Extended Longevity

Scientific White Paper

Telogenic Formula, increases telomere length. Includes phytotherapeutic extracts of: *Astragalus membracanus, Centella asiatica and Salix Alba*. Biological Actions, Molecular Mechanisms, and Their Effects.

Steven M Schorr
Extended Longevity, Inc., *Department of Scientific Research. P.O. Box 448 Puunene, HI 96784 USA*

Telogenic Formula is designed to increase the length of telomeres and contains extracts of: ***Astragalus membranaceus, Centella asiatica and Salix alba.*** This formula is a synergistic herbal analog providing support for Telomere rejuvenation.

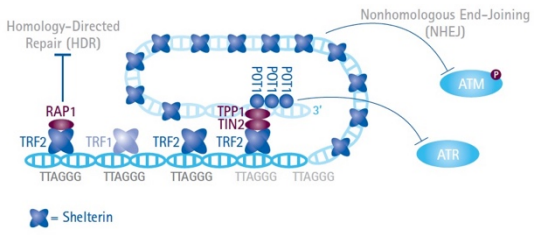
Telomeres

Telomeres are the protective caps found at the ends of your chromosomes that protect our genetic information during cellular division. For our bodies to heal and function properly, cells must divide to produce new cells to replace old, worn-out cells. Telomeres allow our cells to divide without damaging or scrambling the cells' genetic information. Telomeres are like the plastic tips on shoelaces, as they keep the chromosome ends from tangling and fraying. When we are born, our telomeres are at their longest. With every cell division throughout the course of our life, our telomeres lose a bit of their DNA. With age and accumulated exposures to various sources of oxidative stress throughout our lifetimes, telomeres gradually shorten, until the cell cannot replicate. This shortening process acts as an aging clock counting down the remaining life of the cell. At a certain point, chromosomes in the cell reach a critical length and can no longer be replicated. When this occurs, the cell enters into a state of growth arrest, known as "cellular senescence," which is the equivalent of aging. Cellular senescence is a primary driver of the aging process, which links cellular damage with the larger, anatomical effects of aging. Senescent cells do not directly cause aging, but instead have a cumulative effect leading to larger, more visible consequences of tissue breakdown, and over time, the signs and symptoms of old age - sagging, wrinkled skin, decreased muscle mass, weakened immunity, etc. This mechanism explains how microscopic changes to our trillions of cells slowly manifest in the gradual, almost invisible process of aging. Senescent cells differ from their younger counterparts. Cells that contain chromosomes with telomeres approaching a critically short length undergo changes that result in further damage to the organism. Whereas young cells secrete proteins that maintain healthy, functioning tissue, cells approaching senescence begin to secrete inflammatory cytokines that break down these proteins.

Telomere Overview

In 1961, American biologist Leonard Hayflick found that in normal cultured fibroblasts, even if the cells were properly grown, the cells would fail when they split to a certain number of algebras, thus making the cell cycle into an irreversible stagnation state (cell senescence). This is the first time that the cell's longevity and proliferative capacity

are limited, namely the Hayflick boundary. Cellular senescence means that the normal physiological function and proliferative capacity of cells will gradually decline over time. This process is important for tumor, tissue regeneration, and body aging. Cellular senescence mainly includes two types of stress-induced senescence caused by telomere shortening and sensation of external stimuli (such as DNA damage, changes in chromatin structure, and overexpression of oncogenes like Ras and Raf). Telomere shortening or telomere structure disruption is the main cause of cellular senescence. When the cells are senescent, the changes in cell



Telomeres are bound by protein complexes of TRF2, shelterin, and RAP1 to inhibit homology-directed repair (HDR). The T-loop structure at telomere ends, also containing TIN2, TPP1, and POT1, blocks DSB repair.

structure mainly include volume enlargement, nuclear sag, nuclear membrane disintegration, chromatin structure changes, and mitochondrial decrease; functional degeneration changes are mainly caused by loss of cell replication ability, cells cyclic stagnation, decreased stressor sensitivity, down-regulation of cell cycle gene expression, up-regulation of cell cycle inhibitors and other senescence-related genes. Usually, the body's aging cells and new cells continue to be replaced, thus maintaining normal life activities, and the overall level of aging is generally considered to be the basis of the body's aging.

Telomerase in somatic cells

Research has shown that telomerase can be reactivated in healthy adult cells during the course of cell aging. the reactivation of telomerase in adult cells that goes beyond the formation of cancer cells, as the transient activation of telomerase in this manner serves to mediate cellular senescence and reduce the risk of cancer. Each of the chromosomes that stores our genetic information has a protective cap at each end known as a telomere, a specific DNA sequence that is repeated thousands of times. The sequence has two purposes: firstly, it protects the coding regions of the chromosomes and prevents them from being damaged, and secondly, it acts as a clock that controls the number of replications that a cell can make. Each time a cell divides, the telomeres become shorter; this telomere attrition is the basis of replicative senescence.

In regular adult cells, once the cell reaches its replicative limit triggered by the telomeres reaching a critical length, the cell stops dividing and dies via a self-destruct mechanism called apoptosis. However, sometimes the cell experiences DNA damage, fails to enter apoptosis, and lingers, sometimes becoming a pro-inflammatory senescent cell. In some special types of cells, such as stem and progenitor cells, telomerase remains active, which means that these cells keep topping up their telomeres as they divide, preventing them from reaching a critical length. This is why stem cells can live for many decades compared to the shorter-lived regular cells that make up the majority of the cells in our body. The telomerase enzyme itself is made up of two parts: the template RNA (TR, telomerase RNA), and the reverse-transcriptase catalytic subunit (TERT, telomerase reverse transcriptase).

A new role for telomerase in aging and transformation.

The reactivation of telomerase in telomerase-deficient cells was able to rescue the cells from death, allowed them to keep dividing,

and reduced the chance of DNA damage occurring. They also showed that this happens in normal human skin cells, which also activate a burst of telomerase as they approach critical telomere length..

Astragalus membranaceus is a small molecule activator of telomerase and is capable of increasing average telomere length and decreasing the percentage of critically short telomeres. Treatment with astragalus extract results in telomerase-dependent elongation of short telomeres and rescue of associated DNA damage, thus demonstrating that the mechanism of action is through the telomerase pathway. Use leads to an improvement of certain health-span indicators including glucose tolerance, osteoporosis and skin fitness, without significantly increasing global cancer incidence. Astragalus contains life-prolonging compounds for human use and is "associated with a significant age-reversal effect in the immune system, in that it led to declines in the percentage of senescent cytotoxic T cells and natural killer cells after six to twelve months of use". Harley, C. B.; et al. (2011). Astragalus root is known for its effects on telomerase, the shortening of telomeres (resulting from such factors as stress and aging). Short telomeres result in chromosome instability.

