

IMPACT OF DYSFUNCTIONAL TELOMERES ON AGING AND CANCER

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ABSTRACT

Telomeres are repetitive DNA sequences that cap the ends of all eukaryotic chromosomes. Telomeres, complex with unique protein components, solve two important problems at chromosome ends: the end replication problem and the end protection problem. Numerous human diseases are associated with defects in telomere end protection, leading to proliferative failure of stem cells, onset of bone marrow failure syndromes and with increased cancer incidence. Telomere length serves as a reliable biomarker for the proliferative history of somatic cells, and is therefore a marker of biological, and not necessarily chronological, aging. While proper telomere maintenance requires the coordinated activities of the enzyme telomerase and associated protein complexes, environmental and lifestyle factors such as diet, nutrition, smoking, exercise (or the lack of) could negatively impact upon telomere length and the rate of telomere loss. Therefore, the ability to monitor telomere length, especially short telomeres, in individual cells should be an important component of the current revolution in personalized medicine. The seminal discovery that the proliferative capacity of somatic cells in mice with short telomeres could be increased by the activation of telomerase offers the possibility that mammalian lifespan could one day be therapeutically manipulated by modulating telomere length.

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in telomere maintenance contributes directly to several inherited human hematopoietic disorders (5). Critical telomere shortening results in proliferative defects in hematopoietic stem cells, leading to the onset of bone marrow (BM) failure syndromes. The best studied of these diseases is Dyskeratosis congenita (DC), a multisystem disorder characterized by the presence of dysplastic nails, BM failure, skin pigmentation abnormalities, hair greying and increased cancer risk. Autosomal dominant and recessive forms of this disease are due to mutations in the TERC and TERT components of telomerase, respectively, while X-linked DC is a result of mutations in DKC1, a gene encoding a small nuclear RNA protein that interacts with TERC (4, 5). Mutations in the gene encoding the shelterin component TIN2 results in a very severe form of DC, with severe telomere dysfunction, premature BM depletion and ultimately BM failure in patients as early as 10 years of age. Although mutations in POT1 has not yet been associated with DC, mouse models have revealed that deletion of the POT1 protein results in phenotypes highly reminiscent of human DC (6).

Recently, whole exome sequencing resulted in the discovery of a new player in inherited BM failure syndromes. Coats plus is an autosomal recessive disorder characterized by bilateral retinal exudative retinopathy (Coats disease), intracranial calcifications, osteopenia, hair graying, BM failure and the presence of critically short telomeres (4). Whole-exome sequencing of Coats plus patients revealed the presence of biallelic missense CTC1 mutations. Coats plus patients display clinical phenotypes that overlap with several other human BM failure disorders resulting from telomere dysfunction, including DC. Hoyeraal-Hreidarsson syndrome manifests as developmental delay, intracranial calcifications and is due to mutations in DKC1, TIN2 and TERT. Patients with Revesz syndrome also exhibit developmental delay, with exudative retinopathy and intracranial calcifications as characteristic features. As this disease progresses, characteristic features of DC emerge, including BM failure and telomere shortening. Therefore, despite their broad clinical spectrum, DC, Hoyeraal-Hreidarsson syndrome, Revesz syndrome and Coats plus are all telomere biology disorders unified by the common molecular pathology of telomere dysfunction and shortening (7).

What are the molecular mechanisms underlying these telomere disorders (termed telomopathies)? Stem cells lacking functional telomerase are just like normal somatic cells, with limited replicative potentials, resulting in the progressive telomere shortening with each cell division and the eventual generation of dysfunctional telomeres. We know

that a single shortest telomere in a cell could trigger a robust DDR in the setting of an intact p53-dependent DDR pathway (8). This in turn leads to the activation of apoptotic and/or cellular senescence programs, two potent cellular growth suppressor mechanisms to stop further cellular proliferation (2). The decline in proliferative capacities of stem cells (especially in highly proliferative compartments like the BM) results in progressive BM failure and onset of disease phenotypes. While studies of human telomere disorders have shed light on how dysfunctional telomeres negatively impact upon stem cell proliferative capacity, the use of mouse models of telomere dysfunction have been instrumental for our understanding of the impact that dysfunctional telomeres play in compromising highly proliferative cellular compartments. For example, mice engineered not to have any telomerase activities display defects in highly proliferative tissues, including the appearance of erosive dermatitis, reduced proliferation of T and B lymphocytes upon mitogenic stimulation, splenic atrophy and complete infertility at late generations due to defects in reproductive germ cells. These results suggest that telomere maintenance is vital in long-term stem cell survival and organ homeostasis. Progressive BM failure, the hallmark of BM failure syndromes, has been observed when we deleted POT1 or CTC1 function in mice (6, 9). These results reinforce the notion that mouse models of telomere dysfunction are extremely valuable for the understanding and future treatment of human telomopathies.

