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Longevity and aging

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Abstract

Research into the biology of aging seeks to understand the basic mechanisms of aging, with the goal of extending the period of life spent free from chronic disease and disability. Aging results from molecular processes that are modulated by genetic and environmental parameters. At least some of these mechanisms of aging are broadly shared across eukaryotic species from yeast to mice, and likely humans, as well. Recent breakthroughs in aging-related research have identified conserved longevity factors, such as components of the insulin-like signaling pathway and the mechanistic target of rapamycin, and have suggested potential paths toward developing the first interventions to slow aging in humans.

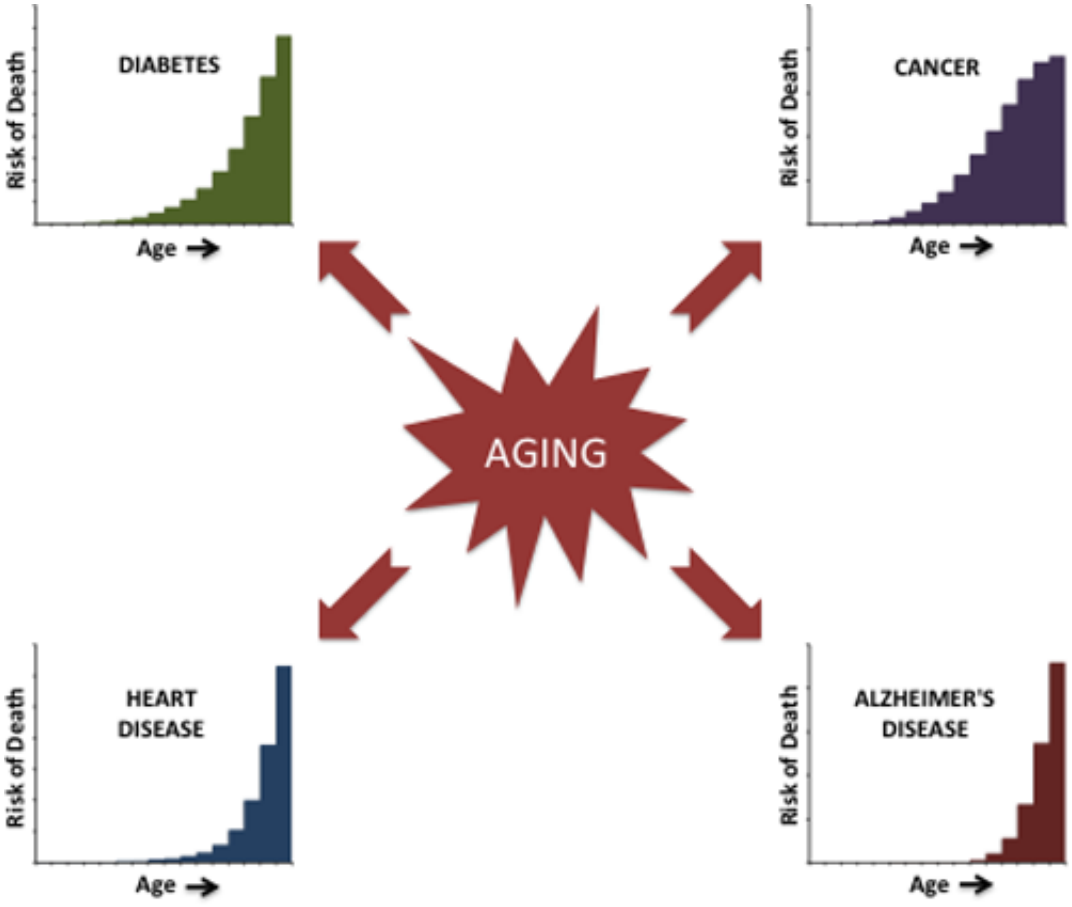
Introduction

Aging drives disease. Nearly every major killer in developed countries shares a common feature: your risk of getting the disease increases dramatically as you get older. For example, the likelihood of being diagnosed with Alzheimer's disease doubles every five years after the age of 65. A similar kind of relationship can be seen for most types of cancer, heart disease, diabetes, kidney disease, and many others (Figure 1). What is it about getting older that simultaneously increases risk for all of these disorders? Are there common molecular changes that cause an organism to switch from youthful and healthy to aged and infirm? Can we intervene in this process to do something about it? These are some of the big questions that scientists who study the biology of aging are interested in answering.

