Aging and the Telomere Connection

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Abstract:

Telomeres are repetitive DNA sequences at the ends of linear chromosomes that serve as essential protective structures that maintain the integrity of chromosomal DNA. Each time a normal human cell divides some telomeric DNA sequences are lost. When telomeres are short, cells enter an irreversible growth arrest state called replicative senescence (or aging). There is mounting evidence that short telomeres correlate with age-associated diseases by limiting the ability of tissues to regenerate. This has led to the idea that telomere length could be a good and highly reliable indicator (a biomarker) of biological (not necessarily chronological) aging. Telomere length measurements, especially measurements of the shortest telomeres, provide a molecular determinant about overall health. It has been shown that environmental stressors can lead to increases in oxidative damage and premature telomere shortening. Smoking, inflammatory disease, lack of modest levels of exercise, and excessive drinking can all contribute to increases in the rate of telomere shortening but in some instances these may be reversed by behavior modification. Just as cholesterol and blood pressure measurements provide an indication of overall health, newly introduced highly quantitative telomere length assays also provide a window into one's overall health. While no one can predict how long any individual person will live, quantitative telomere tests are scientifically proven biological assays that correlate with the ability of human cells to proliferate and replenish tissues.

Background:

Aging is associated with the gradual decline in the performance of organ systems, resulting in the loss of reserve capacity, leading to an increased chance of death (1). In some organ systems, this loss of reserve capacity with increasing age can be attributed to the loss of functional cells (2). Chronic localized stress to specific cell types results in increased cell turnover, focal areas of replicative (aging) senescence (3) followed by predictable alterations in patterns of gene expression. This results in reduced tissue regeneration, culminating in many of the clinical pathologies that are largely associated with increased age.

Evidence that telomere shortening leads to replicative senescence:

Telomere length is a record of the history of and the potential for replication of human cells (4). Telomere dynamics in proliferating tissues might provide insight into not only the biology of aging but also the pathology of age-related diseases. A body of epidemiological and clinical data suggests that relatively short telomeres and accelerated telomere attrition are linked to factors that define aging and diseases of aging in humans (5-12).

There is also experimental support that most human proliferative tissues and organs including most somatic cells (even stem cells of renewal tissues) undergo progressive telomere shortening throughout life (13). While there have been many studies demonstrating correlations between telomere shortening and proliferative failure of human cells, the evidence that it is causal has now been directly demonstrated (14). Telomerase is a ribonucleoprotein enzyme that functions to lengthen telomere length during early fetal development and in some proliferative adult stem cells. However, almost all adult tissues do not have detectable telomerase activity and telomere lengths

decrease throughout life. Introduction of the telomerase catalytic protein component into normal human cells results in detection of telomerase activity (14). Normal human cells stably expressing transfected telomerase demonstrate telomere maintenance and extension of life span, providing direct evidence that telomere shortening controls cellular aging. The cells with introduced telomerase maintain a normal chromosome complement and continue to grow in a normal manner (15). These observations provide the first convincing evidence for the hypothesis that telomere length determines the proliferative capacity of human cells.

Evidence that telomere shortening is important in organ or tissue ageing:

There is a relationship between replicative aging and skin pressure ulcers (16) that are believed to be caused initially by decreased circulation, leading to localized areas of necrosis. This is followed by attempts of the skin cells to regenerate. In the areas of pressure ulcers there is dramatic shortening of telomeres compared to adjacent normal areas. Other examples include patients with advanced and progressive human immunodeficiency virus infections who have specific T-cell deficiencies, patients with liver cirrhosis, patients with muscular dystrophy, and patients with bone-marrow exhaustion in myeloproliferative diseases. In spite of a diverse etiology, a common pathological mechanism is an increased turnover of stem-like cells leading to cellular senescence and then to a disease state. Thus, in patients with progressive AIDs there is increased turnover of certain types of mature T-cells and at least initially the patient regenerates more T-cells. Proliferative failure due to telomere erosion may ultimately result in the patient having low T-cell counts, leading to opportunistic infections. In Duchenne muscular dystrophy, children are capable of walking for a few years but, because they have inherited a mutated dystrophin gene, their muscle fibers degenerate. To compensate, their muscle stem-like cells (satellite cells) regenerate

new muscle fibers but these also degenerate and eventually the stem cells cannot keep dividing and the muscle is replaced with fat (adipocytes). More recently alterations in key genes involved in maintaining telomeres have been demonstrated to be involved in human genetic diseases such as dyskeratosis congenita, sporadic bone marrow failure and idiopathic pulmonary fibrosis (17-21). In summary, there is mounting evidence that in some aged-related disorders telomere decline in specific tissues and organs may contribute to aging and cancer vulnerability (22).

