Biology of telomeres: importance in etiology of esophageal cancer and as therapeutic target

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The purpose of this review is to highlight the importance of telomeres, the mechanisms implicated in their maintenance, and their role in the etiology as well as the treatment of human esophageal cancer. We will also discuss the role of telomeres in the maintenance and preservation of genomic integrity, the consequences of telomere dysfunction, and the various factors that may affect telomere health in esophageal tissue predisposing it to oncogenesis. There has been growing evidence that telomeres, which can be affected by various intrinsic and extrinsic factors, contribute to genomic instability, oncogenesis, as well as proliferation of cancer cells. Telomeres are the protective DNA-protein complexes at chromosome ends. Telomeric DNA undergoes progressive shortening with age leading to cellular senescence and/or apoptosis. If senescence/apoptosis is prevented as a consequence of specific genomic changes, continued proliferation leads to very short (ie, dysfunctional) telomeres that can potentially cause genomic instability, thus, increasing the risk for activation of telomere maintenance mechanisms and oncogenesis. Like many other cancers, esophageal cancer cells have short telomeres and elevated telomerase, the enzyme that maintains telomeres in most cancer cells. Homologous recombination, which is implicated in the alternate pathway of telomere elongation, is also elevated in Barrett's-associated esophageal adenocarcinoma. Evidence from our laboratory indicates that both telomerase and homologous recombination contribute to telomere maintenance, DNA repair, and the ongoing survival of esophageal cancer cells. This indicates that telomere maintenance mechanisms may potentially be targeted to make esophageal cancer cells static. The rate at which telomeres in healthy cells shorten is determined by a number of intrinsic and extrinsic factors, including those associated with lifestyle. Avoidance of factors that may directly or indirectly injure esophageal tissue including its telomeric and other genomic DNA can not only reduce the risk of development of esophageal cancer but may also have positive impact on overall health and lifespan. (Translational Research 2013;162:364–370)

Abbreviations: HR = homologous recombination; BAC = Barrett's esophageal adenocarcinoma; ALT = alternative lengthening of telomeres; DSB = DNA double strand breaks; ITRs = interspersed telomeric repeats; QFISH = quantitative fluorescent in situ hybridization; hTERT = catalytic subunit of telomerase; hTR = RNA component of telomerase; LCM = laser capture microdissection

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DNA at each chromosome end is comprised of multiple nucleotide repeats of "TTAGGG." A number of proteins (including TRF1 and TRF2[1]) interact with these repeats and play specific roles in the maintenance of chromosome ends. It has been proposed that the interaction of TRF2 with terminal "TTAGGG" repeats helps formation of a looped structure at each chromosome end.[2] The protective structure formed by association of "TTAGGG" repeats with specific proteins, at each chromosome end, is called a telomere. Telomeres protect chromosomes from degradation by nucleases and unscheduled or unnecessary inter-telomeric recombination and/or fusion.[3] Telomeres, therefore, play a critical role in the maintenance of genomic integrity and the preservation of vital genetic information. In the normal cellular environment, the inability of replication machinery to completely synthesize telomeric deoxyribonucleic acid (DNA) leads to a gradual shortening of telomeres with age.[4,5] When telomeres shorten beyond a certain length, the protective structure falls apart leading to complete or partial loss of the chromosome and subsequently, the induction of cellular senescence and/or apoptotic death.[6-11] Therefore, the length of telomeric DNA may influence the overall lifespan of a cell in culture and an organism *in vivo*.[12] Telomere shortening can also be expedited by various intrinsic or extrinsic factors, which may induce damage to telomeric DNA.[13] Excessive telomere shortening is not only associated with reduced lifespan but also with genomic instability that can lead to oncogenesis.[14-16]

Telomeres, the DNA-protein complexes at chromosome ends, form a looped structure that caps the chromosomal DNA, thus, protecting it from degradation, allowing the recognition of DNA damage, and/or preventing interchromosomal fusion.[17] However, the length of telomeric DNA in most normal somatic cells shortens with each cell division. When telomere length in a cell reaches the critical length required to support its protective function, the cell undergoes growth arrest and replicative senescence and/or apoptosis.[18-20] Short and dysfunctional telomeres can also be recognized as DNA damage leading to p53-dependent apoptosis.[21] As a normal cellular process, telomeres undergo a gradual and progressive shortening with age, thus, limiting the replicative potential and lifespan of normal cells.[22,23] Although telomere length and the rate of its shortening may vary among different tissues in the body, telomere length negatively correlates with age.[5,22-26] The rate of telomere shortening is also affected by various intrinsic and extrinsic factors including genetic and epigenetic signals, oxidative metabolites, environmental exposures, and individual lifestyle.[23,27-33] For example, smoking, lack of exercise, and consumption of an unhealthy diet (marked by excessive fat and processed meats with the reduced intake of fruits, vegetables, fiber, and antioxidants) can accelerate telomere shortening, which in turn can predispose to the early onset of a number of age-related health issues including heart disease, cancer, and reduced lifespan.[15,16,34-41] In summary, telomeres preserve chromosomal integrity but shorten with age, thus, limiting the number of doublings a cell can go through in culture or *in vivo*. The lifespan of normal cells depends on telomere length and the rate of its shortening. Unhealthy diet and lifestyle can increase the rate of telomere shortening leading to early onset of age-associated diseases.

The progressive telomere shortening that occurs as a normal process in most somatic cells is prevented in the germ-line and in a subset of stem cells by telomerase, the enzyme that adds "TTAGGG" repeats to existing telomeres.[42,43] Telomerase activity, which is absent or weakly detected in normal somatic cells, is elevated in the majority of immortal cells and cancer cells.[44-46] Telomerase is an enzyme with 2 distinct components, the protein or catalytic subunit (hTERT) and the ribonucleic acid (RNA) subunit (hTR), which carries the telomeric sequence information. The catalytic subunit of the enzyme copies telomeric sequences from the template hTR and reverse transcribes them for incorporation into telomeres.[47] Certain cancer cells and immortal cells do not have detectable telomerase activity and elongate their telomeres using an alternative mechanism known as the alternative lengthening of telomeres (ALT) pathway.[48] ALT can be defined as homologous recombination-mediated extension of telomeric DNA and requires the activity of several DNA repair and recombination proteins. Unlike telomerase positive cells, ALT cells have long heterogenous telomeres. Electron microscopy has revealed that the 3′ single-stranded part of telomeric DNA undergoes looping and invasion into the adjacent double-stranded DNA. The process is similar to recombinase (RAD51)-mediated homologous pairing and the resulting structure has similarity with Holliday junctions. The invaded DNA strand in this structure is positioned for elongation by the homologous recombination, which is deregulated in cancer. In normal cellular environment, homologous recombination is extremely precise and regulated and, therefore, does not extend telomeres. Moreover, there are mechanisms that prevent unscheduled or unnecessary recombination at telomeric sequences in normal cells; these mechanisms seem to be disrupted in ALT cells.

Shorter telomeres lead to the induction of replicative senescence and/or apoptosis.[18,20] However, in the absence of p53 function or with similar defects in