Mutations, Cancer and the Telomere Length Paradox

Abraham Aviv* †, James J Anderson‡, and Jerry W Shay§

†The Center of Human Development and Aging, New Jersey Medical School, Rutgers, Newark, NJ 07103, USA
‡Center for Statistics and the Social Sciences and Center for Studies in Demography and Ecology, University of Washington, Seattle, WA 98105, USA
§Department of Cell Biology, UT Southwestern Medical Center, Dallas TX, 75390, USA

Abstract

Individuals with short telomeres should be at increased risk for cancer, since short telomeres lead to genomic instability—a hallmark of cancer. However, individuals with long telomeres also display an increased risk for major cancers, thus creating a cancer-telomere length paradox. The two-stage clonal expansion model we propose is based on the thesis that a series of mutational hits (1st Hit) at the stem-cell level generates a clone with replicative advantage. A series of additional mutational hits (2nd Hit) transforms the expanding clone into cancer. By proposing that the 1st Hit is largely telomere length-independent, while the 2nd Hit is largely telomere length-dependent, we resolve the paradox, highlighting a regulatory role of telomeres in cancer.

Keywords
telomeres; cancer; evolution; stem cells; clones; mutation

The Cancer-Telomere Length Paradox and the Two-stage Model of Carcinogenesis

Two recent papers offered competing mathematical models that examined the potential roles of mutations arising from proliferative tissues in the development of human cancers [1,2]. An additional report described the accumulation of mutations in benign nevi as their cells experienced transformations through proliferation into incipient and then malignant melanomas [3]. Telomere length (TL), a key element in proliferative potential and hence mutation accumulation and cancer, was not mentioned in these papers. The frequent
omission of TL’s role in proliferation-mediated mutations and cancer might reflect a struggle of cancer epidemiologists and biologists to distinguish between the constitutive TL, i.e., TL in normal somatic tissues of individuals stricken with cancer, versus TL in their cancerous tissues.

In this opinion article we propose a solution that reconciles apparently conflicting, yet connected observations about TL dynamics and cancer in humans. First, most solid cancers that originate from proliferative tissues display short telomeres [4]. Second, given that cancer incidence increases with age, older individuals, whose telomeres in somatic cells are typically shorter than in younger ones, have increased propensity to major cancers. These findings therefore suggest that individuals with constitutively short telomeres should be at increased risk for cancer. In fact, it is widely believed that short telomeres in combination with other oncogenic changes might lead to genomic instability, which is often displayed in many cancers [5]. However, while earlier studies (perhaps due to suboptimal study design and small sample sizes) generated mixed findings [6], recent studies have shown that in the general population individuals with constitutively long telomeres are also at a higher risk for major cancers [7–14].

The solution we offer for these seemingly conflicting findings is based on “condensing” the multistage theory of carcinogenesis offered by Armitage and Doll more than half a century ago [15] into a two-stage model. While the solution can be incorporated into multistage models of carcinogenesis, complexity beyond a two-stage model is not necessarily required to illustrate the role of TL dynamics (TL and its shortening) in cancer development. This perspective aligns with Armitage [16], who concluded: “In the construction of mathematical models for biological phenomena it is not uncommon to find that theories of quite disparate types provide good fits to the same data. Discrimination between models must then depend partly on general biological plausibility and partly on the ability of the models to explain new data.” There was little information about telomeres at the time that this statement was written. The present understanding of telomere biology and epidemiology provides sufficient confidence in the plausibility of the two-stage telomere model of carcinogenesis we now propose.

Our model, whose mathematic formulation is provided under Supplementary Information, is built on the concept that TL plays little or no role in the first stage of carcinogenesis that takes place at the stem cell level. However, TL is a key determinant in the progression towards cancer in the second stage—the clonal expansion. First, however, we briefly review telomere biology from evolutionary and epidemiological perspectives, underscoring findings suggesting there is a tradeoff between cancer and degenerative disease, driven by TL-mediated replicative potential.

**Evolutionary and Epidemiological Perspectives**

Somatic cells undergo far more replications for growth and maintenance in large compared to small mammals. Given that replications of somatic cells increase the organismal mutation burden and hence cancer risk, Peto reasoned four decades ago that large mammals should have more cancer than small ones, yet there is no evidence that cancer risk scales with body
Large mammals, who are by and large long-living, have developed an array of anti-cancer defenses, a few of which might be unique for a given species, while others are shared across species.

