JCI The Journal of Clinical Investigation

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J Clin Invest. 2019;129(9):3474-3481. https://doi.org/10.1172/JCI120851.

Review Series

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Long telomeres and cancer risk: the price of cellular immortality

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The distribution of telomere length in humans is broad, but it has finite upper and lower boundaries. Growing evidence shows that there are disease processes that are caused by both short and long telomere length extremes. The genetic basis of these short and long telomere syndromes may be linked to mutations in the same genes, such as the telomerase reverse transcriptase (*TERT*), but through differential effects on telomere length. Short telomere syndromes have a predominant degenerative phenotype marked by organ failure that most commonly manifests as pulmonary fibrosis and are associated with a relatively low cancer incidence. In contrast, insights from studies of cancer-prone families as well as genome-wide association studies (GWAS) have identified both rare and common variants that lengthen telomeres as being strongly associated with cancer risk. We have hypothesized that these cancers represent a long telomere syndrome that is associated with a high penetrance of cutaneous melanoma and chronic lymphocytic leukemia. In this Review, we will synthesize the clinical and human genetic observations with data from mouse models to define the role of telomeres in cancer etiology and biology.

Introduction

Telomeres may at first glance simply appear as geographic boundaries at chromosome ends. However, there is mounting evidence that disturbances in telomere length, at short and, as we will discuss here, long extremes, are linked to disease susceptibility. Telomere DNA is a repetitive hexamer of TTAGGGs that is bound by a specialized protein complex known as shelterin (1–3). Telomerase synthesizes new telomere DNA to offset the shortening that normally occurs during DNA replication (4, 5). Telomerase has two essential components: the telomerase reverse transcriptase (TERT) uses a template within an intrinsic telomerase RNA component, TR (also known as TERC), to add telomere repeats onto the 3′ ends of chromosomes (6–9). Shelterin proteins prevent chromosome ends from fusing and from being recognized as double-strand breaks (3). They also regulate telomerase access to the telomere, and promote telomere repeat addition processivity by allowing TERT to use a relatively short template in TR to iteratively synthesize longer telomere tracks (10–12). As we will discuss here, disorders of telomere length are increasingly appreciated as causing clinically recognizable disease processes (13). The short telomere syndromes are now phenotypically and genetically well characterized (14–19). This knowledge is increasingly integrated into clinical algorithms, especially for patients with lung disease and bone marrow failure (20, 21). Here, we focus on emerging evidence, from cancer-prone families as well as from population-based studies, linking germline variants that promote telomere lengthening to cancer susceptibility. We contrast the genetic basis of the two extreme telomere length phenotypes and highlight how recent human-focused studies provide critical insights into the fundamentals of cancer etiology.

Telomerase is limiting

The foundational understanding of the role of telomeres and telomerase in disease has been rooted in curiosity-driven science, in simple systems and model organisms (22). One major theme that emerges at the intersection between this fundamental science and disease genetics is that relatively small, subtle changes affecting telomerase abundance or function can influence telomere length and, in turn, disease risk (23). The exquisite sensitivity of telomere length to these small changes is related to the fact that telomerase is in very low abundance and its activity is tightly regulated. In yeast, mice, and humans, the number of telomere ends exceeds the number of telomerase molecules (refs. 24, 25, and reviewed in refs. 23, 26). The low levels of telomerase set up a system wherein not all telomeres are elongated during a given cell cycle even when telomerase is normally expressed (27). There are at least three additional limits on telomerase activity. The first is that the essential telomerase components, TERT and TR, are expressed at very low levels relative to other proteins and RNAs (e.g., refs. 24, 28). Even other factors involved in telomerase biogenesis, such as nuclear assembly factor 1 (*NAF1*), which promotes shuttling of TR to the nucleolus for assembly with TERT, show haploinsufficiency for telomere length (28). Thus, although only 10% of human genes are estimated to show haploinsufficiency, many of the telomerase and related genes that have been heretofore linked to Mendelian disease, including *TERT*, *TR*, and *NAF1*, do so (14, 28–30). A second limit is that telo-

Conflict of interest: EJM is currently employed by and has stock options in SQZ Biotech, a cell therapy company.