Centella asiatica

Centella asiatica extract was able to trigger an almost 9-fold increase in telomerase activity compared to the untreated cells, suggesting that it could be a strong natural telomerase activator with important anti-aging effects. It is a widely used Ayurvedic medicine and traditional Chinese medicine, which has been shown to be effective in improving cognitive ability, increasing antioxidant response, as well as treating wound healing disturbances. In investigating the effect of Centella asiatica on cognitive ability, as well as mitochondrial and antioxidant response, it was shown that treatment enhanced cognitive ability in led to higher expression of mitochondrial and antioxidant genes in the brain and liver, which could contribute to cognitive improvement. It has also been suggested that it can heal wounds due to the specific plant chemicals that it contains, known as triterpenoid saponins.

Salix alba Study

In the recent study, published in the journal *Oncotarget*, researchers from Concordia and the Quebec-based biotech company Idunn Technologies conducted more than 10,000 trials screening for plant extracts believed to increase the lifespan of yeast. Yeast is the most common organism studied in anti-aging research. From a cellular viewpoint, yeast ages in a similar way to people. It is one of the best models to understand how aging occurs. "In total, we found six new groups of molecules that decelerate the chronological aging of yeast," Vladimir Titorenko, senior author of the study and professor in the Department of Biology at Concordia, said in a press release. The extracts were derived from *Cimicifuga racemosa*, *Valeriana officinalis L.*, *Passiflora incarnata L.*, *Ginkgo biloba*, *Apium graveolens L.* and *Salix alba*.

Salix alba Tests

Researchers described an extract of willow bark (*Salix alba*), as "the most potent longevity-extending pharmacological interventions yet described in the scientific literature." In particular, the study found that the willow bark extract increased the average chronological lifespan of yeast by 475% and maximum chronological lifespan 369%.

Active Herbal Ingredients

Astragalus membranaceus, is a small molecule activator of telomerase and is capable of increasing average telomere length and decreasing the percentage of critically short telomeres. It contains life-prolonging compounds for human use and is "associated with a significant age-reversal effect in the immune system, in that it led to declines in the percentage of senescent cytotoxic T cells and natural killer cells after six to twelve months of use". Harley, C. B.; et al. (2011). Astragalus root is known for its effects on telomerase, the shortening of telomeres (resulting from such factors as stress and aging). Thus, short telomeres result in chromosome instability.

Centella asiatica, extract was able to trigger an almost 9-fold increase in telomerase activity compared to the untreated cells and is a strong natural telomerase activator with important anti-aging effects. It is a widely used Ayurvedic medicine and traditional Chinese medicine, which is effective in improving cognitive ability, increasing antioxidant response, as well as treating wound healing.

Salix alba. A research group screened 37 different plant extracts to see what effect they might have on slowing aging and extending life. Willow bark was found to be the most potent life extension substance ever found. A specific extract of white willow bark (Salix alba) is the most potent longevity - extending pharmacological intervention ever described in the scientific literature. In testing, the white willow bark extract increased the average chronological lifespan of yeast by 475 percent and the maximum chronological lifespan by 369 percent.

CELL CYCLE

As published in BT*i* October 2005

Telomere length: the biological clock reviewed

by Dr Sofie Bekaert

As eukaryotic cells divide, the protective ends of the linear chromosomes, the telomeres, gradually shorten with each cell division. When a critical telomere length is reached, the cells are signalled into senescence, an irreversible state of quiescence. Thus, telomere length has emerged as a replicative clock within each population of cells and the tissues and organs they form *in vitro*. Consequently telomere length has become accepted as a biomarker for biological ageing *in vivo*. Although chronological ageing *per se* does not parallel biological ageing, there are no accurate and reliable biomarkers to distinguish between both types of ageing. The question remains whether telomere dynamics is a determinant or merely a predictor of human biological age over and above chronological ageing.

Telomeres - tandem-repeated TTAGGG hexamers at the termini of mammalian chromosomes - are associated with specific proteins to form protective caps that prevent the chromosome ends from being recognised as double strand breaks by the DNA repair machinery of the cell. When telomeres become either shorter than a critical length or are missing completely, the normal cell cycle is halted and repair mechanisms are induced. Although the repair of cells that form our tissues and organs is of course crucial to maintain a healthy state, the investment in such repair decreases. From an evolutionary point of view, this concept is expressed as the "Disposable soma theory", which postulates that the key to ageing is determined by the equilibrium between the energy devoted to reproduction and that devoted to somatic cell repair. For example, humans have a much longer survival rate than field mice and devote more energy to repair and maintenance, whereas the field mouse, which suffers from a high level of predation, devotes most energy to early reproduction. For long-living organisms ageing is not a well regulated process, but rather the result of erroneous and diminished repair. Such lack of maintenance increases the chance of the occurrence of ageing disorders such as cancer or cardiovascular disease.

Cell reproduction *in vitro*

When a closer look is taken at ageing at the cellular level, some interesting observations can be made. Replication of normal human cells in culture is not infinite; proliferation only continues for about 40-60 population doublings (PDs). Subsequently the cell cycle is halted and the cell shows senescence (which is a form of cellular ageing) and eventually dies. This ageing phenomenon is known as the 'Hayflick-limit' [1].

One outcome from these *in vitro* studies is the observation that the number of PDs that cells can undergo is inversely proportional to the age of the donor from which the cells were taken in the first place. Telomeres are the basis for this limited replicative capacity of cells. Telomere shortening is in fact a kind of molecular clock that determines the proliferative capacity and hence the life span of cells [Figure 1]. At the Hayflick limit (mortality stage 1 = senescence) one or more telomeres become critically short. They are recognised within the cell as chromosome breaks and the cell cycle is irreversibly arrested. The signal that induces replicative senescence is not the shortened telomere sequence *per se*, but rather the loss of the protective telomeric cap (telomere specific proteins binding to the telomere), which creates dysfunctional telomeres. At this stage cell cycle checkpoints are activated and replicative senescence or programmed cell death (apoptosis) is induced. However, if these checkpoint systems are absent, the cells continue to proliferate and telomere erosion gradually continues until nearly all the telomeres reach a critical length and the cells enter crisis (mortality stage 2). This is characterised by chromosomal instability because of erroneous DNA damage repair and the propagation of genomic errors and DNA breaks. At this point the number of cell divisions is counterbalanced by an equal number of cell deaths. Chromosomal end fusions and other cytological abnormalities accumulate [2].

