Proof of Concept

Potential Therapeutic Applications of Telomere Biology

I. INTRODUCTION

Telomeres are structures at the end of our chromosomes that shorten every time a human cell divides. The length of an individual's telomeres is closely associated with their biological age[1] , and research suggests that control of telomere length has the potential to treat many diseases associated with aging, and possibly to allow humans to live at a physiologically "young" age beyond the current theoretical maximum human lifespan of 125 years.

This paper will provide a background on the history of telomere biology, discuss the most important proof-of-concept experiments demonstrating the anti-aging potential of telomere biology, and list some of the potential disease targets for therapies based on telomere length regulation.

II. HISTORY OF TELOMERE BIOLOGY

Telomere biology is a relatively new science. Although the initial discovery of the telomere took place in 1938, research into telomere biology did not proceed to the point where proof of concept experiments on humans became possible until the cloning of human telomerase in 1997.

Telomeres were first discovered and named in 1938 by geneticist Hermann Müller in the fruit fly *Drosophila melanogaster*, who observed that they served as protective caps that could protect DNA from damage.[2] Two years later, geneticist Barbara McClintock discovered that, without telomeres, chromosomes would fuse to each other, causing cell death.[3] Müller and McClintock did not discover that telomeres shorten progressively or that they were associated with human aging.

In 1961, Leonard Hayflick, a researcher at the Wistar Institute in Philadelphia, observed that there was a limit to the number of times a human cell could divide, and that cells from younger individuals could divide more times than cells from older individuals.[4] This was the first real evidence that human cells contained a "clock" from which their age could be determined.

The nature of telomere shortening was first proposed independently by Soviet Scientist Alexei Olovnikov and American scientist James Watson in 1971 and 1972, respectively.[5] Based on their studies of the mechanisms of DNA replication, Olovnikov and Watson realized that it was impossible for a chromosome to replicate itself completely, and that some DNA must be lost with every replication. Olovnikov tied this finding to Hayflick's research on limited somatic cell division, and proposed for the first time that telomere shortening was the mechanism that limited the number of times a cell could divide. Watson and Olovnikov believed that cells must have some strategy to re-lengthen their telomeres, but were unable to describe it.

In 1975, Elizabeth Blackburn and Joseph Gall, biologists at The University of Yale, discovered that the telomeres of the ciliate *Tetrahymena thermophila* consisted of

repetitive DNA in a six-base pattern.[6] Follow-up research by Blackburn and Jack Szostak, a geneticist at Harvard Medical School, demonstrated that yeast was able to re-lengthen its telomeres.[7] Blackburn and Szostak theorized that the yeast's telomeres were being lengthened by an unknown enzyme, which would later come to be known as telomerase.

In 1984, Blackburn and her graduate student Carol Greider were able to discover and isolate telomerase in Tetrahymena.[8] For their work in discovering the structure and mechanisms of telomeres and telomerase, Blackburn, Szostak and Greider were awarded the Nobel Prize in Medicine in 2009.

Although telomeres and telomerase had been described in microorganisms, it would be impossible to do proof of concept experiments in humans until the telomerase enzyme was successfully cloned in human cells. In 1997, scientists including Bryant Villeponteau, Junli Feng, Walter Funk, and William H. Andrews, working at Geron Corporation, successfully cloned the RNA component of human telomerase, for which they were awarded second place for National Inventor of the Year. The subsequent discovery of the protein component of human telomerase followed shortly thereafter.[9] [10]

III. PROOF OF CONCEPT EXPERIMENTS

1. A. Telomerase can make a normal line of human cells immortal.

In 1998, a team at Geron Corporation added the gene for telomerase to normal human cells by use of a plasmid, and using these cells, created a line of telomerase-positive cells. They found that cells from this line were able to divide indefinitely, without entering replicative senescence as an unmodified cell culture would.[11] This demonstrated that normal human cell lines could be made immortal.