Reference information: *J Clin Invest*. 2019;129(9):3474–3481.
https://doi.org/10.1172/JCI120851.

merase expression is also tightly regulated. After early embryonic development, the *TERT* promoter is repressed in most somatic cells, likely through promoter hypomethylation (31–33). The repressive effect of this hypomethylation on *TERT* expression is counter to effects in most other contexts. The timing of *TERT* silencing leaves a small window during early development for telomeres to be elongated (34), and makes telomere length highly heritable and, in great part, influenced by parental telomere length (27, 35, 36). A third check on telomere elongation is that even when expressed, the timing for telomere repeat addition is cell cycle–regulated and restricted to late S phase (reviewed in ref. 37). For all these reasons, telomeres shorten even in telomerase-expressing somatic cells, such as hematopoietic progenitors and T cells (26). These checks favor a system where telomere shortening is an overall general default, and as we discuss here, evidence linking long telomeres to the risk of multiple cancers underscores the tumor-suppressive advantages of these checks.

Telomere length has definable upper and lower boundaries

One of the major advances in understanding the role of telomere length in human disease has been the standardization of telomere length measurement methods that more robustly define absolute "short" and "long" telomere thresholds (20, 38). Relying on a method that measures telomere length in distinct leukocyte lineages using combined flow cytometry and fluorescence in situ hybridization (flowFISH), there is outstanding concordance and reproducibility across laboratories (20, 38, 39). These flowFISH telomere length measurements show that human telomere length has a definable normal range with discrete upper and lower boundaries (20). This type of telomere length analysis has the advantage of establishing age-, percentile-adjusted values rather than relative comparisons of "longer" and "shorter" descriptors. This advance has made interpretation of telomere length for precision medicine use possible and analogous to other clinical measurements (e.g., white blood cell count) wherein the normal range is broad, but extreme values, relative to healthy controls, may be associated with the risk of certain pathologies. For this Review, "telomere length" refers to the mean length as measured in leukocytes by flowFISH and reported as an age-adjusted percentile. Within each cell, the shortest telomere(s) signal the DNA damage response associated with cellular senescence and apoptosis (40). Remarkably, however, the mean telomere length, in defined and limited clinical contexts, is an outstanding surrogate and can generally distinguish individuals with germline defects in telomere maintenance from their relatives (15, 41). As such, the mean telomere length as measured in leukocyte subsets by flowFISH is used widely as a diagnostic and prognostic tool in patients suspected to have short telomere syndromes (18, 20, 42).

The human short telomere syndrome phenotype

The cancer-prone state associated with telomere lenghtening contrasts with that of short telomere syndromes. To facilitate these comparisons, we will first briefly review the better-described short telomere diseases. Short telomere syndromes encompass a continuum of clinical presentations that manifest from infancy to late adulthood (26). They are caused by mutations in telomerase and telomere maintenance genes. Their onset is determined in great part by the severity of the short telomere defect (20, 43). Short telomere syndromes generally have two primary clinical presentations. A more severe form manifests in infants and children; it causes disease in high-turnover tissues and primarily recognized as immunodeficiency, bone marrow failure, and enteropathy (16, 19, 20, 44). Adult-onset short telomere syndromes are more common and account for at least 90% of presentations (45). They manifest most frequently as idiopathic pulmonary fibrosis (IPF) and other telomere-related lung disease (45). These telomere-related lung disorders in the vast majority show autosomal dominant inheritance and may appear as emphysema in smokers (45, 46). IPF affects 100,000 individuals in the United States alone, and at least 50% of IPF patients have telomere length in the lowest decile of the population (45). In one-third of families with pulmonary fibrosis, a mutation in telomerase or telomere-related genes is detectable (45). The high frequency of telomere defects in IPF and the prevalence of this disease make IPF the most common of the human short telomere phenotypes. A subset of adult IPF patients show extrapulmonary short telomere syndrome features including bone marrow failure, immunodeficiency, and liver disease; their recognition is critical for the diagnosis and management of these patients (18, 21, 41–43, 47).

Cancer is relatively rare in short telomere syndromes

Cancer is an overall relatively rare complication of short telomere syndromes and affects approximately 10% to 15% of patients (48). This rate is far lower than in other common cancer-prone syndromes, such as Li-Fraumeni, which have lifetime risks around 90% (49). The short telomere cancer spectrum is also restricted to mostly hematologic cancers, the most common being myelodysplastic syndrome, an age-associated clonal disease of the bone marrow. This low cancer incidence lies in contrast to predictions from cell-based models, which reported spontaneous immortalization and transformation of cells after short telomere–induced senescence (50). These clinical observations suggest that, in the presence of an intact DNA damage response, as is the case in most patients with short telomere syndromes, degenerative disease is the predominant phenotype and leads to progressive failure of hematopoiesis, T cell immunity, and end-stage lung-liver disease. Below we will highlight how these clinical findings support what has been documented in nearly all tumor-prone mouse models.

