grew old forever.

During the Great Depression concern arose that chronic dietary restriction might lead to premature deaths. Studies of dietary restriction in rats were conducted, but these surprisingly revealed that dietary restriction lengthened lifespan, and this finding was later reproduced in yeast, worms, flies, mice, and in one of two studies of rhesus monkeys (Kenyon, 2010). It is not known if dietary restriction prolongs lifespan or healthspan in humans, though members of the Dietary Restriction Society are practicing dietary restriction with that expectation (Fontana and Klein, 2007). Dietary restriction is defined as the reduction of calorie consumption to below the level of usual *ad libitum* intake but not so low as to cause malnutrition. Most studies of dietary restriction range from 20% to 40% reduction. Across the species used in laboratory settings, dietary restriction is the most robust intervention for aging and longevity research (Baur, 2012). For the purposes of this protocol, I am using the term "dietary restriction" as interchangeable with "calorie restriction.

Another landmark in aging research was the identification of long-lived strains of the nematode *Caenorhabditis elegans* (*C. elegans*) by Klass in 1983 (Klass, 1983). The findings of this study suggested that a component of lifespan had a genetic and, therefore, molecular basis. Ten years later, Cynthia Kenyon and colleagues reported that mutations in the *C. elegans* daf-2 gene, an ortholog of the insulin/IGF-1 receptor doubled the lifespan of the mutant worms, and this longevity phenotype was dependent on activity of a downstream transcription factor daf-16, a member of the FOXO family (Kenyon, 1993). This finding together with similar studies in flies and mice established that aging is not simply a random process of time-dependent degeneration, but instead is regulated by known or knowable signaling and transcriptional pathways (Kenyon, 2010).

The use of dietary restriction in genetically modified model organisms has permitted the delineation of regulatory pathways involving signaling molecules and transcription factors that mediate the longevity effects of dietary restriction. Such

studies have suggested potential drug targets for the purpose of mimicking dietary restriction and landmark studies are reviewed below.

Hallmarks of Aging

In an attempt to provide a conceptual framework for future aging studies, Serrano and colleagues last year published a categorization of the hallmark features of aging. These authors identified 9 "hallmarks of aging" (Lopez-Otin, 2013).

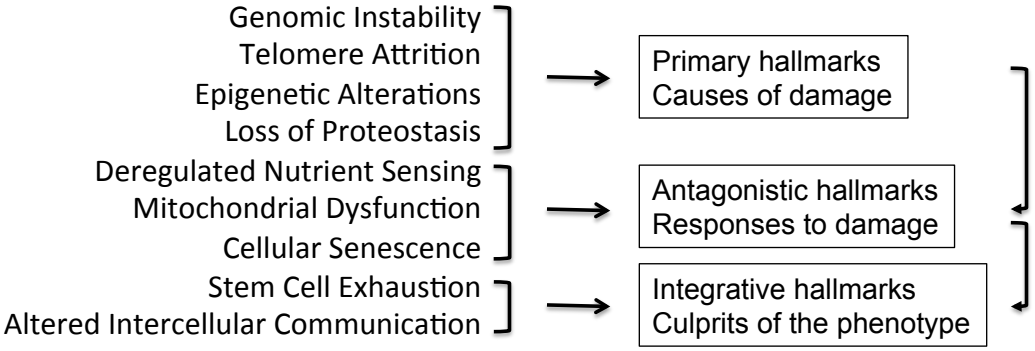

Figure 2. The Hallmarks of Aging. Adapted from Lopez-Otin et al. 2013.

According to the authors, each hallmark (1) should occur during normal aging, (2) when experimentally promoted should hasten aging, and (3) when experimentally inhibited should prolong healthspan. The review by Lopez-Otin et al. provides extensive, fully referenced detail about each of the Hallmarks.

Genomic instability can occur due to defects in nuclear DNA repair, as in some human progerias, due to somatic accumulation of mitochondrial DNA damage, or due to defects in the nuclear lamina.

Telomere attrition refers to the progressive shortening of the protective telomere structures that cap the ends of chromosomes, which occurs during normal aging in mice and humans. Pathological telomere shortening is associated with premature aging, and enforced expression of telomerase in mice delays aging.