The perspective that most age-related disorders share a common underlying biology is a departure from traditional biomedical science, one that potentially offers a more powerful approach towards improving human health. Rather than focus on curing the individual disease, interventions that target the molecular processes of aging can simultaneously delay the onset and progression of most age-related disorders. Such an intervention is predicted to have a much larger effect on life expectancy than can be attained by treating individual diseases [1-3]. This is because even if one disease is cured, the relationship between age and all the other disorders of aging still holds. For example, it has been estimated that curing cancer will lead to only a 3-5 year increase in survival for an average 50 year-old woman, while slowing aging to an extent that is routine in laboratory organisms has about a 5-10-fold greater impact on life expectancy [1-3]. Importantly, these added years from slowing aging are spent largely free from chronic disease and disability, while the relatively small gains in survival by curing cancer (or any other individual disease of aging) are still associated with the inevitable age-related declines in function of every other bodily system. This concept of extending the period of life spent free from chronic disability and disease, referred to as healthspan, is a critically important idea in the field of aging-related research.

Although the average human lifespan in developed nations has increased dramatically over the past century, there is little evidence that the rate of aging has been slowed [4]. As a consequence, nearly every developed nation in the world is experiencing a growth in the number of elderly living longer, but they are living longer with multiple age-associated disorders [5]. The ability to

Figure 1. Aging drives disease



Aging is the greatest risk factor for the leading causes of death in developed nations. Risk of death from Alzheimer's Disease, diabetes, heart disease, and cancer increase dramatically with age. Graphs represent data taken from the United States Center for Disease Control database for deaths in 2010.

provide care for this expanding population of elderly is predicted to have dramatic social and economic consequences over the next few decades, a so-called "silver tsunami" [6]. From a public health perspective, successful intervention into human aging must be accompanied by compression of morbidity, where the majority of lifetime illness is compressed into a shorter period of time near the end of life [7]. Advances in aging-related research have the potential to alleviate these stresses by delaying the onset of age-related morbidity and allowing elderly people to retain high functionality and productivity for a greater proportion of their lives.

The first molecular theory of human aging to gain prominence was the free radical theory of aging, proposed by Denham Harman more than 50 years ago [8]. This theory posited that oxidative damage from free radicals, produced as a by-product of metabolism and environmental insults, results in damage that, over time, ultimately causes the pathological consequences of aging at the cellular, tissue, and organismal level. Although this theory is now recognized as insufficient to explain all aspects of aging, and the relevance of oxidative stress as a general cause of aging is currently debated [9], the idea that the biological process of aging can be defined by a relatively small number of specific molecular processes has become generally accepted. Here, I will discuss how recent work in humans and model organisms has begun to elucidate these molecular processes, has demonstrated the existence of broadly conserved longevity pathways, and, for the first time, offers real hope of intervening to enhance healthy aging.

Model organisms and conserved mechanisms of aging

The relatively long lifespans of humans make direct mechanistic studies of aging in people particularly challenging. There are currently no reliable biomarkers

for quantifying the rate of aging, making it impossible to validate claims that specific genetic polymorphisms, lifestyle choices, or pharmacological interventions impact the aging process itself. Although it is possible through correlative studies to establish the effects of specific factors on mortality, it is important to understand that such effects may or may not be relevant for the basic mechanisms of aging. This is particularly true when increased risk of death is correlated with a specific factor, because there are many ways to enhance your risk of dying without accelerating the normal aging process.