In fact this stochastic mechanism of cell maintenance dilutes the build up of irreparable cells, yet paradoxically this accelerated cell turnover also increases the chance of mutations. Only a few rare cells can escape this crisis, doing so by the activation of the specialised telomere-synthesising enzyme, telomerase. This enzyme synthesises the required telomeric repeats onto the 3' overhangs of the telomere. In this way an equilibrium is established at this short telomere length, the cells can continue to proliferate and thus they become immortal. This telomere length equilibrium regulated by telomerase is present in single-celled organisms such as yeast, in stem cells and reproductive cells. Telomerase activity is also detected in about 90% of all tumours in humans.

As a result of this observation, much attention has been focussed over the past few years on telomerase inhibition as a surrogate cancer treatment. However, such telomerase inhibition therapy is not without risk. Blocking telomerase can impair fertility, wound healing and the production of blood cells and immune system cells. In addition, some cells induce alternative telomere lengthening mechanisms (ALT) resulting in very long and heterogeneous telomeres.

Telomere length and regulation

Nevertheless, telomerase is still considered the missing link proving the existence of a telomere-based mitotic clock. Ectopically expressed telomerase in mortal cells results in an increase in average telomere length with a consequent rise in the replicative potential of the cells [3].

The appreciation of the implications of telomere biology both for ageing and ageing disorders such as cancer, has increased steadily. After the identification of the TTAGGG telomeric sequence, several quantitative assays

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JCB: Review

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Telomere-driven diseases and telomere-targeting therapies

Paula Martínez and Maria A. Blasco

Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre, Madrid E-28029, Spain

Telomeres, the protective ends of linear chromosomes, shorten throughout an individual's lifetime. Telomere shortening is proposed to be a primary molecular cause of aging. Short telomeres block the proliferative capacity of stem cells, affecting their potential to regenerate tissues, and trigger the development of age-associated diseases. Mutations in telomere maintenance genes are associated with pathologies referred to as telomere syndromes, including Hoyeraal-Hreidarsson syndrome, dyskeratosis congenita, pulmonary fibrosis, aplastic anemia, and liver fibrosis. Telomere shortening induces chromosomal instability that, in the absence of functional tumor suppressor genes, can contribute to tumorigenesis. In addition, mutations in telomere length maintenance genes and in shelterin components, the protein complex that protects telomeres, have been found to be associated with different types of cancer. These observations have encouraged the development of therapeutic strategies to treat and prevent telomere-associated diseases, namely aging-related diseases, including cancer. Here we review the molecular mechanisms underlying telomere-driven diseases and highlight recent advances in the preclinical development of telomere-targeted therapies using mouse models.

Telomeres, telomerase, and shelterins

Telomeres form a special heterochromatic structure at the end of linear chromosomes that protects them from degradation and DNA repair and recombination activities. Thus, telomeres are essential to ensure chromosome stability (Blasco, 2005; Palm and de Lange, 2008). Mammalian telomeres comprise several kilobases, between 10 and 15 kb in humans and 25 and 50 kb in mice, of tandem TTAGGG DNA repeats (Blasco, 2005). Telomeres are characterized by the presence of a 30–400-nucleotide-long 3′ overhang of a G-rich strand, known as the G-strand overhang. The G-strand overhang can fold back and invade the double-stranded telomeric region, forming the so-called T-loop and generating a displacement loop, or D-loop. The T-loop structure has been proposed to protect chromosome ends from degradation and DNA repair activities as well as from telomerase activity (Fig. 1, A and B; Griffith et al., 1999; Doksani et al., 2013). Telomeres are bound by a specialized complex known as shelterin that has crucial functions in telomere length regulation and in the protection of telomeres from the DNA damage response (DDR) by masking the chromosome ends from the DNA repair machinery through repression of the ATM and ATR signaling pathways (Palm and de Lange, 2008; Fig. 1 C). The shelterin complex is composed of six proteins: telomeric repeat binding factors 1 and 2 (TRF1 and TRF2), TRF1-interacting protein 2 (TIN2), protection of telomeres protein 1 (POT1), TIN2, POT1-interacting protein (TPP1), and repressor/activator protein 1 (RAP1; Fig. 1 C; de Lange, 2005; Martínez and Blasco, 2010, 2011).

Telomeres shorten with each cell division as a result of the incomplete replication of linear DNA molecules by conventional DNA polymerases, which is called the end-replication problem (Watson, 1972; Olovnikov, 1973). Telomerase compensates for telomere attrition through de novo addition of TTAGGG repeats onto chromosome ends in those cells where it is normally expressed, such as pluripotent stem cells and adult stem cell compartments (Liu et al., 2007; Flores et al., 2008; Marion et al., 2009). Telomerase is composed of a reverse transcriptase subunit (TERT) as well as an associated RNA component (*Terc*), which is used as a template for the de novo addition of telomeric repeats (Fig. 1 C; Greider and Blackburn, 1985). Although telomerase is expressed in adult stem cell compartments, this is not sufficient to counteract telomere attrition associated with cell division throughout life, and therefore telomeres shorten with age in vitro and in vivo (Harley et al., 1990; Hastie et al., 1990; Lindsey et al., 1991; Collado et al., 2007; Liu et al., 2007; Flores et al., 2008; Marion et al., 2009). This progressive telomere shortening eventually leads to critically short telomeres that can impair the regenerative capacity of tissues and has been proposed as one of the molecular hallmarks of aging (López-Otín et al., 2013). In mice, it has been shown that the rate of increase in the percentage of short telomeres, rather than the rate of telomere shortening throughout life, is a significant predictor of life span (Vera et al., 2012). Shortened telomeres induce a DDR that leads to a growth arrest, during which cells attempt to repair the damage and, if DNA damage is irreparable, triggers replicative senescence (Zou et al., 2004; Fumagalli et al., 2012). Senescent cells progressively accumu-

Correspondence to Maria A. Blasco: mblasco@cnio.es

Abbreviations used: 6-thio-dG, 6-thio-2′-deoxyguanosine; AAV, adeno-associated vector; ALT, alternative lengthening of telomeres; BM, bone marrow; DC, dyskeratosis congenita; DDR, DNA damage response; HHS, Hoyeraal-Hreidarsson syndrome; HSPC, hematopoietic stem/progenitor cell; IPF, idiopathic PF; MC, mitotic catastrophe; PF, pulmonary fibrosis; TPE, telomere position effect.