Epigentic alterations in DNA methylation, histone modifications and chromatin remodeling occur with aging. Increased or decreased acetylation and methylation of histones H3 and H4 on specific lysine residues, known as histone "marks," constitute an epigenetic signature of aging.

Loss of proteostasis may be due either to failure to adequately stabilize properly folded proteins, for example with heat shock proteins, or failure to degrade damaged or misfolded proteins by the autophagy/lysosomal or the ubiquitin/proteosomal systems. Aggregates of proteins accumulate in some aging-associated diseases, such as Alzheimer's disease. At autopsy supercentenarians are often found to have healthy organs but are also found to have major vessels lined with obstructive aggregates of transthyretin, a condition called senile systemic amyloidosis and a common cause of death among the extremely old (Coles and Young, 2012).

Deregulated nutrient sensing involves signaling pathways that are activated by anabolic conditions (insulin-IGF-1 signaling and mTOR), or by nutrient poor

conditions (AMP-kinase and sirtuin signaling). In general, genetic, nutritional or pharmacological manipulations that promote the anabolic pathways accelerate aging and those that promote the nutrient restricted pathways foster increased healthspan and/or lifespan. Such manipulations will be discussed below.

Mitochondrial dysfunction manifested as decreased respiratory efficiency is associated with aging in most species. Interestingly, the excess production of reactive oxygen species (ROS) is not initially damaging to the cell, but rather ROS activates homeostatic responses in compensation. Only when ROS levels reach a critical threshold do they begin to contribute to aging-associated damage. An emerging concept is "mitohormesis," according to which mild damage to mitochondria engenders a repair response that makes the mitochondria and cell healthier than before the damage occurred. Thus, drugs that cause mild chronic damage to mitochondria may improve mitochondrial function over time.

Cellular senescence occurs when cells arrest in the cell cycle and manifest specific phenotypes, including DNA damage, expression of p16, and senescence-associated beta-galactosidase staining. Senescent cells increase during aging, but this may be due to failure of the immune system to clear senescent cells coupled with inadequate recruitment of progenitor cells as replacement, rather than increased cell senescence per se. Cellular senescence may actually be a beneficial response to cell damage that is adaptive until the progenitor cell capacity is exhausted. Interestingly, two papers published last year in the same issue of *Cell* established that cell senescence is a required during mammalian development for proper embryonic growth, patterning and tissue remodeling (Storer, 2013; Munoz-Espin, 2013).

Stem cell exhaustion occurs with aging in most if not all stem compartments, and in the hematopoetic compartment it is responsible for reduced adaptive immune function, increased anemia, and myeloid malignancies. Parabiosis experiments in which young mice have been coupled to old mice suggest that circulating factors in young mice can promote some stem compartments in old mice (Conboy, 2005). This line of research presents an exciting therapeutic avenue for the rejuvenation of stem cells in the elderly through the potential identification of such circulating factors. GDF11 has been identified as being one such circulating "youth" factor that can rejuvenate old stem cells and improve heart, muscle, and brain function (Loffredo, 2013; Katsimpardi, 2014).

Altered intercellular communication that arises during aging illustrates that aging is not a cell-autonomous process, but rather involves endocrine and neuroendocrine signaling, as well as the activation of inflammatory pathways and the action of inflammatory cytokines. The aging-associated imbalance between inflammatory and anti-inflammatory pathways results in chronic low-grade inflammation that has been termed "infammaging" (Franceschi, 2007).

In this conceptual framework of Serrano and colleagues, the primary hallmarks of aging (genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis) damage cells and tissues, which then leads to the homeostatic responses of the "antagonistic hallmarks" (deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence). These homeostatic responses begin as adaptive and beneficial but over time progress/persist to the

point of being harmful to the aging organism. The integrative hallmarks manifest themselves when tissues start to decompensate under the burden of the other hallmarks (Lopez-Otin, 2013).

Notably, key molecular mediators of aging pathways and longevity in model organisms either contribute to or protecting from the hallmarks of aging. A selection of those mediators that have suggested pharmcotherapeutic strategies to prolong healthspan and/or lifespan are discussed below.