Gradual shortening of telomeres coincide with the long term aging process:

Under normal conditions most tissues can last a typical life span. However, with the improvement in sanitation, the development of antibiotics, vaccines, and modern pharmaceutical drugs, humans are living longer. A proliferative capacity for good maintenance and repair for 80-100 years would not have been selected for in evolutionary terms, when the average human lived at most 30-40 years. Today there is an increase in aged-related cellular decline in normal people who live to an exceptionally old age, while in the past problems from a limited cellular proliferative capacity was only observed in disease states (23). However in older individuals without diseases, there is an increased incidence of immunological deficiencies, chronic ulcers, wearing down of the vascular endothelium leading to arteriosclerosis, proliferative decline of retinal pigmented epithelial cells leading to age-related blindness and cancer. In order to track telomere lengths and potentially slow down or reverse the increased probability of telomere associated diseases, it is fair to ask if telomere tests would have utility as a clinical diagnostic assay.

Are telomere tests ready for prime time?

We still know little about the dynamics of telomere length changes over multiple years in large

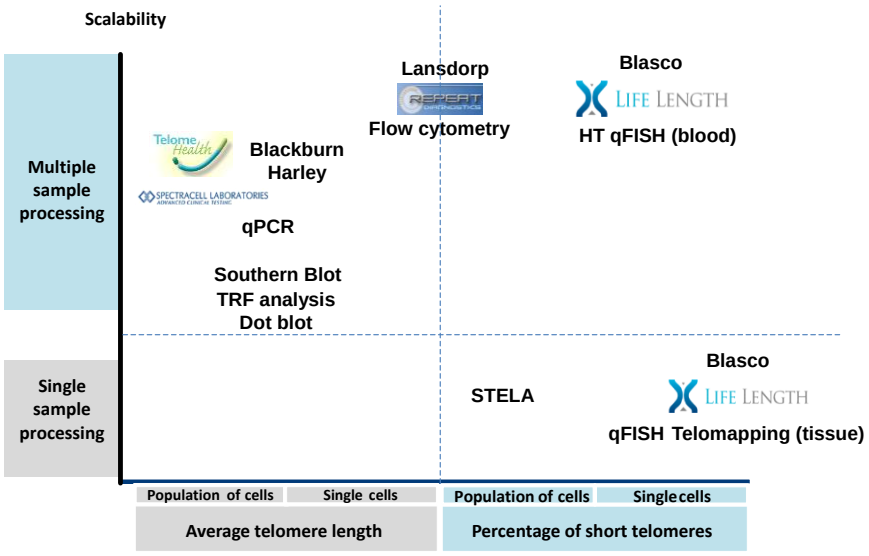
human populations. It would be difficult for government funding agencies in today's financially challenged environment to support large scale longitudinal telomere research studies. Thus, the private sector has taken the lead and developed a series of telomere tests to determine if the multiple associations of diseases with telomere length measurements hold up in placebo controlled studies. Since there are emerging classes of natural products (telomerase activators) (24) and genetic manipulations that may influence telomere biology and aging (25-26), we need rigorous scientific telomere tests to prove the mechanism of actions. The ability of the private sector to start large scale longitudinal telomere measurements, including patients completing detailed questionnaires, will permit the scientific community to assemble databases that will allow for determining statistically relevant sub-populations of patients that may benefit from telomere length modifications.

Telomere length measurement tests:

The basic laboratory research telomere test is known as TRF (terminal restriction fraction). This test is at present not suited for high throughput scale up, but provides a visual representation of the distribution of populations of cells using a Southern blot electrophoresis approach. Other laboratory assays being developed include a modification of the TRF assay using a dot blot approach which is similar but does not provide visualization of the range of telomere lengths (Figure 1). Neither the TRF nor dot blot method provides information about the shortest telomeres in individual cells. However, as long as one can get sufficient DNA, data can be obtained. Another newly developed method is called STELA (single telomere length analysis). This is very low throughput but the advantage is that it can provide information on the shortest telomeres in a population of cells. It cannot easily be adapted to a commercial test at the present

time due to turnaround is more than a week per assay, and this method does not provide information about the shortest telomeres in individual cells.