PROGRESSIVE TELOMERE SHORTENING IS A CONSEQUENCE OF NORMAL HUMAN AGING.

Humans in developed countries are living longer, with increased life expectancies coming from improved sanitation, childhood vaccinations and the use of modern pharmaceutical drugs. However, our limiting telomere length ultimately restrains how long we could live. Increased aged-related cellular decline due to telomere attrition could be found in stem cells and tissues with high rates of proliferative capacities in normal people who live to an exceptionally old age. For example, an increased incidence of immunological deficiencies due to decreased T and B-cell function, chronic ulcers, diminished vascular endothelium function leading to arteriosclerosis, proliferative decline of retinal pigmented epithelial cells leading to age-related blindness and cancer have all been attributed to progressive telomere attrition. In addition, exposure to environmental and life-style factors such as cigarette smoking, reduced exercise, and excessive drinking could directly lead to increased telomere erosion, exacerbating cellular proliferative defects. While we cannot yet slow down normal telomere attrition in humans, a recent report suggesting that turning on telomerase in mouse cells with short

telomeres could actually rescue their proliferative defects gives us great hope that we could one day slow down or even reverse telomere shortening in aging human tissues (10). This intriguing possibility makes it imperative to have proper tests available to allow accurate telomere length measurements in human tissues. In addition, knowing one's overall telomere length could lead to lifestyle modifications that reduce/eliminate the contributions of environmental factors' adverse impact on telomere length.

ASSAYS TO MEASURE TELOMERE LENGTHS.

Several different methods exist to measure telomere length. The oldest is the terminal restriction fragment (TRF) assay, a test that relies on size fractionation of telomere fragments and Southern blotting with a telomere probe to detect telomeres. This qualitative test is not scalable for high throughput analysis and is often hard to interpret due to the heterogeneity of telomere length in human cells, appearing as a smear of variable intensity. In a mixed cell population, it is not possible to tell which cell types have the shortest telomeres. Despite its shortcomings, TRF Southern are routinely performed on human samples due to its simplicity. A modification of the TRF assay using a dot blot approach is even more problematic than the TRF method, in that it does not provide visualization of the range of telomere lengths (Figure 2). Neither the TRF nor dot blot method provides information about the shortest telomeres in individual cells.

The quantitative PCR method of telomere detection is fast and scalable for high throughput analysis. Its main disadvantage is that it gives information only as an average of telomere lengths and provides no information on the length of the shortest telomeres. Two companies (Telomehealth.com; Spectracell.com, Figure 2) provide this technology commercially. Another PCR-based approach is called STELA (single telomere length analysis). This is very low throughput method that analyzes telomere lengths

from only a few specific chromosomes. However, it has the advantage that it can provide information on the shortest telomeres in a population of cells. It is not yet a viable commercial test due to the long turnaround time, and it cannot provide information about the shortest telomeres in individual cells.

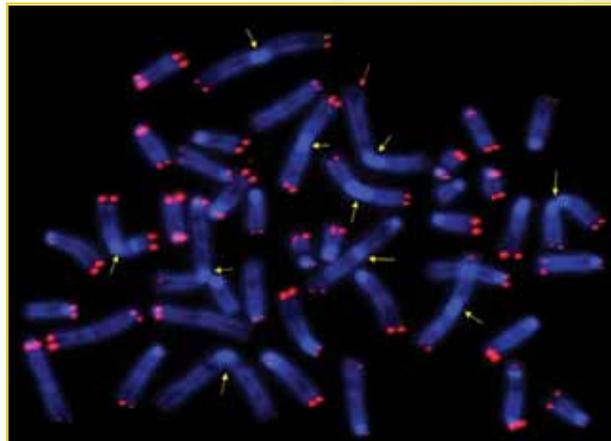
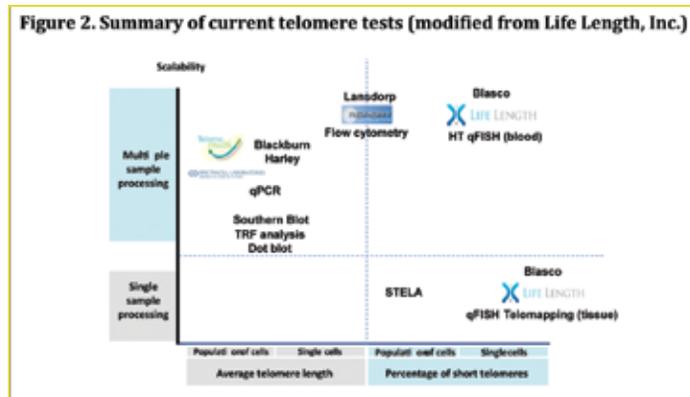