Dietary Restriction and Aging in Non-Human Primates

Dietary restriction promotes healthspan and/or lifespan in multiple species, including yeast, worms, flies, and mice. This observation has permitted the genetic dissection of potential longevity pathways in which the phenotype of transgenic overexpression of a specific gene or knockout of the gene is assayed for lifespan and for the incidence of age-associated diseases or for biomarkers of these diseases. Such mechanistic studies are not possible in primates, but the effects of different regimens of dietary restriction have been measured in rhesus monkeys, which display many of the same age-related phenotypes and diseases as aging humans (Colman, 2014). Two long-term studies are underway, one at the University of Wisconsin (UW) (Colman, 2014) and the other at the National Institute of Aging (NIA) (Mattison, 2012). These two studies have had conflicting interim results. The UW study has reported significant improvement in age-related and all-cause mortality with dietary restriction (calorie intake restricted 30% below measured usual intake versus ad libitum feed controls), but no effect on non-age-related deaths. The NIA study failed to demonstrate a survival benefit of dietary restriction, though it did find significant gender- and age-dependent improvements in some metabolic parameters, in neoplasia rates, and in immune responses. The designs of these two studies differ in significant ways. First, the UW study initiated the experiment only in adult monkeys, whereas the NIA study started with monkeys of a broad age range including young monkeys. The UW study is using only monkeys of Indian origin, and the NIA study is using monkeys of both Indian and Chinese origin. The UW study employs a semi-purified diet that is high in sucrose, and control monkeys are fed ad libitum. In contrast, in the NIA study, the diet is “healthier” with much lower sucrose content, and the diet is not purified but rather has a natural ingredient base. Most importantly, the control monkeys in the NIA study are fed a fixed amount and are not fed *ad libitum*. The NIA control monkeys weigh less than both the UW control monkeys and the national average for body weight in non-experimental captive rhesus monkeys. Thus, the NIA control monkeys may be subject to “modest” dietary restriction, thereby minimizing the differences with the dietary restricted group. Though the jury is still out, similar to smaller organisms, dietary restriction may improve lifespan and healthspan in non-human primates, so looking for pharmacological mimetics of dietary restriction to improve lifespan and healthspan in humans remains viable.

Dietary Restriction and Aging in Humans

No long-term study in humans like the UW and NIA dietary restriction studies of rhesus monkeys has been conducted, nor due to the expense and

impracticality of such a study is one likely to ever be started. Shorter-term randomized controlled trials of 6-12 months duration have been reported. These trials included from 12 to 19 nonobese subjects who decreased their calorie intake from 20-25% (reviewed in Fontana and Klein, 2007). Predictably, the calorie-restricted groups lost body fat. One trial found beneficial metabolic effects such as a reduced TNFα/adiponectin ratio, reduced glucose and insulin, and improved insulin sensitivity, but also detected unfavorable effects including reduced bone density and reduced muscle mass. Unfortunately, there are no universally accepted biomarkers of aging, so the field has relied on surrogate markers of age-related diseases in these human studies. Some have proposed endocrine changes as biomarkers (with the rationale being that fasting insulin rises and DHEA-S and IGF-1 fall with aging), as well as maximal oxygen consumption and markers of oxidative damage (Redman and Ravussin, 2011). The discovery and validation of robust biomarkers of aging and longevity will be essential for rigorous trials of anti-aging therapeutics in humans.

Candidate Targets for Anti-Aging Therapeutics

The FDA does not recognize aging as a disease. Thus, if any drugs that slow aging and prolong lifespan are to be approved, such drugs must be safe and effective for the treatment of aging-associated diseases. Mutations that delay the progression of aging in model organisms also delay the progression of age-associated diseases, so it is at least possible that therapeutics directed at these mutant pathways may also ameliorate the diseases of aging.