A few molecular and hormonal changes that occur during aging have been proposed as potential predictors of individual longevity. Among these are declines in serum dehydro-epiandrosterone sulfate (DHEAS), growth hormone, and telomere length [10-12], the latter two of which have achieved popular notoriety as "causes" of human aging. Growth hormone therapy is even recommended by some "anti-aging doctors" as a treatment for aging in otherwise healthy individuals, and several companies are actively developing telomerase activators to help maintain telomere length during aging. Unfortunately, the actual benefits, if any, of such treatments are currently unclear, and the potential risks, particularly increased risk of cancer, warrant caution [11,13]. Importantly, none of these, or other correlative markers, can currently be used reliably to predict either individual life expectancy or biological (as opposed to chronological) age.

Due to the challenges of defining basic mechanisms of aging in humans directly, rodent models served for many decades as the organisms of choice for these kinds of studies; however, even mice and rats tend to live 2-3 years, making the pace of progress slow relative to other areas of research. This all changed in the mid-1990s as simpler eukaryotic systems became widely used in the field. Along with rodent models, the budding yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis elegans*, and the fruit fly *Drosophila melanogaster* have served as the primary model organisms for developing a biochemical and genetic framework for understanding aging [9,14,15]. In each of these model systems, multiple single gene mutations have been identified that substantially extend both median and maximum lifespan, and in both yeast and nematodes the number of such mutations is now in the hundreds. These discoveries have further supported the idea that aging is a defined biological process with a strong genetic component. They have also provided insight into molecular mechanisms of aging, as well as possible targets for interventions that could slow aging.

A particularly important outcome from the use of yeast and invertebrate species in aging research is the discovery of conserved genetic pathways that modulate longevity across broad evolutionary distance [15-17]. Insulin-like signaling and the mechanistic target of rapamycin (mTOR), in particular, are now known to modulate the pace of aging from simple eukaryotes through to mammals. In general, insulin-like signaling and mTOR activity are highest under conditions favoring growth, where reproduction is maximized and aging occurs most rapidly. When nutrients and growth cues are scarce, signaling through these pathways is reduced, fecundity is reduced or absent altogether, and longevity is maximized. This is consistent with the idea that aging and reproduction are evolutionarily coupled, such that both processes are simultaneously slowed in order to allow organisms to withstand periods of resource scarcity, then resume faster reproduction (and faster aging) when times are good again.

Dietary restriction and rapamycin

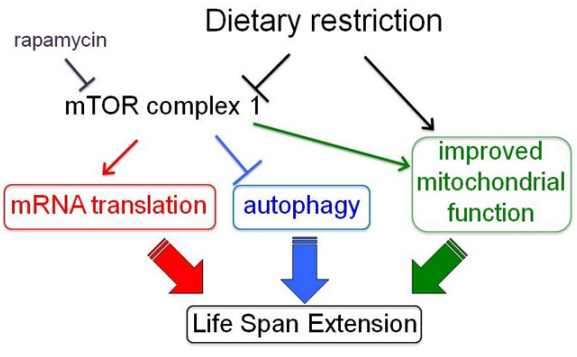
The best-characterized intervention for modulating aging is dietary restriction (also referred to as caloric restriction or calorie restriction). Dietary restriction, which can be defined as a reduction in nutrient availability in the absence of malnutrition, was first found to extend lifespan in rats more than 70 years ago [18]. Since then, hundreds of studies have shown that a reduced calorie regimen can increase lifespan and delay the onset of multiple age-related phenotypes in a diverse range of organisms, including all of the major model systems used in biomedical research [19,20].