The genetic causes of short telomere syndromes

Understanding the genetic mechanisms by which short telomere syndromes arise is particularly relevant for our Review, because mutations in some of the same genes have also been linked to a cancer-prone state, which we hypothesize is long-telomere mediated. Thirteen genes have been implicated to date in Mendelian short telomere syndrome genetics; they explain 50% to 70% of cases (Figure 1A). Two of these genes are also mutated in cancer-prone families. In general, the vast majority of mutations cause telomere shortening by depleting the abundance of telomerase, disturbing its catalytic functions/processivity, or interfering with its recruitment to the telomere. They affect the telomerase holoenzyme itself (*TERT*, *TR*, *DKC1*), adaptors of the dyskerin complex (*NHP2*, *NOP10*), genes that affect TR

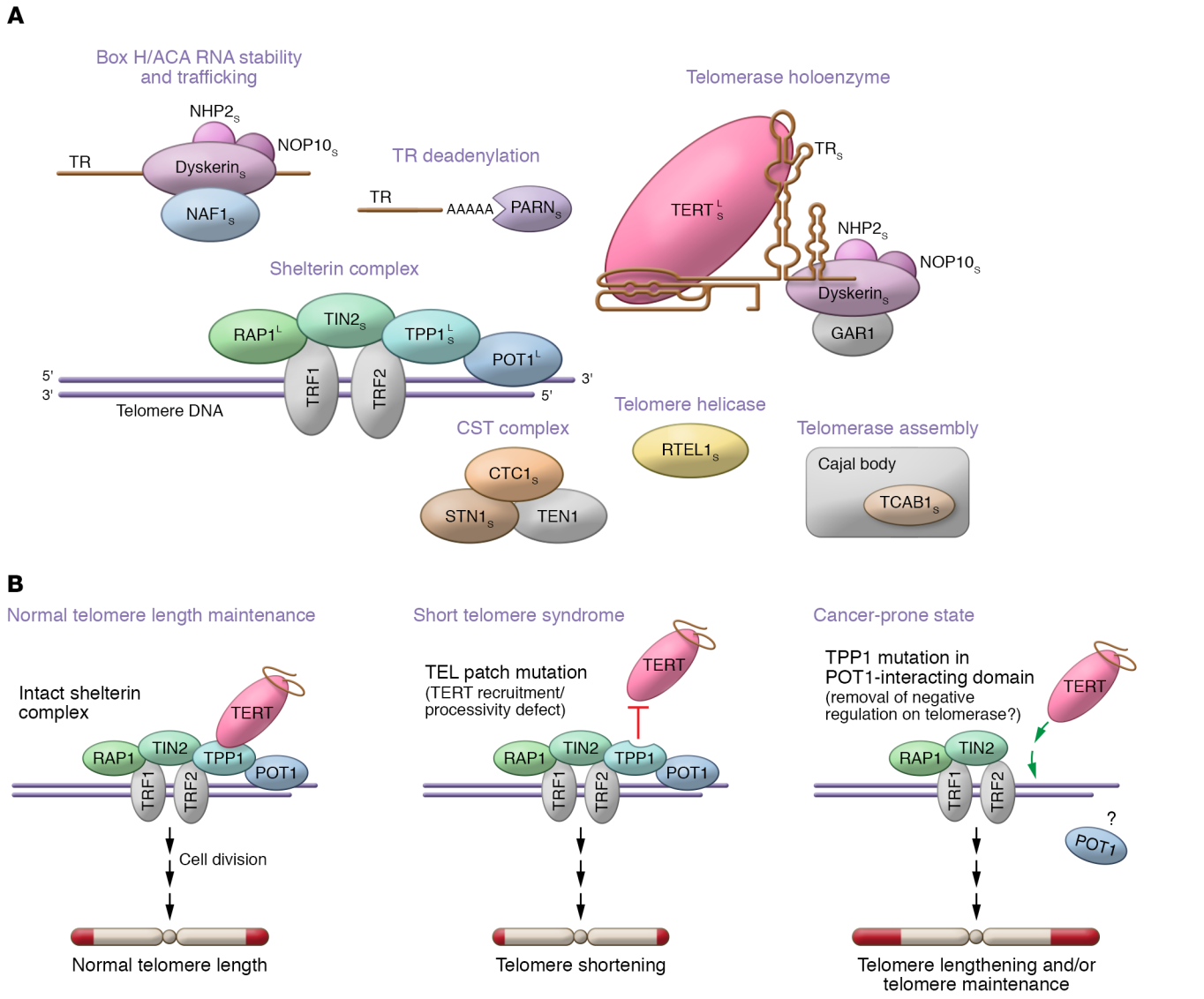

Figure 1. Schematic of mutant telomerase and telomere genes in Mendelian short and long telomere syndromes and model for *TPP1* allele-specific effects on telomere length. (**A**) Components with known mutations are shown in color, and their telomere function is indicated above each group. Thirteen genes have been identified, with the short telomere syndrome associations marked by a subscript S. Four genes are associated with long telomere syndrome phenotypes and are marked by a superscript L. Adapted with permission from *Current Opinion in Genetics & Development* (13). (**B**) The left panel shows the state of telomere length maintenance normally. The middle panel shows how in-frame deletions in the TEL patch interfere with