IMPACT OF DYSFUNCTIONAL TELOMERES ON AGING AND CANCER

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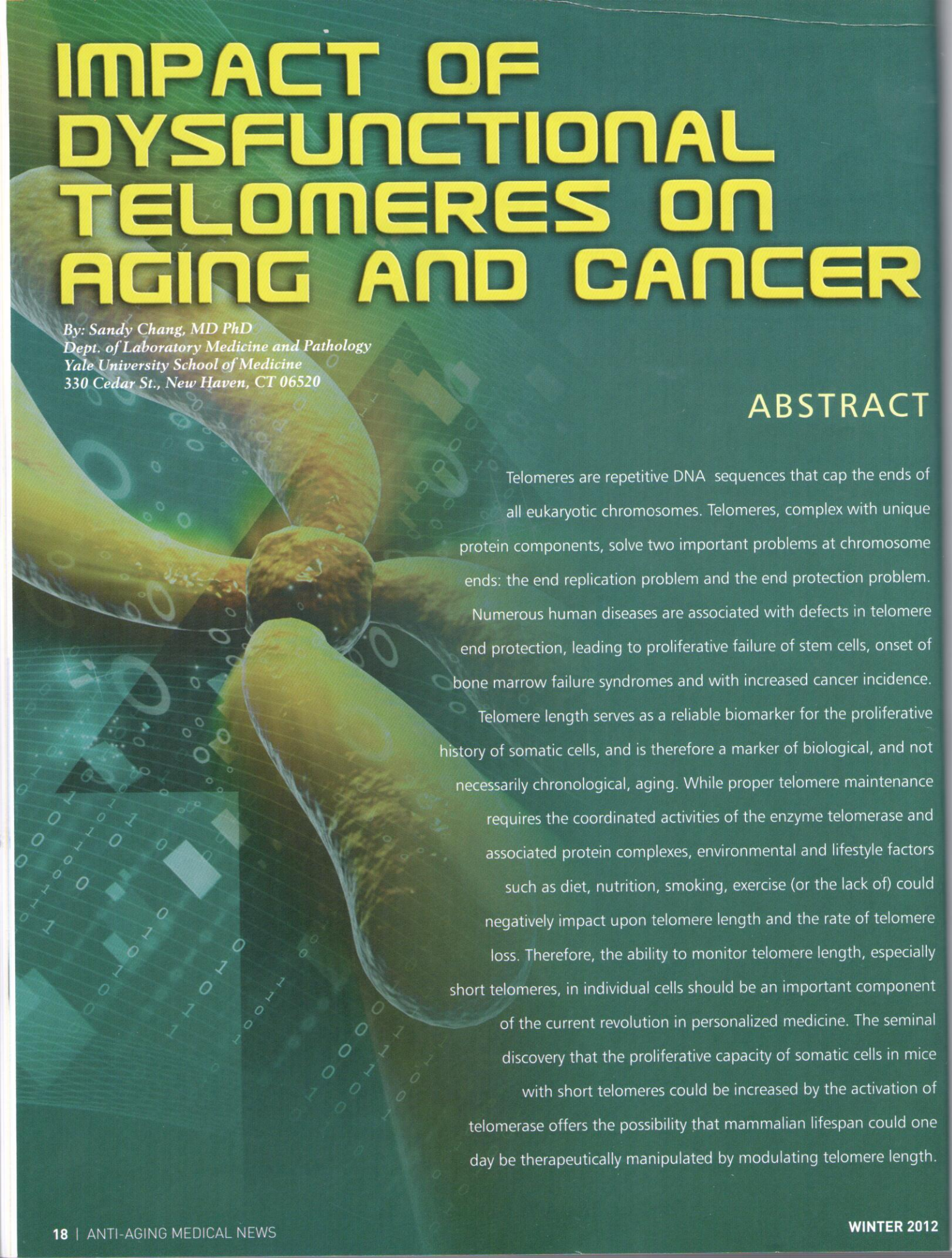

ABSTRACT

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.

TELOMERES ARE NEEDED TO MAINTAIN CELLULAR FUNCTION. More than 50 years ago, Dr. Leonard Hayflick conducted a ground breaking experiment (1). He took primary human diploid fibroblasts and continuously passaged these cells in culture. What he found greatly surprised him - his cells would invariably stop dividing after 60-70 passages (now named the Hayflick limit). This result suggested that human primary fibroblasts cannot divide forever (they are mortal), and that they contained a signal telling them to stop dividing after a defined number of cell divisions. His data contrasted with those observed in human cancer cell lines, which do not display this growth checkpoint. Cancer cells are immortal and could be passaged indefinitely, while normal somatic cells experience cellular aging, or replicative senescence, after a set number of divisions. It was a great puzzle, then, as to why these two cell types were so different.