Studies of dietary restriction in non-mammalian systems have led to important advances in our understanding of the fundamental relationship between diet and aging. One example is the observation in both *C. elegans* and *D. melanogaster* that, in addition to food consumption, food sensing can also reduce longevity. Simply being exposed to food odorants can attenuate the beneficial effects of dietary restriction in both organisms [21,22]. A second example is that dietary restriction appears to be effective at reducing mortality even when initiated late in life [21,23]. Demographic analysis of several thousand flies indicates that dietary restriction causes a nearly instantaneous shift in the mortality trajectory, as if the risk of death is immediately reduced, without molecular memory of the prior fed state [23]. Return to a control diet again shifts the mortality trajectory back to the original state, or nearly so, and similar effects are also seen in *C. elegans* [21]. One interpretation of these data is that dietary restriction, at least in invertebrates, is not really slowing the rate of aging, but is instead causing individuals to become more resistant to the damage associated with aging. Whether a similar phenomenon occurs in mammals is currently unclear; however, limited

studies seem to indicate that there is a diminishing longevity benefit when dietary restriction is initiated in older animals relative to younger animals, but that late-onset dietary restriction still induces a robust anti-cancer effect in mice [24].

The molecular mechanisms by which dietary restriction achieve such remarkable effects across a diverse range of organisms is an area of active research. Not surprisingly, dietary restriction modulates the activity of multiple cellular factors, several of which have been implicated in longevity and healthspan. These include sirtuins, key metabolic regulators such as AMP kinase, antioxidant enzymes, DNA damage response enzymes, and others [25]. Among these, however, the mTOR pathway, in particular, has repeatedly emerged as a central player in the pro-longevity effects of dietary restriction in yeast, nematodes, and fruit flies [26,27]. In response to nutrient depletion, mTOR activity is reduced and this results in a cascade of downstream events that have been shown to promote longevity and enhance resistance to stress. In particular, reduced synthesis of new proteins via inhibition of mRNA translation, enhanced degradation of damaged proteins and other macromolecules via autophagy, and altered carbon metabolism and mitochondrial function all contribute to lifespan extension from dietary restriction in simple eukaryotes [28] (Figure 2). Similar responses also occur in mammals in response to dietary restriction or mTOR inhibition; however, there is less direct evidence for their involvement in lifespan extension. In addition to these cellular adaptations, it is likely that several broad physiological responses to dietary restriction also play an important role in promoting longevity and health in mammals. Reduced inflammation, decreased levels of growth-promoting hormones, enhanced glucose homeostasis, decreased adiposity, protection from a variety of cancers, and preservation of stem cell function have each been proposed to be an important part of the "dietary restriction effect".

Figure 2. Reduced mTOR signaling extends lifespan in response to dietary restriction



Dietary restriction reduced mechanistic target of rapamycin (mTOR) complex I activity, and studies in yeast, nematodes, and flies have implicated at least three distinct mTOR complex I- regulated processes in lifespan extension from dietary restriction: reduced mRNA translation, increased autophagy, and improved mitochondrial function. The mTOR complex I inhibitor rapamycin has also been shown to extend lifespan in each of these organisms.

Other than dietary restriction, the only non-genetic intervention that has been similarly found to extend lifespan in yeast [29,30], nematodes [31], fruit flies [32], and mice [33] is treatment with the drug rapamycin. Rapamycin is a specific inhibitor of mTOR [34], and has been proposed to act as a "dietary restriction mimetic" by inducing the pro-longevity response to dietary restriction under high nutrient conditions [35]. Recently the pro-longevity effects of mTOR inhibition by rapamycin were also extended to the plant kingdom, with a study showing that rapamycin slows aging in an *Arabidopsis* strain engineered to be sensitive to the drug [36]. The first study showing that rapamycin could extend lifespan in mice was particularly noteworthy for at least two reasons. First, it was carried out in a genetically heterogeneous strain background, alleviating a common concern that many longevity studies are performed in laboratory-adapted inbred mouse lines. Second, the drug was not given to the mice until they had reached 600 days of age, roughly equivalent to 60 years of age in a person [37]. Since then, the pro-longevity effect of rapamycin in mice has been replicated, including initiating the treatment early in life, which yields a slightly greater extension of median longevity than late-life treatment alone [38-40]. Interestingly, initial analysis of end-of-life pathology indicates that rapamycin does not significantly alter the spectrum of causes of death in mice, but instead delays the age-related declines in a variety of parameters including alterations in heart, liver, adrenal glands, endometrium, tendon, and spontaneous activity [38,41]. These data are consistent with the idea that rapamycin is slowing the aging process in mice such that many normal causes of morbidity and death are delayed. It is important to note that these studies have largely been performed using a single dose of rapamycin and the observed effects on longevity and healthspan may be different at higher or lower doses of the drug.