Insulin/IGF-1/FOXO Signaling

As noted in the Introduction, the first landmark genetic studies of aging in a model organism were in the nematode *C. elegans* and implicated the insulin/IGF-1/FOXO pathway (DAF-2/DAF-16 in the worm) (Kenyon, 1993). Loss of function mutations of *daf-2* were associated with a doubling of lifespan. These mutant worms not only lived longer but also remained young in appearance and behavior well after wild-type worms looked old. This landmark study established that aging in *C. elegans* is a hormonally regulated phenotype. Attenuation of signaling through the insulin/IGF-1/FOXO pathway results in gene expression changes that promote stress resistance, including antioxidant, chaperone, antimicrobial programs (Kenyon, 2005). In mice and humans mutations in multiple different genes of the insulin/IGF-1/FOXO pathway have been associated with longevity. For example, there are 9 long-lived human cohorts worldwide who have mutations in FOXO3A or FOXO1. In one German cohort FOXO3A variants are more common in centenarians than in nonagenarians, which suggests an effect on longevity (reviewed in Kenyon, 2010).

Laron syndrome is a rare growth disorder in humans that results from a mutation in the growth hormone receptor gene. Affected individuals have low IGF-1 levels and very short stature (average 1.2 meters in height). Mice engineered to harbor the same mutation are also small, but display metabolic benefits including increased insulin sensitivity, protection from diabetes, reduced cancer rates, and increased lifespan. Of the approximately 300 affected individuals in the world,

about 100 reside in a region of Ecuador. A multidisciplinary team of basic and clinical researchers have been studying this population over the past decade. In comparison to unaffected individuals in the community, those with Laron syndrome, like the mutant mice, are protected from diabetes and cancer, but they do not appear to have increased longevity, perhaps due to a high rate of accidental deaths and alcohol-related deaths.

Targeting the insulin/IGF-1/FOXO pathway for the purpose of longevity and aging has not been straightforward. For example, a recent manuscript reported results for a novel inhibitor of the pathway, NT219, which inhibits IGF-1 receptor autophosphorylation and targets insulin responsive substrates 1 and 2 (IRS-1 and IRS-2) for degradation (El-Ami, 2014). In *C. elegans*, this compound effectively protected against neurodegeneration due to Aβ- and polyQ-mediated proteotoxicity but had no effect on longevity.

TOR

TOR is a kinase of the phosphoinositide 3-kinase family that serves as an integrator of growth and starvation signaling in cells. TOR orthologs are expressed in unicellular eukaryotes, such as yeast, and are well conserved in higher organisms. TOR was originally identified in yeast as the "target of rapamycin," an immunosuppressive drug used clinically to prevent graft rejection but that also inhibits proliferation and growth of mammalian cells in general, thereby suggesting a potential role as an antineoplastic agent (Lamming, 2013). In mammals TOR is known as mammalian TOR or mechanistic (mTOR). mTOR is found in two multi-protein complexes, mTORC1 and mTORC2. A detailed account of mTOR signaling is beyond the scope of this protocol, but excellent recent reviews are available (Lamming, 2013; Zoncu, 2011). Briefly, mTORC1 receives signaling inputs from amino acids (especially leucine), glucose, oxygen, cAMP, and insulin/IGF-1 signaling, among others, and has downstream effects to promote anabolic programs (protein translation and ribosome biogenesis) and to inhibit catabolic programs (autophagy and lysosome biogenesis).

In 2003 Muller and colleagues reported that knockdown of the TOR ortholog in *C. elegans* extended lifespan independently of FOXO, thereby implicating another nutrient sensing pathway in aging (Vellai, 2003). Similar findings were subsequently made in yeast, flies, and mice, which has raised the possibility that mTOR inhibitors, such as rapamycin or other "rapalogs," might pharmacologically prolong lifespan. In 2009 Harrison, Miller and colleagues reported that rapamycin treatment of genetically heterogeneous, older mice (starting at age 600 days, equivalent to human age 60) increased both median and maximal lifespan in both males and females (Harrison, 2009). The mean lifespan for males increased 9% and for females 13%. This study was the first demonstration of a positive durg effect on maximal lifespan in mammals. This study did not address whether rapamycin slowed age-related changes in organs or prevented age-related diseases, but this possibility was tested a recent study by Ehninger and colleagues (Neff, 2013). These investigators confirmed the positive effects of rapamycin on lifespan in mice, but by extensive structural and functional phenotyping of the mice they found that only a small subset of aging-related

phenotypes were improved. Moreover, the same effects on these phenotypes seen in old mice were also seen in young mice, from which the authors concluded that rapamycin was not having a purely anti-aging effect. Other studies, however, have shown positive effects of rapamycin on musculoskeletal, neurocognitive, vascular and other aging phenotypes (reviewed in Richardson, 2013). Older mice treated with rapamycin do not have a greater disease burden at death than control mice, even though they live up to an additional decade in human years, so "they do not exhibit the Tithonus phenotype" (Richardson, 2013). Thus, rapamycin and other rapalogs may merit testing in clinical trials to address aging-related diseases.