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Extended Longevity

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REVIEW

Role of Telomeres and Telomerase in Aging and Cancer

Jerry W. Shay[1,2]

ABSTRACT Telomeres progressively shorten throughout life. A hallmark of advanced malignancies is the ability for continuous cell divisions that almost universally correlates with the stabilization of telomere length by the reactivation of telomerase. The repression of telomerase and shorter telomeres in humans may have evolved, in part, as an anticancer protection mechanism. Although there is still much we do not understand about the regulation of telomerase, it remains a very attractive and novel target for cancer therapeutics. This review focuses on the current state of advances in the telomerase area, identifies outstanding questions, and addresses areas and methods that need refinement.

Significance: Despite many recent advances, telomerase remains a challenging target for cancer therapy. There are few telomerase-directed therapies, and many of the assays used to measure telomeres and telomerase have serious limitations. This review provides an overview of the current state of the field and how recent advances could affect future research and treatment approaches. *Cancer Discov; 6(6); 584–93. ©2016 AACR.*

INTRODUCTION

Historical Background

Telomere terminal transferase (telomerase) enzyme activity (not the identification of the genes encoding the components of telomerase) was discovered in 1985 in the single-cell organism *Tetrahymena* (1). Almost a decade later, telomerase was described as an almost universal marker in advanced human cancers (2, 3), but it was not until 1997 that the catalytic protein component was isolated, first in yeast (4) and shortly thereafter in humans (5, 6). It is well recognized that telomeres progressively shorten with increased age *in vitro* and *in vivo* (7–14) and, in combination with a series of oncogenic changes, cells with short telomeres escape senescence and become immortal (Fig. 1), generally by activating or upregulating telomerase. Most human tumors (85%–90%) not only constitutively express telomerase (2) but also have short telomeres, whereas telomerase activity is absent in most normal tissues or is highly regulated in normal transit-amplifying stem-like cells, making the inhibition of telomerase an attractive target for cancer therapeutics (2).

[1]Department of Cell Biology, The University of Texas Southwestern Medical Center, Dallas, Texas. [2]Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia.

Corresponding Author: Jerry W. Shay, The University of Texas Southwestern Medical Center, Department of Cell Biology, 5323 Harry Hines Boulevard, Dallas, TX 75930-9039. Phone: 214-648-4201; Fax: 214-648-5814; E-mail: Jerry.Shay@utsouthwestern.edu

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Telomerase is a reverse transcriptase that adds new DNA onto the telomeres that are located at the ends of chromosomes (1, 15–17). Although the importance of telomeres has been recognized for a long time (18–19), the DNA sequence of telomeres was a somewhat more recent discovery (20–21). Telomeres in mammals consist of long tracts of the hexameric TTAGGG nucleotide repeat and an associated protein complex termed shelterin (22–23). The shelterin complex protects chromosomes from end-to-end fusions and degradation by forming special t-loop–like structures (24), thus masking the very ends of chromosomes from being recognized as double-strand DNA breaks. The TTAGGG repeats shorten with each cell division due to the "end replication problem" (25, 26), oxidative damage, and other still poorly understood end-processing events. When a few telomeres become critically shortened, there is a growth arrest state, at which time a DNA-damage signal and cellular senescence is normally triggered (27–29). In the absence of other changes, cells can remain in a quiescent/senescent state for years, which can be considered a tumor suppressor mechanism at least for long-lived species such as humans. It is a common misconception that normal senescent cells undergo apoptosis and die. It is now recognized that senescent cells can secrete factors that can influence age-associated diseases (30) and remain viable but not dividing for long periods of time. Thus, with increased age it is believed that there is a gradual accumulation of senescent cells that may affect some aspects of aging.

In contrast, human carcinomas (tumors derived from epithelial tissues) almost universally bypass cellular senescence and DNA damage–induced inhibitory signaling pathways by upregulating telomerase. Regulated telomerase activity is present in

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Telomere length & MORTALITY

Cawthon RM, et al. **Association Between Telomere Length in Blood and Mortality in People Aged 60 Years or Older.** *Lancet.* 2003 Feb 1;361(9355):393-5.

This study assessed the association between blood leukocyte average telomere length and mortality in 143 normal unrelated individuals over the age of 60 years. Individuals with shorter telomeres had poorer survival, attributable in part to a 3.18-fold higher mortality rate from heart disease (95% CI 1(.)36-7.45, p=0.0079), and an 8.54-fold higher mortality rate from infectious disease (1.52-47.9, p=0.015). These results support that telomere shortening in human beings contributes to mortality in many age-related diseases.

Needham BL, et al. **Leukocyte Telomere Length and Mortality in the National Health and Nutrition Examination Survey, 1999–2002.** *Epidemiology*. 2015 July; 26(4): 528–535.

This study examined the association between leukocyte telomere length and mortality in US adults aged 50–84. (n=3,091). A decrease of 1 kilobase pair in telomere length at baseline was marginally associated with a 10% increased hazard of all-cause mortality (HR: 1.1, 95% CI: 0.9, 1.4) and a 30% increased hazard of death due to diseases other than cardiovascular disease or cancer (HR: 1.3, 95% CI: 0.9, 1.9).

Njajou OT, et al. **Association Between Telomere Length, Specific Causes of Death, and Years of Healthy Life in Health, Aging, and Body Composition, a Population-Based Cohort Study.** *Health ABC study. J Gerontol A Biol Sci Med Sci.* 2009.

Health ABC study, a community-based cohort of 3,075 healthy, well-functioning, men and women aged 70–79 years. Average Telomere Length, as measured by Q-PCR, was assessed to see if those with the shortest ATL have poorer survival, shorter life span, and fewer years of healthy life (YHL).

Longer telomere length was associated with more years of healthy living. Longer ATL was positively associated with longer years of healthy life (p = .03). Findings suggest that ATL may be an informative biomarker of healthy aging.

Telomere length & DIABETES

Zhao J, et al. **Association Between Telomere Length and Type 2 Diabetes Mellitus: A Meta-Analysis.** *PLOS ONE.* 2013, November 2013 | Volume 8 | Issue 11 | e79993.