For instance, a recent study showed that African elephants have 40 copies of $Tp53$ compared with 2 copies of the gene in humans. This gene has evolved in multi-cellular organisms, and it encodes a transcriptional regulator protein that serves as a powerful tumor suppressor through participating in multiple cell-cycle checkpoints. Further analysis revealed that not only African elephants but also other elephants, including the extinct woolly mammoth, have multiple $Tp53$ retrogenes and that several of these genes transcribe the tumor suppressor protein. Another recent study found that while the bowhead whale does not have extra copies of $Tp53$, the genome of this longest-living mammal displays the signature of positive selection of genes engaged in DNA repair and cell-cycle regulation, which might augment cancer resistance.

On average, one in three persons in middle and high income countries is stricken with cancer during her/his life course. This high cancer incidence in contemporary humans is attributed to increased mutation burden brought about by rapid environmental changes caused by pollution, lifestyle and migration. That said, from the evolutionary perspective, humans, who are middle-size but the longest-living terrestrial mammals, share comparatively short telomeres and repressed activity of telomerase in somatic tissues with African elephants, bowhead whales and other long-living large mammals. Short telomeres and repressed telomerase would thus diminish the probability of attaining a critical number of replication-mediated mutations required for cancer development. That is because cells with short telomeres are prone to telomere-based senescence after fewer replications compared to cells with long telomeres. This paradigm has led to the concept of evolutionary tradeoff (i.e., cost): Cancer resistance due to repressed telomerase and short telomeres might limit regenerative capacity, thus increasing the likelihood of age-dependent degenerative diseases, particularly as animals get older and their telomeres undergo further shortening.

The cancer-degenerative disease tradeoff may apply not only across mammalian species, but also within the human species. TL, which is a highly heritable complex genetic trait, displays wide variation across the population, a phenomenon that is already observed at birth. While individuals with constitutively long telomeres are prone to major cancers, those with constitutively short telomeres are prone to atherosclerotic cardiovascular disease, perhaps the most common human degenerative disease. Moreover, the cancer-atherosclerosis tradeoff is also displayed at the genomic level, since alleles associated with long telomeres increase the risk of major cancers; while alleles associated with short telomeres increase the risk of atherosclerotic cardiovascular disease. Evolutionary forces ostensibly account for some of the variation in TL and TL-related disease outcomes across humans. Support for this notion is provided, for instance, by findings that polygenetic adaptation might partially explain the shorter TL in individuals of European ancestry compared to those of African ancestry. Such an adaptation might have attenuated the increased risk of depigmented skin to sporadic melanoma.
The Potential Resolution of the Cancer-Telomere Length Paradox

Why do individuals with constitutively long telomeres have a higher risk for major cancers, yet the overwhelming majority of cancerous tissues have short telomeres [4]? Moreover, if increased replicative potential and constitutively long telomeres augment cancer risk, why do the incidences of major cancers increase with age in the face of progressive age-dependent TL attrition and hence shorter telomeres in replicating somatic tissues of older persons?

With rare exceptions [35], carcinogenesis is believed to arise from a series of driver mutations and selection of cells with replicative advantage [36]. The rate of accumulation of somatic mutations is much faster in children than in adults [37], perhaps because during growth and development a faster stem cell replication pool serves not only maintenance activities but also the building of somatic tissues. We propose that the first stage in the two-stage model of carcinogenesis entails one or more mutational hits when tissue-specific, multipotent stem cells undergo replications (1st Hit), which may take place during any period in the life course, including childhood (Fig. 1, Key Figure). The resulting mutated clones have replicative or survival advantages compared with surrounding cells. These still benign clones are at a higher risk of cancerous transformation in the event of second-stage series of driver mutations (2nd Hit), i.e., mutations that engender positive selection at the clonal level [38]. Thus, more replications of the expanding clones would heighten the risk of additional mutations and incipient cancer. Here is where TL might become important.

The model we propose comprises two key premises: In the first-stage, a random process describes the probability of the 1st Hit upon stem cell division and explains the rise with age in the incidence of many types of cancers. This stage is TL independent. In contrast, the 2nd Hit is TL dependent, such that in individuals with constitutively long telomeres, clones primed by the 1st Hit at the stem cell level are capable of undergoing more replications before reaching replicative senescence (scenarios a, b, c in Figure 1, Key Figure). A larger clonal expansion due to long telomeres would thus increase the hazard of acquiring the 2nd Hit driver mutations that ultimately cause malignant transformation. Notably, although stem cells at the top of the somatic cell hierarchy experience age-dependent TL attrition, TL in most stem cells is still longer than that in most differentiated somatic cells down the hierarchy. Thus, TL becomes limiting primarily in highly proliferative somatic cells down the hierarchy, i.e., at the 2nd Hit, during clonal expansion.