It is now known that telomeres, protein-DNA complexes that cap the ends of all chromosomes, serve as mitotic clocks that keep track of the number of cell divisions during a cell's lifetime. Because the DNA polymerase machinery cannot completely replicate lagging chromosome strands, each cell division results in progressive erosion of chromosomal ends. It is estimated that up to 200 base pairs of genomic DNA are lost with each round of DNA replication, resulting in a total loss of ~10 kb of DNA over the lifetime of long-lived organisms like humans. This degree of erosion could result in the loss of vital genetic information, eventually adversely affecting cellular homeostasis. So how do cells protect important genes from being lost through erosion?

The DNA portion of telomeres consist of long stretches of TTAGGG repeats that act as a buffer of non-coding sequences that prevent more important genes from being lost. Most importantly, higher eukaryotes have an enzyme called telomerase that functions to add TTAGGG repeats to chromosome ends, preventing them from being whittled away. Telomerase is a unique ribonucleoprotein complex that includes an RNA template (TERC) and a reverse transcriptase catalytic subunit (TERT). Telomerase therefore solves the "end replication problem" that plagues all organisms carrying linear chromosomes. Telomerase is expressed only in certain cells in our body, including stem cells. They are also highly expressed in most human cancer cells. Telomerase-positive cells therefore do not experience telomere shortening with increased cell division, making them immortal. Somatic cells, on the other hand, do not express telomerase. Their telomeres gradually shorten with each round of cell division, until their telomeres become so short that they are no longer protective. These "dysfunctional" telomeres act as damaged DNA, which in turn activates a potent p53-dependent cellular DNA damage response (DDR) to stop further cell division. These results indicate that continued maintenance of telomere length by telomerase is essential for cellular immortality. In addition, dysfunctional telomeres often stick to each other, resulting in increased chromosomal fusions and the formation of an unstable genome that promotes cancer initiation and progression (2).

MANY PROTEINS ARE REQUIRED FOR TELOMERE FUNCTION. Besides telomerase, maintenance of telomeres also require six telomere-specific binding proteins which forms a complex, termed Shelterin, that protects telomeres from inappropriately activating DDR checkpoints (Figure 1)(3). Three proteins, TRF1, TRF2 and RAP1, bind specifically to the double-stranded portion of telomeres. In addition, the protein POT1 binds to the very ends of telomeres, which exist as single-stranded DNA. POT1 forms a heterodimer with another protein TPP1, and this complex in turn interacts with TRF2 through the adapter protein TIN2. Deletion of these telomere binding proteins results in the rapid activation of a DDR and end-to-end chromosome fusions that result in genome instability. In addition to telomerase and shelterin, several other

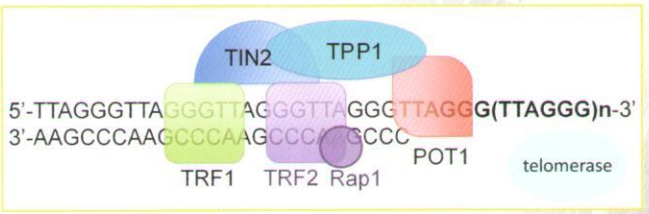

Figure 1. Schematic of a telomere. The telomere repetitive DNA sequences, six telomere binding proteins and telomerase are illustrated.

accessory proteins are required for the maintenance of telomere homeostasis. While it is not possible to document them all in this brief review, the STN1-TEN1-CTC1 protein complex has been shown to be required to recruit telomerase to telomeres, and is also critically important for telomere replication (4). Therefore, the maintenance of proper telomere function requires the orchestration of a large number of proteins at the ends of our chromosomes: telomerase to elongate telomeres after each round of cell division, CST complex to replicate telomeres, and shelterin to constantly stand guard and protect telomeres from being recognized as broken DNA by our DNA damage surveillance machinery.

DYSFUNCTIONAL TELOMERES PROMOTE HUMAN DISEASES. Given the large number of essential proteins required for telomere maintenance, it should not come as a surprise that several human diseases are due to mutations of these proteins. Accumulating evidence show that defects

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