In meta-analysis of 5,759 cases and 6,518 controls in nine cohorts, shortened telomere length was significantly associated with type two diabetes mellitus (OR: 1.291; P,0.001) with heterogeneity (I2 = 71.6%). When three cohorts responsible for the heterogeneity were excluded, the pooled OR for the remaining cohorts indicated a significant association remained (OR: 1.117; P = 0.045).

Gmail by Google

Steven Schorr <sms@intelligentremedies.com>

[New post] Telomeres: The Longer the Better

1 message

Josh Mitteldorf <donotreply@wordpress.com> Tue, Oct 29, 2019 at 7:04 AM
To: sms@intelligentremedies.com

New post on **Josh Mitteldorf**

Telomeres: The Longer the Better

by Josh Mitteldorf

Mice have much longer telomeres than we do, long enough that telomeres never get critically short in a mouse lifetime. Yet, when designer mice were engineered to have even longer telomeres (hyper-long by any standard, longer than we can account for the use of them), these mice lived longer and were healthier in every way than mice with normal-long telomeres. Lab mice usually die of cancer, and these with the longer telomeres were protected from cancer, along with every other ailment that was looked at.

First, I ask your indulgence if I harp on the obvious: this result is not consistent with the prevailing theory of telomeres. In most vertebrates, telomerase is rationed so that telomeres are allowed gradually to shorten over a lifetime, and this is explained by most evolutionary biologists and geroscientists as an anti-cancer program. According to theory, in each species, telomere length has been optimized by natural selection as a compromise between longer telomeres (allowing stem cells to last longer without senescing) and shorter telomeres (which provide a firewall against cancer, a drop-dead signal when unchecked cell growth might be life-threatening). In contrast, experiments have frequently shown that longer telomeres lead to a ***lower*** cancer rate. Blasco's new result is a clear case. We can't explain telomere dynamics as a cancer prevention program.

(For background on what telomeres are and how they function, I refer you to my early blogs on the subject.)

But beyond this, there remain many mysteries. This study highlights the truth that we don't understand the mechanisms. How exactly are hyper-long telomeres working on a biochemical level? What can a hyper-long telomere do that an extra-long (regular mouse) telomere can't do?

Known mechanisms include:

- Senescent cells. Much of the literature has focused on the importance not of ***average TL*** but on the ***shortest*** because a few cells run out of telomere and

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The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence

Bruno Bernardes de Jesus[1], **Kerstin Schneeberger**[1], **Elsa Vera**[1,2], **Agueda Tejera**[1], **Calvin B. Harley**[3], and **Maria A. Blasco**[1]

[1]Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Melchor Fernández Almagro 3, Madrid, E-28029, Spain

[2]Life Length, S.L., Agustín de Betancourt 21, Madrid, E-28003, Spain

[3]Telome Health, Menlo Park, CA, 94025, USA

Abstract

Here, we show that a small-molecule activator of telomerase (TA-65) purified from the root of *Astragalus membranaceus* is capable of increasing average telomere length and decreasing the percentage of critically short telomeres and of DNA damage in haploinsufficient mouse embryonic fibroblasts (MEFs) that harbor critically short telomeres and a single copy of the telomerase RNA *Terc* gene (G3 $Terc^{+/-}$ MEFs). Importantly, TA-65 does not cause telomere elongation or rescues DNA damage in similarly treated telomerase-deficient G3 $Terc^{-/-}$ littermate MEFs. These results indicate that TA-65 treatment results in telomerase-dependent elongation of short telomeres and rescue of associated DNA damage, thus demonstrating that TA-65 mechanism of action is through the telomerase pathway. In addition, we demonstrate that TA-65 is capable of increasing mTERT levels in some mouse tissues and elongating critically short telomeres when supplemented as part of a standard diet in mice. Finally, TA-65 dietary supplementation in female mice leads to an improvement of certain health-span indicators including glucose tolerance, osteoporosis and skin fitness, without significantly increasing global cancer incidence.

Keywords

telomerase activation; TA-65; telomere length; aging; mouse

Introduction

Progressive attrition of telomeres is one of the best understood molecular changes associated with organismal aging in humans (Harley *et al*. 1990) and in mice (Flores *et al*. 2008). Telomeres are specialized structures at the ends of chromosomes, with an essential role in protecting the chromosome ends from fusions and degradation (Blackburn 2001; de Lange 2005). Mammalian telomeres consist of TTAGGG repeats bound by a six-protein complex known as shelterin (de Lange 2005). A minimum length of TTAGGG repeats and the integrity of the shelterin complex are necessary for telomere protection (Blackburn 2001; de

#To whom correspondence should be addressed: mblasco@cnio.es.

Author contributions M.A.B conceived the idea. B.B. performed most of the experiments of the paper. K.S. performed the TA-65 administration, and performed Fig 1d-g and Fig. 5d. E.V. performed telomere length determinations (Fig. 3). A.T. performed the TRAP assays (Fig 1b and Sup. Fig. 1). M.A.B and B.B. wrote the paper.

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Discovery of potent telomerase activators: Unfolding new therapeutic and anti-aging perspectives

DIMITRIS TSOUKALAS[1-3*], PERSEFONI FRAGKIADAKI[3,4*], ANCA OANA DOCEA[5*], ATHANASIOS K. ALEGAKIS[3], EVANGELIA SARANDI[1,3], MARIA THANASOULA[1], DEMETRIOS A. SPANDIDOS[6], ARISTIDIS TSATSAKIS[3,4], MAYYA PETROVNA RAZGONOVA[7] and DANIELA CALINA[2]

[1]Metabolomic Medicine, Health Clinics for Autoimmune and Chronic Diseases, 10674 Athens, Greece; [2]Department of Clinical Pharmacy, University of Medicine and Pharmacy, Faculty of Pharmacy, 200349 Craiova, Romania; [3]Laboratory of Toxicology, Medical School, University of Crete, 71003 Heraklion; [4]Spin-Off Toxplus S.A., 71601 Heraklion, Greece; [5]Department of Toxicology, University of Medicine and Pharmacy, Faculty of Pharmacy, 200349 Craiova, Romania; [6]Laboratory of Clinical Virology, School of Medicine, University of Crete, 71003 Heraklion, Greece; [7]SEC Nanotechnology, Far Eastern Federal University, Vladivostok 690950, Russia