1. B. Telomere loss causes symptoms of aging.

In a 1999 experiment at the National Centre of Biotechnology ("CNIO") in Spain led by Maria Blasco, researchers deleted the telomerase gene in mice. Mice naturally express telomerase and age by a different mechanism than humans: the prevailing theory is that they age as a result of oxidative stress. By deleting the telomerase gene, the CNIO team created a line of telomerase-deficient mice. After breeding these mice with each other for six to seven generations, with each generation having shorter telomeres than its parents, they successfully produced mice that showed many of the classic hallmarks of human aging: graying hair, frailness, spontaneous malignancies, and a reduced capacity for wound healing.[12] This was a very strong indication that these symptoms in humans - our "natural aging process" - are the result of telomere shortening.

1. C. Telomerase can make old skin young again.

In 2000, Geron Corporation published a study that for the first time offered a strong indication that telomerase-based therapies could not only prevent, but also reverse, the aging process in humans. A team at Geron cultured human skin cells until they were near their replicative limit, and then used those skin cells to grow human skin on the backs of immunodeficient mice. As expected, the skin showed the hallmarks of "old skin," such as fragility and subepidermal blistering.

The team then added the telomerase gene to the old skin cells, grew them for another 25 generations (which took them past the theoretical limit of unaltered cells), and grew the resulting human skin cells on mice. Geron then performed DNA array analysis on their artificially "telomerized" skin, as well as unaltered young and old skin.

Not only did the telomerized skin visually resemble the young skin, but it also shared virtually the same gene expression profile as the young skin. By all available measurements, "telomerizing" the skin had actually made it younger.[13]

1. D. Telomerase could theoretically make an old animal young again.

The CNIO team performed a follow-up study to their 1999 research with telomerase-deficient mice. In 2001, they bred mice with critically short telomeres with normal mice, thereby re-introducing the gene for telomerase. They found that this re-lengthened the telomeres in the resulting offspring. Those offspring had chromosomes with detectable telomeres, and did not show any chromosomal instability or premature aging. In addition, the symptoms of old age that had formerly been seen in the telomerase-deficient mice were not present; the offspring were young and healthy.[14]

The rejuvenation of these mice took place across generations; however, these mice had been altered specifically so they could not produce young and healthy offspring, and the introduction of the telomerase gene reversed this condition. Thus, this experiment lent some support to the idea that introducing telomerase into an "aged" organism could lead to a "young" organism and suggested that therapies based on telomere biology could reverse the aging process in a living mammal.

1. E. Short telomeres cause us to die of old age.

In 2003, a team led by Richard Cawthon at the University of Utah studied 143 individuals over the age of 60 who had donated blood between 1982 and 1986, measured the telomere lengths in that donated blood, and then examined the relationship between those telomere lengths and the mortality of those individuals in the intervening two decades. They found that the mortality rate of individuals with shorter telomeres was nearly twice as high as that of individuals with longer telomeres, and that mortality as a result of heart disease was over three times higher in individuals with shorter telomeres. This provided solid evidence of a correlation between telomere shortening and death from old age or age-related diseases in humans.[15]

1. F. Telomerase can extend the lifespan of an organism.

In 2008, the CNIO in Spain published a study on a line of mice whose cells had been engineered to produce ten times more telomerase than wild mice. Telomerase overexpression allowed these mice to live an average of 38% longer than normal. Further, the mice stayed healthier and athletic longer. In the control group, approximately half of all mice had lost the ability to walk a tightrope by the time they were 116-160 weeks old; the telomerase-positive sample showed no loss in this ability at all.[16]

This represented the first time that the lifespan of a multicellular organism has ever been extended through telomere therapy.

1. G. Telomerase production can be induced by a nutraceutical or pharmaceutical.

In 2006, Geron Corporation discovered a nutraceutical derived from *Astragalus membranaceus* that generated telomerase activity at very low levels. This nutraceutical was licensed to TA Sciences and introduced into the market in 2007 as TA-65.

A year later, in 2007, Sierra Sciences discovered a synthetic drug-like molecule that induced cells to produce telomerase at significantly higher levels,[17] and, over the next several years, identified a total of 39 drug families that caused telomerase induction.

Before these discoveries, it was unknown whether it would be possible to activate telomerase in adult human cells without resorting to gene therapy, which has been proven to carry an unacceptably high risk of cancer.