TERT recruitment and processivity, provoking telomere shortening. The right panel shows a model for how cancer-associated mutations may promote telomere maintenance in cancer-prone families. *TPP1* deletions or missense mutations in the POT1-interating domain are predicted to affect POT1's telomere-binding capacity, allowing TERT to elongate more efficiently. The latter is hypothesized to have a net effect of telomere lengthening and/or telomere maintenance.

biogenesis and localization (*PARN*, *NAF1*, *TCAB1*), and regulation of telomerase recruitment to the telomere as well as processivity by shelterin (*TPP1*, also known as *ACD*, and likely *TINF2*; ref. 51). There are also mutations in genes that are thought to affect telomere replication (*RTEL1*) and telomere lagging strand synthesis (*CTC1*, *STN1*). The genetic basis of short telomere syndromes has been reviewed elsewhere (13, 28). As further discussed below, for *TERT* and *TPP1*, mutations that predict telomere lengthening are also associated with high-penetrance familial cancer syndromes (13).

Familial cancers caused by telomere-lengthening mutations

Evidence that long telomere length confers a longevity advantage came initially from studies of primary cultured fibroblasts in which cells with longer telomeres had longer replicative potentials (52). Moreover, exogenous TERT expression was sufficient to bypass cellular senescence and immortalize primary cells (53). In humans, evidence that telomerase upregulation confers a risk of familial cancer was first documented in a five-generation autosomal dominant family with cutaneous malignant melanoma