Intrinsic to the model is the long-held notion that telomeres undergo progressive erosion during the clonal expansion until telomerase is activated by mechanisms that might include telomerase reverse transcriptase gene (TERT) promoter mutations, transcriptional changes, epigenetic/chromatin modifications and splicing alterations [39]. Thus, telomerase activation during clonal expansion is permissive to the development of most cancers, as recently illustrated for the accumulating mutations in expanding nevi while they undergo successive stages of transformation into malignant melanoma [3]. That said, a small subset (~10%) of adult cancers [40] displays long TL, perhaps due to early activation of TERT by promoter mutations. Such exceptions hardly represent the cancer-TL nexus in the majority of the population. In addition, other potential mechanisms might explain short telomeres in...
cancerous tissues. These include dysregulation during carcinogenesis in the shelterin proteins [41]. In vitro studies also showed that engineered telomere elongation in prostate cancer cells promoted their differentiation, suggesting selection of cancer cells [42].

However, these alternatives do not contradict the premise that individuals with constitutively long TL are more likely to sustain the 2nd Hit, which is crucial for carcinogenesis in the two-stage telomere model we propose.

The model might also explain the decline in the incidence of cancer after the 8th decade of life [43] (see mathematic formulation in Supplementary Information). While very old persons have accumulated first-stage mutational hits at the stem cell level, because of their short telomeres, they are less likely to sustain through clonal expansion the 2nd Hit. This might explain the age-dependent increase of carcinomas in situ, or ‘covert cancers’ [44], which suggests that in some old persons, due to short telomeres, incipient cancers have exhausted their replication capacity prior to becoming malignant.

We note that the partition of the 1st Hit at the stem cell level from the 2nd Hit at the clonal level does not imply that most cancers arise from stem cells. The premise of TL-independent 1st Hit is meant to reconcile findings that older individuals, whose telomeres are relatively short, are at a higher risk of cancer. Accumulation of mutations at the stem cell level might explain such findings, but in most cases stem cell mutations appear insufficient to trigger cancer. In line with this premise are findings that while driver mutations in the TERT promoter robustly activated telomerase in cultured somatic cells, the same mutations in pluripotent stem cells increased only modestly TERT transcription without an apparent increase in telomerase activity [45]. Perhaps the same may apply to multipotent stem cell in vivo.

Cancer risk is high in diseases with deleterious, highly penetrant germ-line mutations in telomere maintenance genes, including dyskeratosis congenita [46]. Patients with these diseases display critically short TL and their propensity to develop cancer appears on the surface to contradict the model. The heightened cancer risk in these patients may indeed result from critically short telomeres causing rearrangements through chromosome breakage-fusion-bridge cycles that bring about chromosomal instability. Based on this concept, it has been assumed that innately short telomeres increase cancer risk due to genomic instability [5]. However, the very short telomeres observed in rare monogenic diseases of telomere maintenance are typically below the lower 1% of the TL distribution in the general population. This means that the genomic instability in monogenic diseases with very short telomeres is constitutive, systemic, and already present at the stem cell level. In contrast, genomic instability associated with cancer in individuals from the general population is acquired at the clonal level and primarily occurs locally at the specific site that experiences clonal expansion. Thus, with respect to cancer susceptibility, what applies for monogenic diseases of critically short telomeres does not seem to apply for the general population.
Concluding Remarks

Our model (see mathematic formulation in Supplementary Information) is clearly an oversimplification of a very complex process, but we believe that it illustrates key ingredients in the cancer-TL connection. Furthermore, it should be straightforward to incorporate the TL-regulation mechanism into other models. The model underscores that constitutively long telomeres do not cause cancer but they might facilitate carcinogenesis in certain instances. It predicts that the 1st Hit is age-dependent in sporadic cancers, but might be largely age-independent in familial cancers due to highly penetrant germ-line mutations (e.g., BRCA1 and BRCA2). This suggests that individuals with familial cancers and comparatively long telomeres would express the disease on average at a younger age. (For further learning about the potential role of TL in cancer and other aging-related diseases, see Outstanding Questions.)