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Abstract. Telomere length, a marker of cellular aging, decreases with age and it has been associated with aging-related diseases. Environmental factors, including diet and lifestyle factors, affect the rate of telomere shortening which can be reversed by telomerase. Telomerase activation by natural molecules has been suggested to be an anti-aging modulator that can play a role in the treatment of aging-related diseases. This study aimed to investigate the effect of natural compounds on telomerase activity in human peripheral blood mononuclear cells (PBMCs). The tested compounds included *Centella asiatica* extract formulation (08AGTLF), Astragalus extract formulation (Nutrient 4), TA-65 (containing *Astragalus membranaceus* extract), oleanolic acid (OA), maslinic acid (MA), and 3 multi-nutrient formulas (Nutrients 1, 2 and 3) at various concentrations. The mean absorbance values of telomerase activity measured following treatment with some of the above-mentioned formulations were statistically significantly higher compared to those of the untreated cells. In particular, in order of importance with respect to telomerase activation from highest to lowest, 08AGTLF, OA, Nutrient 4, TA-65, MA, Nutrient 3 and Nutrient 2, triggered statistically significant increase in telomerase activity compared to the untreated cells. 08AGTLF reached the highest levels of telomerase activity reported to date, at least to our knowledge, increasing telomerase activity by 8.8 folds compared to untreated cells, while Nutrient 4 and OA were also potent activators (4.3-fold and 5.9-fold increase, respectively). On the whole, this study indicates that the synergistic effect of nutrients and natural compounds can activate telomerase and produce more potent formulations. Human clinical studies using these formulations are required to evaluate their mode of action. This would reveal the health benefits of telomerase activation through natural molecules and would shed new light onto the treatment of aging-related diseases.

Correspondence to: Professor Aristidis Tsatsakis, Laboratory of Toxicology, Medical School, University of Crete, P.O. Box 1393, 71003 Heraklion, Greece
E-mail: tsatsaka@med.uoc.gr

*Contributed equally

Abbreviations: CAD, cardiovascular disease; BMI, body mass index; TLDP, telomere length database project; COPD, chronic obstructive pulmonary disease; TERT, transcriptase catalytic subunit; TERC, telomerase RNA component; DKC, Dyskeratosis congenita; 08AGTLF, *Centella asiatica* extract formulation; MA, maslinic acid; OA, oleanolic acid; PBMCs, peripheral blood mononuclear cells; CAG, cycloastragenol

Key words: telomerase activity, natural molecules, telomere length, PBMCs

Introduction

Several studies have indicated that short telomere length is associated with aging-related diseases, including cardiovascular diseases (CADs), stroke, cancer, arthritis, osteoporosis, cataracts, diabetes type 2, hypertension, mental diseases, chronic obstructive pulmonary disease (COPD) and dementia (1). Telomere shortening can be affected by environmental factors, including physical activity, body mass index (BMI), hormone replacement therapy, smoking, chronic inflammation, oxidative stress, dietary antioxidants and vitamins (2-5). For instance, DNA-damage caused by various environmental factors triggers a DNA-damage response at telomeres that protects them from instability and shortening (6,7). Moreover, Vakonaki *et al* demonstrated an association between telomere length and drug abuse, which leads to premature biological aging (8). Telomere length has been proposed to be a biomarker of somatic cell aging, while the rate of increase of short telomeres has been

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In vitro and *in vivo* immunomodulating and immunorestorative effects of *Astragalus membranaceus*

William Chi Shing Cho [a,*], Kwok Nam Leung [b]

[a] *Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong, SAR, China*
[b] *Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong, SAR, China*

Abstract

Astragalus membranaceus is a common traditional Chinese medicinal plant widely used as a tonic to enhance the body's natural defense mechanisms. In this study, bioactive fractions were isolated from the roots of *Astragalus membranaceus*. One of these fractions, designated as AI, was found to be the most potent with respect to its mitogenicity on murine splenocytes. Effects of AI on both specific and nonspecific immunity in mouse models were examined. Results showed that AI could exhibit mitogenic and co-mitogenic activities on mouse splenocytes, both *in vitro* and *in vivo*. Experiments in human cell culture demonstrated that AI was also active on human lymphocytes. It was found that AI was mitogenic to T cell depleted population but virtually inactive on B cell depleted population. Intraperitoneal injection of AI into mice markedly augmented the antibody response to sheep red blood cells. Besides, both the influx of macrophages into the peritoneal cavity and the phagocytic activity of macrophages were found to be enhanced by AI *in vivo*. On the other hand, AI could significantly increase the interleukin-2 receptor expression on mouse splenocytes *in vitro*. In terms of immunorestorative activity, it was found that AI could restore the lymphocyte blastogenic response of the older mice to values that are normally found in the younger mice. Moreover, administration of AI *in vivo* could partially restore the depressed immune functions in tumour-bearing mice and cyclophosphamide-treated mice. Collectively, the results clearly showed that AI could exhibit immunomodulating and immunorestorative effects, both *in vitro* and *in vivo*.

Keywords: *Astragalus membranaceus*; Huangqi; Immunomodulating effect; Immunorestorative effect

1. Introduction

Astragalus membranaceus (Fisch.) Bunge (AM), Maxim of the Leguminosae family, is a traditional Chinese medicinal herb originated in Northern China. The dried root of AM, Huangqi, contains 2′4′-dihydroxy-5,6-dimethoxyisoflavone, kumatakenin, choline, betaine, polysaccharides, saponins, glucuronic acid, sucrose, amino acids, traces of folic acid and astraisoflavanin (Bensky and Gamble, 1993; Ma et al., 2002; Wu and Chen, 2004). Huangqi is the Chinese name for the root of AM. It is a widely used Chinese medicinal herb that is well-known for its vital-energy tonifying, skin reinforcing, diuretic, abscess-draining and tissue generative actions. In traditional Chinese medicine, Huangqi is often combined with other herbs, such as angelica and ginseng, in various complex prescription formulas. Such herbal formulas have been used for centuries in Asia to treat cancers, diabetes, kidney infections, strokes and many other diseases (Zhang et al., 2006; Li et al., 2007). Although Huangqi is usually combined with other herbs, it can be taken separately by itself. Recommended oral dose for decoction is 3–6 g of Huangqi per 350 mL water. Huangqi, which was found to be effective in treating a wide variety of diseases, has been extensively used as a tonic to enhance the body's defense system (Anon., 2003; Kusum et al., 2004; Liu et al., 2004; Yin et al., 2004). Evidences have indicated the importance of AM polysaccharide fractions in the modulation of immune functions both in human and experimental animals (Chen et al., 1981; Chu et al., 1988a,b; McKenna et al., 2002; Wang et al., 2002; Block and Mead, 2003; Tan and Vanitha, 2004).