In addition to rapamycin, several other compounds are being actively studied for their potential to delay age-related disease by targeting key aging-related pathways. The most publicized of these is undoubtedly resveratrol, a chemical found in red wine that, like rapamycin, was first reported to increase lifespan in yeast and has also

been proposed to act as a dietary restriction mimetic [42]. Unlike rapamycin, however, the initial studies reporting robust lifespan extension from resveratrol in yeast and invertebrate species have proven controversial [43,44], and attempts to extend lifespan of mice with resveratrol have not been successful [38]. Interestingly, resveratrol does appear to enhance at least some measures of healthspan in mice, in particular improving metabolic function in the context of a high fat diet [45]. Resveratrol also extends survival of mice fed a specific high fat diet formulation [46], although the relevance of this to normal aging is unclear. Dozens of additional compounds are also currently being, or have already been, tested for effects on lifespan by the National Institute on Aging's Interventions Testing Program [47] and individual research groups. Any compound that significantly extends lifespan or healthspan in mice will be of particular interest for future studies in people.

From model organisms to humans

A major unanswered question is whether the longevity interventions identified in model organisms will have similar effects on longevity and healthspan in humans. There are at least three different schools of thought on this issue. The first argues that human aging is fundamentally different from aging in short-lived, laboratory-bred organisms, including rodents. This is based largely on theoretical arguments and cannot be ruled out, but experimental evidence is mounting against this idea (discussed further below). The second viewpoint argues that humans have evolved to have exceptional longevity, and that any additional gains in maximum lifespan are likely to be minimal; however, interventions that significantly extend lifespan in model organisms have the potential to extend healthspan in humans, resulting in a compression in morbidity. The third viewpoint recognizes that the evolutionary distance between yeast and mice is much greater than the distance between mice and humans, and, since longevity interventions have already been identified that span the larger evolutionary distance, there is a good chance these same interventions will have a similar effect on longevity in people. Clearly, the jury is still out on this question, but there is accumulating evidence that interventions, such as dietary restriction, that affect aging in model organisms can also impact age-related diseases in people.

One major limitation of most aging-related studies in model organisms is that they have been performed on inbred or laboratory-bred strains of animals. This creates at least two potential complications: (1) lab strains generally have minimal genetic heterogeneity and (2) lab strains have generally been artificially selected for life in the lab, which usually means rapid reproduction under minimally stressful conditions and an over-abundance of food. While this latter condition (too much food) may in fact better reflect life in most developed countries, it has been speculated that laboratory selection may cause laboratory strains to show a robust response to some interventions, such as dietary restriction, that would not be seen in natural populations. In partial support of this idea, one study in mice found that a wild strain was longer-lived than a standard inbred lab strain under laboratory conditions and failed to show significant median lifespan extension from dietary restriction [48]. Dietary restriction did reduce late life mortality in the wild strain, suggesting at least a partial benefit from dietary restriction [48]. In another study, the question of genetic heterogeneity was addressed by examining the effect of dietary restriction across 41 inbred lines of mice. Surprisingly, although some lines showed the expected lifespan extension from dietary restriction, many showed either no effect or a substantial lifespan reduction [49]. Taken together, these studies suggest that genotype will play a large role in determining individual response to dietary restriction and other interventions that impact aging in people.