On the other hand, the toxicities of rapamycin in humans are well-known from its use in cancer treatment, and these adverse effects include hyperlipidemia, hypertension and insulin resistance, all of which one would hope to avoid in any anti-aging therapy.

AMP-Kinase

The commonly used antidiabetic drug metformin has been reported to increase both lifespan and healthspan when given to middle aged mice (de Cabo, 2012). Metformin improved healthspan parameters such as insulin sensitivity, cholesterol, cataract formation, mitochondrial function, inflammation, and physical performance, and led to increases in mean lifespan of 5.83% and 4.15% in two different strains of male mice. Further, metformin treatment resulted in patterns of hepatic gene expression similar to those of dietary restriction, despite food intake being increased. Metformin indirectly activates AMP-kinase through LKB1 but the mechanism for this activation is not fully understood. Since metformin has been used clinically for years and is known to be safe for those with adequate renal function, it may be a viable candidate to prevent or attenuate aging-related diseases in humans.

The Sirtuins and NAD^+

In 1999 Guarente and colleagues identified the protein SIR2 (silent information regulator 2) as a mediator of extended lifespan in *Saccharomyces cerevisiae* (Kaeberlein, 1999). Subsequently, the same lab determined that SIR2 is a NAD^+-dependent histone deacetylase (Imai, 2000). Seven SIR2 orthologs are present in mammals, SIRT1-SIRT7. These proteins, known collectively as sirtuins, differ from one another in tissue expression, subcellular localization, and substrate targets, which include not only histones but also key regulatory proteins in metabolism, inflammation, cell cycle, circadian control and other essential processes. An important regulator of sirtuin activity is the availability of its NAD^+ cofactor. NAD^+ levels rise in fat, liver and muscle during exercise, calorie restriction, and fasting. The most-studied member of the family, SIRT1, is the mammalian ortholog of yeast SIR2 and is activated under conditions of dietary restriction that are associated with increased lifespan. Early work in yeast, worms and flies was consistent with the notion that SIRT1 orthologs mediate the life-extending effects of dietary restriction, but this model has been a subject of many publications and considerable controversy (reviewed in Imai and Guarente, 2014). More recent work supports a pivotal role for SIRT1 in the determination of lifespan

and aging. For example, mice that overexpress SIRT1 only in the brain (BRASTO mice) live longer and exhibit delayed onset of aging-related conditions (Satoh, 2013). BRASTO female mice had a 16% increase in median lifespan, and males had a 9% increase. In a previous study of SIRT1 overexpression driven by its own promoter, mice were protected from aging- and metabolic syndrome-associated cancer but did not live longer than controls (Herranz, 2010).

Another means of probing the functions of SIRT1 is with chemical compounds that potentiate its deacetylase activity. The most famous of these compounds, which are known collectively as STACs (sirtuin-activating compounds), is resveratrol. Resveratrol is a naturally-occurring compound present a low concentrations in red wine. Resveratrol is available as a supplement and is taken by many to slow aging, despite much scientific debate as to whether it activates SIRT1 or not. Some negative studies of resveratrol's efficacy may reflect its limited bioavailability. Last year Sinclair and colleagues identified a common allosteric mechanism by which resveratrol and other STACs activate SIRT1 deacetylase activity (Hubbard, 2013). One such STAC, SRT1720 was recently reported to have positive effects on lifespan and healthspan in male mice fed a standard diet (Mitchell, 2014). The increase in mean lifespan was 8.8%, with a trend toward an increased median lifespan and no difference in 90th%ile survival. Specific health benefits were noted in cataract formation, glucose metabolism, and rotorod performance, which tests balance, coordination and motor strength. Whole genome microarrary analysis in liver and muscle demonstrated that SRT1720 treatment was associated with a reduction in pro-inflammatory gene expression, which would address one of the hallmarks of aging (altered intercellular communication).