Nevertheless, the mechanisms whereby AM components exerting their immunomodulating effects have not been fully elucidated. In this study, the bioactive components from AM

* Corresponding author. Tel.: +852 2958 5441; fax: +852 2958 5455.
E-mail address: chocs@ha.org.hk (W.C.S. Cho).

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A study on the immune receptors for polysaccharides from the roots of *Astragalus membranaceus*, a Chinese medicinal herb

Bao-Mei Shao,[a] Wen Xu,[a] Hui Dai,[a] Pengfei Tu,[b] Zhongjun Li,[c] and Xiao-Ming Gao[a,*]

[a] *Department of Immunology, School of Basic Medical Science, Peking University Health Science Center, Beijing, China*
[b] *Department of Natural Medicine, School of Pharmaceutical Science, Peking University Health Science Center, Beijing, China*
[c] *Department of Organic Chemistry, School of Pharmaceutical Science, Peking University Health Science Center, Beijing, China*

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Abstract

The immunopotentiating effect of the roots of *Astragalus membranaceus*, a medicinal herb, has been associated with its polysaccharide fractions (*Astragalus* polysaccharides, APS). We herein demonstrate that APS activates mouse B cells and macrophages, but not T cells, in terms of proliferation or cytokine production. Fluorescence-labeled APS (fl-APS) was able to selectively stain murine B cells, macrophages and a also human tumor cell line, THP-1, as determined in flow cytometric analysis and confocal laser scanning microscopy. The specific binding of APS to B cells and macrophages was competitively inhibited by bacterial lipopolysaccharides. Rabbit-anti-mouse immunoglobulin (Ig) antibody was able to inhibit APS-induced proliferation of, and APS binding to, mouse B cells. Additionally, APS effectively stimulated the proliferation of splenic B cells from C3H/HeJ mice that have a mutated TLR4 molecule incapable of signal transduction. These results indicate that APS activates B cells via membrane Ig in a TLR4-independent manner. Interestingly, macrophages from C3H/HeJ mice were unable to respond to APS stimulation, suggesting a positive involvement of the TLR4 molecule in APS-mediated macrophage activation. Monoclonal Ab against mouse TLR4 partially inhibited APS binding with macrophages, implying direct interaction between APS and TLR4 on cell surface. These results may have important implications for our understanding on the molecular mechanisms of immunopotentiating polysaccharides from medicinal herbs.

Keywords: *Astragalus membranaceus*; Polysaccharides; Receptor; TLR4; Immune cells

Polysaccharides from natural sources are a class of macromolecules that can profoundly affect the immune system and therefore have the potential as immunomodulators with wide clinical applications [1]. For example, polysaccharides purified from certain mushrooms have anti-tumor activities via macrophage activation [2,3]. β-Glucans (glucose polymers) from the cell walls of plants, fungi, and bacteria exhibit anti-tumor and anti-infection activities [4–6]. Polysaccharides from various traditional medicinal herbs have been shown to be immunopotentiating both in vivo as well as in vitro [7–19]. The roots of *Astragalus membranaceus* (Huangqi) are amongst the most popular health-promoting herbs in China, their use dates back more than 2000 years, and were recorded in *Shen Nong's Materia Medica* written in the Han dynasty. Scientific investigation in the last two decades has revealed much insight into the pharmacological functions of different components of Huangqi, especially its polysaccharide fractions [18–21]. However, molecular mechanisms for the immunobiological function of *Astragalus* polysaccharides (APS) are far from clear. This study was designed to identify and characterize APS-binding cellular receptors expressed by immune cells.

Materials and methods

Animals and antibodies. Female BALB/c mice were purchased from the Experimental Animal Division of Peking University Health Sciences Center, Beijing, China. Female C3H/HeJ mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of

* Corresponding author. Fax: +86-10-82801156.
E-mail address: xmgao@bjmu.edu.cn (X.-M. Gao).

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Review Article

Recent Updates in Neuroprotective and Neuroregenerative Potential of *Centella asiatica*

Yogeswaran Lokanathan[1], Norazzila Omar[1], Nur Nabilah Ahmad Puzi[1], Aminuddin Saim[2], Ruszymah Hj Idrus[1,3]

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[1] *Tissue Engineering Center, UKM Medical Center, 56000 Cheras, Kuala Lumpur, Malaysia*

[2] *Ear, Nose and Throat Consultant Clinic, Ampang Puteri Specialist Hospital, 68000 Ampang, Selangor, Malaysia*

[3] *Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia*

Abstract

***Centella asiatica*, locally well known in Malaysia as pegaga, is a traditional herb that has been used widely in Ayurvedic medicine, traditional Chinese medicine, and in the traditional medicine of other Southeast Asian countries including Malaysia. Although consumption of the plant is indicated for various illnesses, its potential neuroprotective properties have been well studied and documented. In addition to past studies, recent studies also discovered and/or reconfirmed that *C. asiatica* acts as an antioxidant, reducing the effect of oxidative *stress in vitro and in vivo*. At the in vitro level, *C. asiatica* promotes dendrite arborisation and elongation, and also protects the neurons from apoptosis. In vivo studies have shown that the whole extract and also individual compounds of *C. asiatica* have a protective effect against various neurological diseases. Most of the in vivo studies on neuroprotective effects have focused on Alzheimer's disease, Parkinson's disease, learning and memory enhancement, neurotoxicity and other mental illnesses such as depression and anxiety, and epilepsy. Recent studies have embarked on finding the molecular mechanism of neuroprotection by *C. asiatica* extract. However, the capability of *C. asiatica* in enhancing neuroregeneration has not been studied much and is limited to the regeneration of crushed sciatic nerves and protection from neuronal injury in hypoxia conditions. More studies are still needed to identify the compounds and the mechanism of action of *C. asiatica* that are particularly involved in neuroprotection and neuroregeneration. Furthermore, the extraction method, biochemical profile and dosage information of the *C. asiatica* extract need to be standardised to enhance the economic value of this traditional herb and to accelerate the entry of *C. asiatica* extracts into modern medicine.**

Keywords: *antioxidant, neuroprotective, neurological disease, neuronal injury, pegaga*