Several groups have attempted to begin to address the question of whether dietary restriction is likely to slow human aging experimentally, either through direct studies of dietary restriction in humans or by studying dietary restriction in non-human primates. As discussed above, such studies in humans are limited to correlative measures due to the lack of validated biomarkers of human aging; however, many of the physiological changes associated with dietary restriction in rodents occur similarly in people practicing a reduced-calorie diet. These include reduced adiposity, enhanced glucose homeostasis, decreased blood pressure, and improved cardiac function [50,51], many of which are predictors of improved health and reduced disease risk. Thus, there is evidence that dietary restriction is likely to extend healthspan in people, although it will be many years before this question is answered definitively, and we may never know whether dietary restriction substantially extends lifespan in people.

The first published study of the effects of dietary restriction on longevity in primates was performed in rhesus monkeys and showed a significant reduction in deaths due to age-related causes in the restricted group relative to the control fed group [52]. More importantly, this study and others also showed that dietary restriction dramatically reduced the incidence of age-related disorders including, sarcopenia, cancer, diabetes, cardiovascular disease, as well as changes in brain structure and function [52-55]. In a more recent report, a second

rhesus monkey study performed at the United States National Institute on Aging failed to detect a significant increase in survival from dietary restriction, although this study did find that dietary restriction appeared to improve some measures of healthspan [56]. The reason for the different outcomes from these two studies remains an active area of investigation, and there are several differences in experimental design that may be involved, including the use of different diet formulations, animals taken from different geographic locations, genetic heterogeneity among the populations, and the age at which dietary restriction was initiated.

Additional support for the idea that human aging shares features with aging in other species comes from direct studies of DNA polymorphisms associated with longevity in people. The best example of this is the Foxo3a gene, which encodes a transcription factor that modulates a variety of cellular processes, including cell death, growth, and stress resistance. Multiple independent studies have identified a variant of Foxo3a that is overrepresented in the longest-lived individuals, including cohorts from Germany, Italy, China, and the United States [57-61]. Foxo3a orthologs in both worms and flies are known to play a central role in modulating lifespan in both species in response to reduced insulin-like signaling [15,62,63]. Thus, although the relationship in people between Foxo3a and aging remains correlative, the finding that this highly conserved longevity factor is associated with human longevity is quite suggestive.

There is also accumulating evidence that, as in other species, mTOR signaling may play a central role in human aging. Activation of mTOR has long been associated with a variety of human cancers, and rapamycin is already clinically approved for treating certain rare forms of cancer [64]. In addition to cancer, aberrant activation of mTOR has also been linked to several additional age-related disorders, including cardiovascular disease, peripheral insulin resistance and diabetes associated with obesity, and kidney disease [34,65]. Thus far, there is little direct evidence in humans that mTOR modulates neurological changes with age in people; however, there is a large body of such literature in rodent models, including recent studies showing that rapamycin improves function in two different Alzheimer's disease models [66,67], as well as delays normal age-association cognitive decline [68,69]. Currently, the NIH clinical trials database (http://clinicaltrials.gov/) lists more than 1,300 clinical trials associated with the search term "rapamycin". The information gained from these trials, along with the continued development and testing of newer and more potent inhibitors of mTOR and other components of the mTOR pathway, may allow for the first effective interventions against human aging.

Conclusion

Aging research has progressed to the point where interventions that modulate human aging are a realistic possibility. In fact, they may already exist among the candidate molecules currently being explored. The potential benefits of such interventions dwarf those that can be attained by traditional disease-centered approaches and are necessary to confront the looming "silver tsunami". Before such benefits can be realized, however, there are challenges that must be overcome. Among these are the need for better methods to confirm and validate putative longevity- and healthspan-promoting interventions, and an improved understanding of the complexities associated with genetically and environmentally heterogeneous populations. Despite these difficulties, there is growing confidence that the next decade will see significant advances in aging research making a profound impact on age-related disability and disease. Such advances can't come too soon. After all, we're not getting any younger.

Abbreviation

mTOR, mechanistic target of rapamycin.

Disclosures

The author declares that they have no disclosures.

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