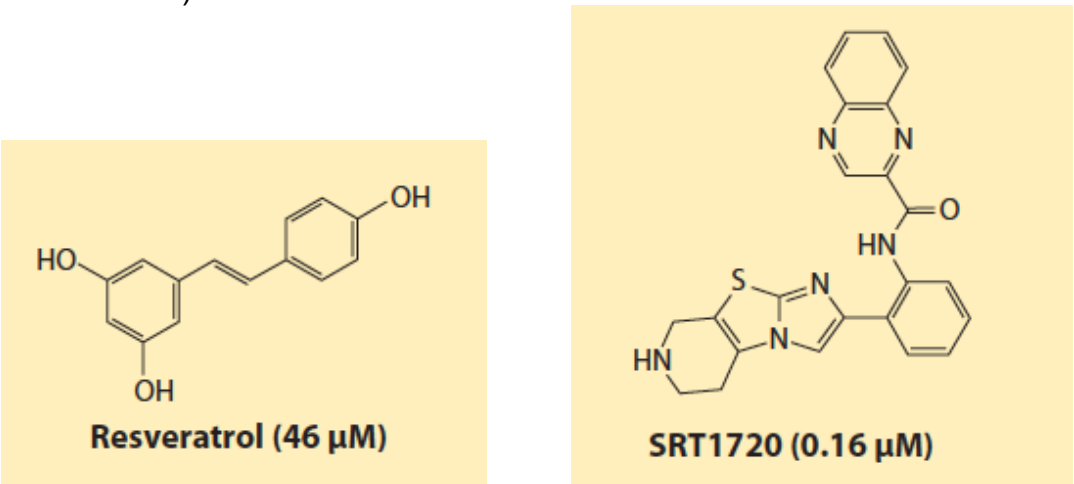

Figure 3. Two STACs (SIRT1-activating compounds)

Schrauwen and colleagues in 2011 reported the results of 30 days treatment of healthy obese men with 150 mg/day resveratrol and found improvements in multiple metabolic readouts that were consistent with a calorie-restriction phenotype (Timmers, 2011). In contrast, a subsequent study of nonobese healthy women dosed with resveratrol at 75 mg/day failed to show any

metabolic improvement (Yoshino, 2012), nor did a later study of high dose (500 mg three times daily) in obese healthy men (Poulsen, 2013).

A new concept is emerging that aging is associated with deficiency of NAD^+, which leads to mitochondrial dysfunction (Gomes, 2013; Mouchiroud, 2013; Prolla and Denu; 2014). An in depth discussion of the studies behind this concept is beyond the scope of this protocol, but suffice it to say that providing the NAD^+ precursor nicotinamide mononucleotide (NMN) for 1 week to 22-month old mice restored muscle mitochondrial function to that of 6-month old mice. This finding suggests that aging may be reversible, at least partially and at least in muscle (Gomes, 2013).

Summary

Over the past 30 years, our understanding of aging has changed from that of a purely random, entropic process of progressive tissue damage to that of a process regulated by classical signaling pathways and transcription factors. Many aging pathways are conserved between species, but the relative importance of each varies according to species and environmental factors. Mutations that delay the progression of aging also delay the progression of age-associated diseases. This observation raises the hope that therapeutics based on our understanding of how aging is regulated will address aging-related diseases, will prolong healthspan, and perhaps prolong lifespan as well. Dietary restriction is the most robust intervention across the species studied in laboratories for aging and longevity effects. Consistent with the beneficial effects of dietary restriction, lifespan-prolonging mutations tend to be in stress and nutrient responsive pathways. It is these pathways that have yielded therapeutic targets and agents that are now in preclinical development or in clinical trials. The best possible outcome of these efforts would be a safe pill that would be started in middle age and would protect against the diseases of aging. Exciting research that has identified a circulating factor (GDF11) in the blood on young mice that can promote the ability of stem cells from old mice to revert to a youthful function offers the possibility of rejuvenation of tissues and of tissue function. Of course, the ethical, economic, and public policy aspects of prolonging healthy life must be considered, and there are always side effects and unintended consequences, but as a physician it is tempting to dream of a world without the “labour and sorrow” of the diseases of aging.