Finally, accurate, reproducible and reliable phenotypic characterizations are critical for precision medicine. However, most epidemiological data about cancer and TL are derived from qPCR-based measurements. These provide information on average telomere DNA content in relative units of amplifiable sequences of telomeres (T) and a single reference gene (S), i.e., the T/S ratio, whose metrics differ across laboratories [47,48]. As illustrated in the Supplemental Information, there is more to understanding the role of telomeres in a given cancer than just knowing whether TL is innately long (or short) in individual’s stricken with the disease. Going forward, knowledge based on precise and reproducible metrics of TL and the extent of the difference (in absolute TL units) between cancer cases and controls and between cancerous and non-cancerous tissues within the individual is crucial for understanding the progression of cancer and the possible role of telomeres in its regulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Telomeres
the region of tandem repetitive nucleotides, (TTAGGG)n, at the ends of chromosomes, which together with the shelterin proteins cap and protect the chromosomes from being recognized as sites of DNA damage

Carcinogenesis or oncogenesis
the process by which normal cells develop stepwise genetic and epigenetic alterations for the initiation and development of cancer

**Genomic instability**

a characteristic of most cancers due to the process of rapid alterations in the genome through mutations, chromosomal rearrangements and altered number of chromosomes

**Stem cells**

the undifferentiated cells at the top of the hierarchy of cells in multicellular organisms; they give rise to progenitor (transit-amplifying) cells that progressively become differentiated cells that make up the tissues of these organisms.

**Somatic cells**

all cells, including stem cells, in the body, except sperm and eggs

**TP53**

a master gene that regulates many other genes in the cell to suppress uncontrolled growth

**Reverse transcriptase**

an enzyme that synthesizes complementary DNA from an RNA template

**Retrogene**

genes copied back from RNAs by reverse transcription

**Positive selection**

the mechanism through which new advantageous alleles (genetic variants) increase in frequency across a population

**Degenerative diseases**

mostly-aging related diseases that are brought about by the inability of the repair mechanisms in the body to catch up with injury

**Polygenetic adaptation**

simultaneous shifts in frequencies of many alleles (genetic variants) influencing an advantageous trait

**Clone**

a cell that is identical to the cell from which it was derived

**Clonal expansion**

the process of expansion through symmetric replication, i.e., a cell giving rise to two identical daughter cells

**Driver mutation**

a mutation that confers a selective growth advantage, thereby promoting carcinogenesis

**Epigenetic**

modification of gene expression by mechanisms independent of alteration of the genetic code

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Carcinoma in situ
a mass of cancerous cells that has not invaded surrounding tissues and did not spread to other parts of the body

Dyskeratosis congenita
a rare disorder caused by a single detrimental germline mutation that results in extremely short telomeres. There are several sub-types of the disease, depending on the specific gene mutant

Constitutive
innate

References


### Outstanding Questions

- Will a catalog of telomere lengths in cancers, their normal tissues of origins and in leukocytes be helpful in obtaining better metrics of cancer risk per unit telomere length?
- Might discoveries of variant telomere maintenance genes enable developing a clinically useful, individual telomere-length genetic risk for a host of aging-related human diseases?
- Might the individual telomere length risk explain in part ethnic differences in the propensity to various cancers and cardiovascular disease over and above traditional environmental and ethnic-related risks?
- Will the individual telomere length risk be useful for early diagnosis of diseases other than cancer, e.g., cardiovascular disease and Alzheimer?
- Might the individual telomere length risk explain some of the inter-individual variation in longevity?
- Can we develop new preventive measures and therapeutic modalities for cancer and other telomere-associated diseases based on the individual telomere length risk?
- Can the differential between cancer and normal tissue TL provide information on the role of senescence in promoting cancer?
Trends Box

- Individuals with constitutively long telomeres and/or variant genes associated with long telomeres exhibit increased risk of major cancers. However, almost all cancers have short telomeres.
- Telomeres are shorter in somatic tissues of older than younger persons, yet older persons are at a higher risk of many cancers.
- Incidence of cancer declines after the eight decade.
- Multistage models of mutation-driven carcinogenesis have not attempted to reconcile the above conflicting findings in regards to telomeres.
- A model based on the premise that mutations acquired at the stem cell level are largely independent of telomere length, while those acquired during clonal expansion are telomere-length dependent provides a solution for these conflicting findings and a new perspective on the role of telomere biology in carcinogenesis.
Fig. 1, Key Figure. Mutation accumulation in the two-stage telomere model of carcinogenesis
The dotted horizontal line partitions the first—stage series of mutational hits (1st Hit) from the second-stage series of mutational hits (2nd Hit). The 1st Hit occurs during replication of stem cells; it primes non-cancerous clones to acquire replicative advantage. The 2nd Hit takes place in the clones, causing further selection, which might ultimately lead to malignant transformation. Telomere length differentially affects the 1st Hit and the 2nd Hit. The 1st Hit is independent of telomere length. The chance of a clone having the 2nd Hit and undergoing malignant transformation scales with the maximal clone size, which depends on telomere length. Thus, the chance for malignant transformation increases in the following order: scenario c > scenario b > scenario a.