1. H. Even a weak telomerase inducer shows some health benefits.

After TA-65 had been available for public consumption for approximately 3½ years, Sierra Sciences, TA Sciences, Geron Corporation, PhysioAge, and the CNIO jointly released a publication on its effects in September 2010. They found that patients taking TA-65 had proportionally fewer immune cells with critically short telomeres than they did at the beginning of the study. In individuals infected with CMV, a virus which prematurely ages the immune system and significantly reduces life expectancy, there was an even more dramatic improvement of the immune system that represented an apparent "age reversal" effect of approximately 5 to 20 years based on one biomarker of immune aging.[18]

1. I. Telomerase doesn't cause cancer (although cancer causes telomerase).

More than 90% of human cancer cell lines express telomerase.[19] For this reason, there has been some speculation that expressing telomerase in normal human cells could increase the risk of cancer. However, studies over the last ten years have consistently refuted this speculation.

Shortly after the discovery of human telomerase, several publications reported that telomerase doesn't cause cancer, including a 1999 paper by Jerry W. Shay and Woodring E. Wright at the University of Texas Southwestern Medical Center[20] and a 1999 paper published by Geron Corporation in collaboration with the Salk Institute and the University of California, San Francisco.[21] In 2002, Calvin Harley at Geron Corporation published a review paper on the relationship between telomerase and cancer, evaluating 86 publications and concluding that telomerase was not a cancer-causing oncogene and did not cause cells to lose growth control and become cancerous.[22]

More recently, scientists have discovered evidence that short telomeres are a significant risk factor for cancer. In 2009, a joint study between Georgetown University and the National Cancer Institutes concluded that short telomeres lead to telomere dysfunction, which leads to chromosome-arm instabilities, which leads to

cancer.[23] The implication is that keeping telomeres long could eliminate this risk factor and prevent the onset of many cancers. In July of 2010, an international team of doctors compared telomere length against incidences of cancer in 787 patients, and found that subjects with the shortest telomeres had three times the incidence of cancer compared to patients with longer telomeres. Further, short telomeres increased the lethality of cancer: mortality rates of the subjects doubled with every one-standard-deviation decrease in telomere length.[24]

This research suggests that keeping telomeres long through telomerase activation could likely prevent cancer and/or increase the chance of survival for individuals who develop cancer.

IV. DISEASE TARGETS

Several studies have shown that mean leukocyte telomere length is a more accurate risk factor for disease than chronological age.[25] [26] In addition, accelerated telomere shortening has been shown to increase mortality, not from any one disease in particular, but from a wide variety of age-related diseases.[27]

It is easily observable that elderly individuals are at a higher risk for a vast range of diseases, both degenerative and infectious, than younger individuals. The hypothesis that control of telomere length could treat this wide array of diseases (that, essentially, it could cause an 80-year-old to be no more susceptible to disease in general, than a 20-year-old would be) has not been proven false, and is supported by the scientific literature.

Below is a discussion of some of the studies on the association between telomere length and various diseases.

1. A. Cardiovascular disease

The relationship between heart disease and telomere length has been studied extensively. A pilot study in 2001 found evidence that patients with severe chronic artery disease had a leukocyte telomere length 303 base pairs shorter than the controls, equivalent in size to individuals 8.6 years older.[28] In a broader study in 2003, the telomeres in patients who had suffered a myocardial infarction were measured; on average, they were as short as controls 11.3 years older. In addition, the study concluded that individuals with shorter than average telomeres had a 2.8 to 3.2 times higher risk of myocardial infarction than individuals in the highest quartile of telomere length.[29]

A 2007 study on 484 individuals with heart disease and 1,058 matched controls showed that mean leukocyte telomere length is a predictor of coronary heart disease; in untreated patients, the risk of individuals with the shortest telomeres was nearly double the risk of individuals with longer telomeres, regardless of their biological age.[30]

Because short telomeres are a risk factor for cardiovascular disease, it stands to reason that therapies controlling telomere length could prevent, and possibly treat, cardiovascular disease.