Introduction

The nervous system, consisting of the brain, spinal cord, and peripheral nerves, is made of complex and specialised structures which are vulnerable to various diseases and injury that reduce sensorimotor and cognitive functions, and may also be the cause of life-threatening problems in acute cases. Unfortunately, spontaneous regeneration and healing processes occur very minimally in damaged tissues due to their high complexity. Our team of researchers at the Tissue Engineering Centre, Universiti Kebangsaan Malaysia has been conducting research on the regeneration of various tissues, and studies on nerve regeneration for development of cell and tissue therapies have been going on for almost eight years (1,2). We have identified various cell sources, ranging from stem cells to adult nerve cells, and developed various scaffolds, ranging from biological tissue to synthetic hollow tubes, to enhance in vitro and in vivo nerve regeneration (3–6). Realising the historical nature of medicinal herbs, we were attracted to scrutinise the pharmacological effects of herb extracts in synergy with the provided cells and scaffolds to further enhance nerve regeneration, and also to improve the utilisation of tissue-engineered nerve grafts and cell therapy in clinical applications for nerve degeneration and injury.

It has been reported that there are 250 000 plant species on the earth, and approximately 5

(NaturalNews) Everybody ages, but not everyone ages equally. The tick of our chronological clock doesn't always match the tick of our biological clock. And although aging is inevitable, a group of Canadian researchers claim to have discovered the six best natural compounds that can slow down the aging process and boost longevity.

In the recent study, published in the journal *Oncotarget*, researchers from Concordia and the Quebec-based biotech company Idunn Technologies conducted more than 10,000 trials screening for plant extracts believed to increase the lifespan of yeast.

Yeast is the most common organism studied in anti-aging research. From a cellular viewpoint, yeast ages in a similar way to people. It is one of the best models to understand how aging occurs.

The impact of extracts

"In total, we found six new groups of molecules that decelerate the chronological aging of yeast," Vladimir Titorenko, senior author of the study and professor in the Department of Biology at Concordia, said in a press release. The extracts were derived from *Cimicifuga racemosa*, *Valeriana officinalis L.*, *Passiflora incarnata L.*, *Ginkgo biloba*, *Apium graveolens L.* and *Salix alba*.

The researchers described one of these groups of molecules, a specific extract of willow bark (*Salix alba*), as "the most potent longevity-extending pharmacological intervention yet described in scientific literature." In particular, the study found that the willow bark extract increased the average chronological lifespan of yeast by 475% and maximum chronological lifespan 369%.

"Rather than focus on curing the individual disease, interventions on the molecular processes of aging can simultaneously delay the onset and progression of most age-related disorders. This kind of intervention is predicted to have a much larger effect on healthy aging and life expectancy than can be attained by treating individual diseases," noted Idunn Technologies founder Eric Simar.

If the results of the study can be replicated in an organism other than yeast, then these compounds could prove to be more effective than anti-aging drugs like rapamycin and

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Discovery of plant extracts that greatly delay yeast chronological aging and have different effects on longevity-defining cellular processes

Vicky Lutchman[1,*], Younes Medkour[1,*], Eugenie Samson[1], Anthony Arlia-Ciommo[1], Pamela Dakik[1], Berly Cortes[1], Rachel Feldman[1], Sadaf Mohtashami[1], Mélissa McAuley[1], Marisa Chancharoen[1], Belise Rukundo[1], Éric Simard[2], Vladimir I. Titorenko[1]

[1]Department of Biology, Concordia University, Montreal, Quebec H4B 1R6, Canada

[2]Idunn Technologies Inc., Rosemere, Quebec J7A 4A5, Canada

[*]These authors contributed equally tothis work

Correspondence to: Vladimir I. Titorenko, **e-mail:** vladimir.titorenko@concordia.ca

Keywords: *yeast, cellular aging, longevity, plant extracts, aging-delaying chemical compounds*

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ABSTRACT

We discovered six plant extracts that increase yeast chronological lifespan to a significantly greater extent than any of the presently known longevity-extending chemical compounds. One of these extracts is the most potent longevity-extending pharmacological intervention yet described. We show that each of the six plant extracts is a geroprotector which delays the onset and decreases the rate of yeast chronological aging by eliciting a hormetic stress response. We also show that each of these extracts has different effects on cellular processes that define longevity in organisms across phyla. These effects include the following: 1) increased mitochondrial respiration and membrane potential; 2) augmented or reduced concentrations of reactive oxygen species; 3) decreased oxidative damage to cellular proteins, membrane lipids, and mitochondrial and nuclear genomes; 4) enhanced cell resistance to oxidative and thermal stresses; and 5) accelerated degradation of neutral lipids deposited in lipid droplets. Our findings provide new insights into mechanisms through which chemicals extracted from certain plants can slow biological aging.

INTRODUCTION

The budding yeast *Saccharomyces cerevisiae* is a unicellular eukaryote amenable to comprehensive molecular analyses [1–3]. The development of various methods of such analyses for *S. cerevisiae* has enabled to uncover mechanisms underlying complex biological processes within individual yeast cells and their populations [1, 4, 5]. In addition, *S. cerevisiae* has relatively short and easy measurable replicative and chronological lifespans [6–13]. Due to these beneficial properties as a model organism for studying mechanisms of aging and longevity, *S. cerevisiae* has been used for the discovery of genes that slow cellular aging and increase healthy lifespan not only in *S. cerevisiae* and other yeasts but also in multicellular eukaryotes [6, 7, 9, 11, 14–16]. Furthermore, using *S. cerevisiae* as a model organism for elucidating mechanisms of cellular aging, several nutrient- and energy-sensing signaling pathways have been revealed; these pathways coordinate an evolutionarily conserved array of longevity-defining cellular processes not only in *S. cerevisiae* and other yeasts but also in eukaryotes across phyla [9, 11, 17–20]. Moreover, *S. cerevisiae* has been a model organism employed for uncovering several low molecular weight molecules that slow aging and extend healthy lifespan in various multicellular eukaryotes [10, 21–27]. All these studies employing *S. cerevisiae* as a model organism have provided evidence that the main features of biological aging have been conserved in the course of evolution [6, 9, 11, 18, 21, 28–31].

Our research is aimed at using *S. cerevisiae* as a model organism to discover chemical compounds that can slow aging and delay the onset of age-related diseases in evolutionarily distant eukaryotic organisms. Some of such geroprotective compounds have been previously revealed in natural products extracted from certain plants [25, 32, 33]. As a first step towards uncovering novel aging-delaying chemical compounds of plant origin, we conducted a screen

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