References

Baur, J.A., Ungvari, Z., Minor, R.K., Le Couteur, D.G., and de Cabo, R. (2012). Are sirtuins viable targets for improving healthspan and lifespan? Nature reviews Drug discovery *11*, 443-461.

Coles, L.S., and Young, R.D. (2012). Supercentenarians and transthyretin amyloidosis: the next frontier of human life extension. Preventive medicine *54 Suppl*, S9-11.

Colman, R.J., Beasley, T.M., Kemnitz, J.W., Johnson, S.C., Weindruch, R., and Anderson, R.M. (2014). Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nature communications *5*, 3557.

Conboy, I.M., Conboy, M.J., Wagers, A.J., Girma, E.R., Weissman, I.L., and Rando, T.A. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature *433*, 760-764.

El-Ami, T., Moll, L., Carvalhal Marques, F., Volovik, Y., Reuveni, H., and Cohen, E. (2014). A novel inhibitor of the insulin/IGF signaling pathway protects from age-onset, neurodegeneration-linked proteotoxicity. Aging cell *13*, 165-174.

Fontana, L., and Klein, S. (2007). Aging, adiposity, and calorie restriction. JAMA : the journal of the American Medical Association *297*, 986-994.

Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M.*, et al.* (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of ageing and development *128*, 92-105.

Gomes, A.P., Price, N.L., Ling, A.J., Moslehi, J.J., Montgomery, M.K., Rajman, L., White, J.P., Teodoro, J.S., Wrann, C.D., Hubbard, B.P.*, et al.* (2013). Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell *155*, 1624-1638.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S.*, et al.* (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature *460*, 392-395.

Herranz, D., Munoz-Martin, M., Canamero, M., Mulero, F., Martinez-Pastor, B., Fernandez-Capetillo, O., and Serrano, M. (2010). Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. Nature communications *1*, 3.

Hubbard, B.P., Gomes, A.P., Dai, H., Li, J., Case, A.W., Considine, T., Riera, T.V., Lee, J.E., E, S.Y., Lamming, D.W.*, et al.* (2013). Evidence for a common mechanism of SIRT1 regulation by allosteric activators. Science *339*, 1216-1219.

Imai, S., Armstrong, C.M., Kaeberlein, M., and Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature *403*, 795-800.

Imai, S.I., and Guarente, L. (2014). NAD and sirtuins in aging and disease. Trends in cell biology.

Kaeberlein, M., McVey, M., and Guarente, L. (1999). The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes & development *13*, 2570-2580.

Katsimpari, L., Litterman, N.K., Schein, P.A., Miller, C.M., Loffredo, F.S., Wojtkiewicz, G.R., Chen, J.W., Lee, R.T., Wagers, A.J., and Rubin, L.L. (2014). Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Sciencexpress.

Kenyon, C. (2005). The plasticity of aging: insights from long-lived mutants. Cell *120*, 449-460.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461-464.

Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504-512.

Klass, M.R. (1983). A method for the isolation of longevity mutants in the nematode Caenorhabditis elegans and initial results. Mechanisms of ageing and development *22*, 279-286.

Lamming, D.W., Ye, L., Sabatini, D.M., and Baur, J.A. (2013). Rapalogs and mTOR inhibitors as anti-aging therapeutics. The Journal of clinical investigation *123*, 980-989.

Loffredo, F.S., Steinhauser, M.L., Jay, S.M., Gannon, J., Pancoast, J.R., Yalamanchi, P., Sinha, M., Dall'Osso, C., Khong, D., Shadrach, J.L.*, et al.* (2013). Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell *153*, 828-839.

Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell *153*, 1194-1217.

Martin-Montalvo, A., Mercken, E.M., Mitchell, S.J., Palacios, H.H., Mote, P.L., Scheibye-Knudsen, M., Gomes, A.P., Ward, T.M., Minor, R.K., Blouin, M.J.*, et al.*

(2013). Metformin improves healthspan and lifespan in mice. Nature communications *4*, 2192.