1. B. Chronic Obstructive Pulmonary Disease (COPD)

A French study in 2009 reported that patients with COPD had significantly shorter mean telomere lengths than either non-smokers or smokers without COPD. [31]Although this established a correlative, rather than causative, relationship, it is difficult to imagine any mechanism by which an individual with long telomeres could acquire a degenerative, non-infectious disease, which would then rapidly accelerate the rate of telomere shortening. The research suggested that short telomeres are a risk factor for COPD, and that control of telomere length could prevent this condition, possibly even in smokers.

1. C. Degenerative Disc Disease

In 2007, a University of Manchester study established that the telomere length of disc cells decreased with every progressive stage of disc degeneration[32] , and hypothesized that degenerative disc disease is caused by cellular senescence as a result of telomere shortening.

1. D. Alzheimer's Disease

Because Alzheimer's is arguably one of the diseases most closely associated with the physical and mental deterioration that occurs during old age, scientists have naturally been interested to see whether telomere shortening plays a role in its incidence.

In 2007, researchers at the University of Adelaide compared the telomere lengths in the white blood cells, buccal cells, and brain tissue of Alzheimer's patients to those of age-matched controls without Alzheimer's. They observed significantly lower telomere lengths in white blood cells and buccal cells of Alzheimer's patients, but a higher telomere length in hippocampus cells.[33] The researchers concluded that Alzheimer's was related to important differences in the mechanisms of telomere maintenance. A 2009 study similarly showed an association between Alzheimer's Disease and shorter mean leukocyte telomere length.[34]

1. E. Other degenerative diseases

In addition to the degenerative diseases discussed above in greater detail, short telomeres have been identified as a risk factor for osteoarthritis[35] , rheumatoid arthritis[36] , osteoporosis[37] , macular degeneration[38] , liver cirrhosis[39] , and idiopathic pulmonary fibrosis.[40]

1. F. Infectious diseases, including AIDS

Cells of the immune system are under enormous proliferative demand; fighting an infection requires cell division on a level rarely seen in other systems in the body.[41] For this reason, chronic or latent infections can cause premature aging of the immune system, reducing its capacity to fight disease.

This phenomenon has been observed in studies of HIV-positive patients, who displayed significantly shorter telomeres in their CD8+ T cells.[42] In monozygotic twin studies, this held true even compared to the patients' HIV-negative twins.[43] AIDS develops from HIV when immune cells lose their ability to fight the HIV infection; these studies suggest that that loss of ability is the result of replicative

senescence in the immune system. Thus, the induction of telomerase in the immune system holds promise of preventing HIV from ever developing into AIDS.

1. G. Diminished regenerative capacity

It is easily observable that the human capacity for wound healing decreases as we age. Wound healing requires cell division, and experiments on telomerase-deficient mice confirm that shortened telomeres reduce an organism's capacity for wound healing.[44] Control of telomere length would almost certainly increase an elderly individual's capacity for wound healing.

For the same reason, therapies that prevent telomere shortening could prove vital to the success of tissue transplants in the elderly. In a proof of concept experiment in 2000, scientists at the Baylor College of Medicine successfully transplanted artificially telomerized bovine tissue into the kidney capsules of immunodeficient mice; these mice survived, with no indications of malignant transformation of the tissue.[45]

Along similar lines, skin aging is thought to be controlled primarily by progressive telomere shortening.[46] It is likely that control of telomere length could arrest skin aging. It is unknown whether such a therapy could reverse skin aging in a living human being, but experiments by Geron Corporation in which skin cells were made youthful by the introduction of the telomerase gene provide evidence that such a therapy may be possible.[47]

1. H. Congenital disorders

Several congenital genetic disorders are characterized by prematurely short telomeres, including muscular dystrophy[48] , progeria[49] , dyskeratosis congenita[50], cri du chat syndrome[51] , Fanconi's anemia[52] , tuberous sclerosis[53] , and Werner syndrome.[54] Maternal telomere length has also been associated with the prevalence of Down syndrome.[55]

It is still unknown whether telomere maintenance therapy could treat and/or cure any or all of these conditions, but it is not unreasonable to hypothesize that such a therapy could be beneficial.

FOOTNOTES

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