Figure 3. Q-FISH analysis of a metaphase spread from mouse spleen cells genetically engineered to possess dysfunctional telomeres. Red dots mark telomeres, while the blue dye stains chromosome bodies. Arrows point to sites of chromosome fusions due to telomere dysfunction. Several chromosome ends have very short telomeres, visualized as very small telomere signals at chromosome ends.

The flow-FISH (fluorescence in situ hybridization) method uses a FACS (fluorescence activated cell sorter) to analyze telomere length in a cell population after hybridization with a fluorescence telomere probe. This method provides only the averages of telomere lengths and not telomere lengths within individual cells. It almost exclusively uses lymphocytes, so is not designed to analyze telomere lengths from solid tissues. Flow-FISH is offered commercially (Repeatdiagnostics.com; Figure 2), and is CLIA certified for measuring telomere lengths as part of genetic counseling.

The high throughput microscopic quantitative (Q)-FISH method is a highly reliable approach to measure telomere lengths. This approach visualizes telomere lengths of hundreds of individual metaphase spreads or cells under a microscope so one can distinguish between subsets of cells containing very short telomeres from those with long 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 (Figure 3) (11, 12). A commercial test (high throughput Q-FISH) was developed the Dr. Maria Blasco at the Spanish National Cancer Research Center and licensed to Life Length (Figure 2). With the exception of HT Q-FISH, no other commercial laboratory method is available which can distinguish a single critically short



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telomere within one cell that may be triggering a DDR. This approach has recently been commercialized into a high throughput method with an accuracy of 5% between tests (www.lifelength.com). Life Length also developed a technique called Telomapping (US Patent No 8,084,203 B2). This is similar to HT Q-FISH but determines the telomere lengths on chromosomes from tissue sections, thus maintaining the spatial topology of the samples. The advantage of this method is that archival formalin embedded paraffin sections can be used to determine if specific cell types within a tissue have short telomeres. This is likely to have important implication in the precancerous detection field. This method is more time intensive and is not scaled up to high throughput analysis at the present time.

In conclusion, reliable telomere tests are now available commercially. While there several options, one needs to ask if the method delivers results that provide measurements of the number of individual cells bearing critically short telomeres, which are universally regarded as the principal cause of replicative cell aging and age-related diseases (4, 8).

SUMMARY AND FUTURE CHALLENGES. The proper maintenance of telomere function is important to delay human cellular aging and prevents the initiation of cancer. Since cancer cells require a high level of telomerase for telomere length maintenance, drugs to inhibit telomerase activity may have some utility in cancer therapeutics. In human diseases of high cellular turnover, for example in inherited BM failure syndromes, reduced telomerase activity resulting in very short telomeres have been found.

Therefore, the ability to lengthen telomeres in BM stem cells by reactivating telomerase might be a therapeutic approach to treat DC patients. Both of these approaches would benefit from the ability to accurately determine telomere length in cells. While the normal aging process is complex and certainly cannot be explained solely on the basis of telomere biology, there is a growing consensus that telomere status plays a fundamental role in determining how an organism ages. Certainly in mouse experimental systems, manipulation of telomere lengths in vivo has shown that decreased telomere length promotes the onset of premature aging phenotypes, while telomere lengthening results in a more robust and fit mouse. In humans, it is hoped that telomere length measurement tests may offer clinicians another piece of data to gauge the health of individual patients. For example, patients with a young chronological age but an advanced biological age, as determined from the increased number of very short telomeres in somatic/stem cells, could lead the physician to recommend behavior modifications to reduce adverse environmental factors such as smoking, which compromise telomere length. While we cannot yet manipulate telomere length in humans, the hope is that future drugs/supplements might offer us this ability in a controlled manner. The challenge will be to increase telomere length in somatic cells to delay cellular aging in humans without increasing cancer initiation.

CONFLICT OF INTEREST:

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