Mattison, J.A., Roth, G.S., Beasley, T.M., Tilmont, E.M., Handy, A.M., Herbert, R.L., Longo, D.L., Allison, D.B., Young, J.E., Bryant, M.*, et al.* (2012). Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature *489*, 318-321.

Minor, R.K., Baur, J.A., Gomes, A.P., Ward, T.M., Csiszar, A., Mercken, E.M., Abdelmohsen, K., Shin, Y.K., Canto, C., Scheibye-Knudsen, M.*, et al.* (2011). SRT1720 improves survival and healthspan of obese mice. Scientific reports *1*, 70.

Mitchell, S.J., Martin-Montalvo, A., Mercken, E.M., Palacios, H.H., Ward, T.M., Abulwerdi, G., Minor, R.K., Vlasuk, G.P., Ellis, J.L., Sinclair, D.A.*, et al.* (2014). The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. Cell reports *6*, 836-843.

Mouchiroud, L., Houtkooper, R.H., Moullan, N., Katsyuba, E., Ryu, D., Canto, C., Mottis, A., Jo, Y.S., Viswanathan, M., Schoonjans, K.*, et al.* (2013). The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. Cell *154*, 430-441.

Munoz-Espin, D., Canamero, M., Maraver, A., Gomez-Lopez, G., Contreras, J., Murillo-Cuesta, S., Rodriguez-Baeza, A., Varela-Nieto, I., Ruberte, J., Collado, M.*, et al.* (2013). Programmed cell senescence during mammalian embryonic development. Cell *155*, 1104-1118.

Neff, F., Flores-Dominguez, D., Ryan, D.P., Horsch, M., Schroder, S., Adler, T., Afonso, L.C., Aguilar-Pimentel, J.A., Becker, L., Garrett, L.*, et al.* (2013). Rapamycin extends murine lifespan but has limited effects on aging. The Journal of clinical investigation *123*, 3272-3291.

Poulsen, M.M., Vestergaard, P.F., Clasen, B.F., Radko, Y., Christensen, L.P., Stodkilde-Jorgensen, H., Moller, N., Jessen, N., Pedersen, S.B., and Jorgensen, J.O. (2013). High-dose resveratrol supplementation in obese men: an investigator-initiated, randomized, placebo-controlled clinical trial of substrate metabolism, insulin sensitivity, and body composition. Diabetes *62*, 1186-1195.

Prolla, T.A., and Denu, J.M. (2014). NAD+ deficiency in age-related mitochondrial dysfunction. Cell metabolism *19*, 178-180.

Redman, L.M., and Ravussin, E. (2011). Caloric restriction in humans: impact on physiological, psychological, and behavioral outcomes. Antioxidants & redox signaling *14*, 275-287.

Richardson, A. (2013). Rapamycin, anti-aging, and avoiding the fate of Tithonus. The Journal of clinical investigation *123*, 3204-3206.

Satoh, A., Brace, C.S., Rensing, N., Cliften, P., Wozniak, D.F., Herzog, E.D., Yamada, K.A., and Imai, S. (2013). Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. Cell metabolism *18*, 416-430.

Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M.C., Di Giacomo, V., Yosef, R., Pilpel, N., Krizhanovsky, V., Sharpe, J.*, et al.* (2013). Senescence is a developmental mechanism that contributes to embryonic growth and patterning. Cell *155*, 1119-1130.

Timmers, S., Konings, E., Bilet, L., Houtkooper, R.H., van de Weijer, T., Goossens, G.H., Hoeks, J., van der Krieken, S., Ryu, D., Kersten, S.*, et al.* (2011). Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell metabolism *14*, 612-622.

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A.L., Orosz, L., and Muller, F. (2003). Genetics: influence of TOR kinase on lifespan in C. elegans. Nature *426*, 620.

Yoshino, J., Conte, C., Fontana, L., Mittendorfer, B., Imai, S., Schechtman, K.B., Gu, C., Kunz, I., Rossi Fanelli, F., Patterson, B.W.*, et al.* (2012). Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell metabolism *16*, 658-664.

Zoncu, R., Efeyan, A., and Sabatini, D.M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. Nature reviews Molecular cell biology *12*, 21-35.