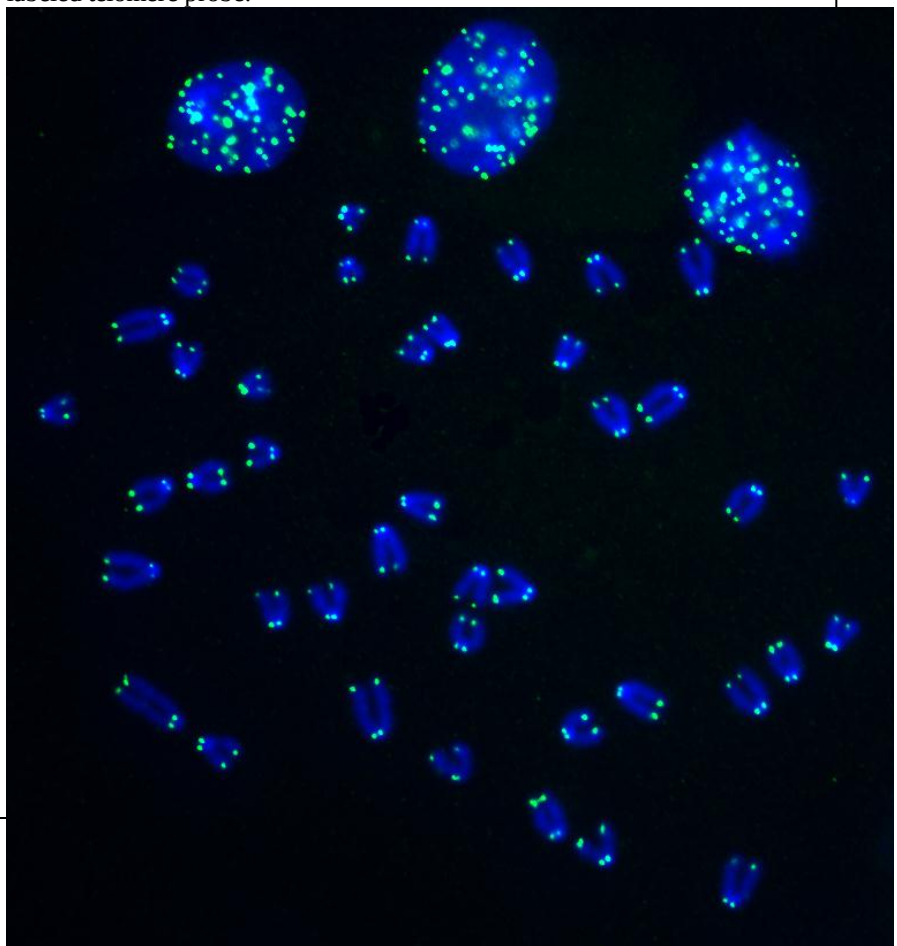
Figure 1. Summary of current telomere tests (modified from Life Length, Inc.)



The qPCR method is not only used in some research laboratories but is also being used in some commercial situations (Telomehealth.com; Spectracell.com). The advantage of the qPCR method is that it is relatively fast and capable of high throughput. The disadvantage is that this test does not give information about individual cells so the results are generally averages of telomere lengths in populations of cells. The flow FISH method almost exclusively uses lymphocytes and is beginning to have some commercial development (Repeatdiagnostics.com; Figure 1). This method uses a FACS (fluorescence activated cell sorter) to analyze cells with different signals after hybridization with a fluorescence telomere probe. This method provides the distribution of telomere lengths one cell at time but only on the average of telomere lengths (not telomere lengths within individual cells). This method is CLIA certified for measuring telomeres as part of genetic counseling. The high throughput microscopic Q-FISH (Quantitative Fluorescence In Situ

Hybridization) method is a highly reliable approach to quantitating telomere lengths and has the advantage over other methods of providing not only average telomere length per cell but also the number and distribution of the shortest telomeres in individual cells (27, 28; Figure 2). This method permits visualization and quantitation in a microscope of individual cells so one can distinguish between a subset of cells containing very short telomeres and those that have very long telomere lengths (Figure 2). A commercial test (HT Q-FISH) has now been developed (Lifelength.com). With the exception of HT Q-FISH, no other commercial laboratory method is available which can distinguish a single critically short telomere within one cell that may be triggering senescence. While initially a laboratory test with relatively low throughput, this approach has recently been commercialized into a high throughput method with an accuracy of 5% between tests (Lifelength.com).

Figure 2. In situ FISH of interphase cells (3 at the top) and one metaphase spread (lower part of figure). Fixed cells were hybridized with a fluorescence labeled telomere probe.



The last method is called Telomapping (Lifelength.com) (US Patent Nº 8,084,203 B2). This is similar to Q-FISH but determines the telomere lengths on chromosomes from tissues (thus maintaining the topology of the samples). The advantage of this method is that archival formalin embedded paraffin sections can be used to determine if specific cells within a tissue have short telomeres. This is likely to have important implication in the precancerous detection field. This method is slightly more time intensive and is not scaled up to high throughput analysis at the present time.

Bottom-line, telomere tests are ready for prime time and while there several options, one needs to ask if the method delivers results that provide insights in critically short telomeres in individual cells which are universally regarded as the principal cause of replicative cell aging and age-related diseases (Dr. Carol Greider, Nobel Prize winner, 2009; 29).

Summary and future challenges:

Telomere biology is important in human aging and cancer. Cancer cells need a mechanism to maintain telomeres if they are going to divide indefinitely, and telomerase solves this problem. Thus, inhibition of telomerase may have utility in cancer therapeutics (30). Since almost all tissues show progressive shortening of telomeres with increased age, in some instances, organ failure may occur in chronic diseases of high cellular turnover. Therefore, telomere manipulations in cells of regenerative tissues may have utility in treating certain disorders in the aging population (24, 28). While the aging process is complex and certainly cannot be explained solely on the basis of telomere biology, there is a growing consensus that in some situations telomere biology and telomere tests may have important utility similar to cholesterol assays or blood pressure

monitoring measurements. This is still a young field and improvements will continue to occur. With longitudinal studies in individuals over many years trends will emerge that are likely to provide important insights into human disease. The challenge is to understand how telomere biology leads to increased aging vulnerability and to learn how to intervene in these processes.

Conflict of Interest:

Dr. Shay is a Scientific Consultant to Life Length, Inc. (www.lifelength.com), Madrid Spain

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