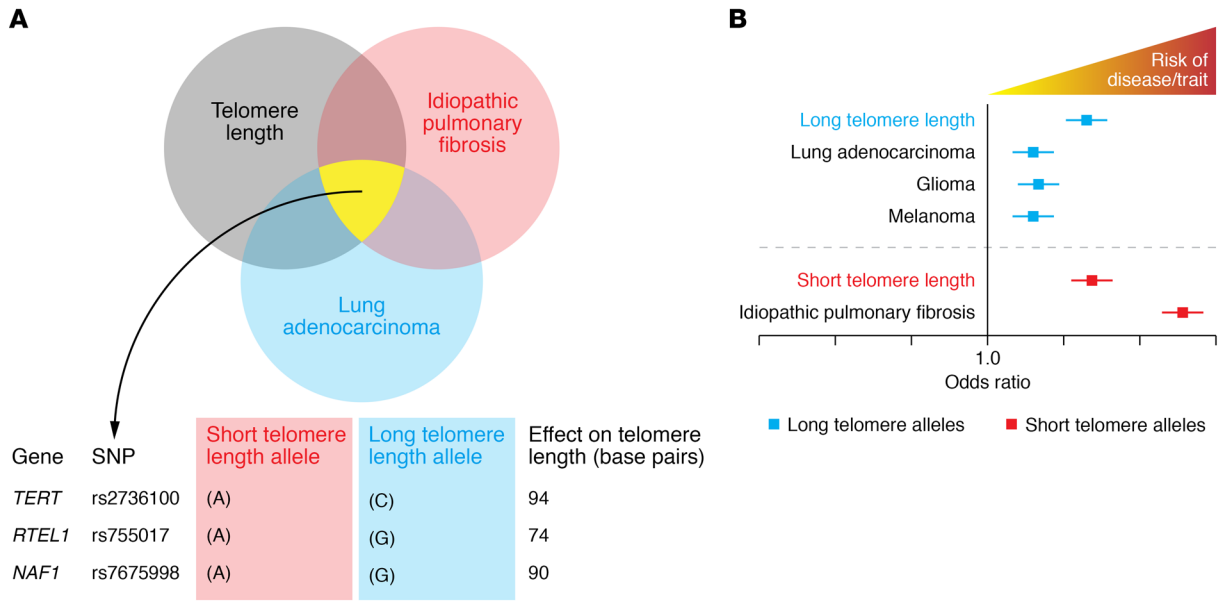

Figure 2. Shared SNPs identified in GWAS near telomere genes are associated with both telomere length and disease risk, but the directionality of the effect is allele-dependent. (**A**) intersection of shared SNPs across GWAS for leukocyte telomere length, lung adenocarcinoma and idiopathic pulmonary fibrosis. The shared SNPs fall near telomere maintenance genes. The alleles for each SNP have differential effects on telomere length with the effect size shown on base pairs. rs2736100 is in intron 2 of TERT. rs755017 is 140 kb downstream of the RTEL1 transcription start site in exon 2. rs7675998 falls 40 kb upstream of the NAF1 transcription start site. (**B**) Schematic forest plot shows the odds ratio of disease risk with short and long telomere SNPs such as those shown in the table in **A**. Data in **B** are adapted with permission from *JAMA Oncology* (88).

(CMM) that was found to carry a mutation in the *TERT* promoter (54). This gain-of-function mutation upregulates *TERT* transcription (54). The mutation, located 57 bases upstream of the *TERT* transcriptional start site, functions similarly to two other common recurrent somatic *TERT* promoter mutations (54, 55). These promoter mutations create a de novo E26 transformation–specific (ETS) transcription factor family binding site that removes the repressive state on *TERT* by allowing interaction with an abundant GA-binding protein (GABP) transcription factor to promote *TERT* transcription (54, 56, 57). A second melanoma family was recently found to carry another *TERT* promoter mutation (58), but overall, the prevalence of germline *TERT* promoter mutations in familial melanoma is less than 1% (58). The importance of telomere maintenance to melanoma susceptibility is, however, highlighted in the fact that germline heterozygous mutations in three other telomere genes, *POT1*, *TPP1*, and *RAP1* (also known as *TERF2IP*), all shelterin components, have been linked to familial melanoma (Table 1). *POT1* mutations are most common, and they account for 2% to 4% of *CDKN2A/CDK4*-negative CMM families (60% of familial CMM cases fall into this category; refs. 59–61). Mutations in *TPP1* and *RAP1* account for another 2% of this familial CMM subset (62). Table 1 summarizes these associations.

Table 1. Telomerase and shelterin genes mutated in familial melanoma

Mutant gene	Mutation type	Effect on telomerase
TERT[A]	Gain of function	↑ *TERT* transcription
POT1	Loss of function	↑ Telomerase access
TPP1/ACD[A]	Loss of function	↑ Telomerase access
RAP1/TERF2IP	Loss of function	↑ Telomerase access

[A]Mutated in short telomere syndromes.

Beyond melanoma, there is also evidence of shelterin gene mutations in other cancer-prone families. Among chronic lymphocytic leukemia (CLL) multigenerational families, mutations in *POT1*, *TPP1*, and *RAP1* are found in nearly 10% of cases (63). Rare *POT1* mutations have also been reported in families with glioma (<1%; ref. 64); cardiac angiosarcoma and Li-Fraumeni–like syndrome (27%, 6 of 22 *TP53*-negative families; refs. 65, 66); colorectal cancer (0.3%, 3 of 1051 families; ref. 67); and Hodgkin lymphoma (5%, 2 of 41 families; ref. 68). These are generally loss-of-function mutations and are predicted to cause telomere lengthening. While these mutations were identified in familial forms of a single cancer, mutation carriers showed other malignancies, suggesting they confer a broader cancer-prone state (54, 58, 66). In support of this idea, a recent study reported an Ashkenazi founder *POT1* mutation that interfered with POT1's DNA-binding capacity; it simultaneously conferred susceptibility to both melanoma and CLL (69). Because the most prevalent cancers in patients with these *TERT* and shelterin mutations are melanoma and CLL, we propose that these cancers define core long telomere cancer phenotypes.

Germline mutations in *TERT* and shelterin are telomere-lengthening

How do mutations in the *TERT* promoter and shelterin genes promote the risk of melanoma and other cancers? Some studies have suggested that the effect is because of telomere deprotection (65),

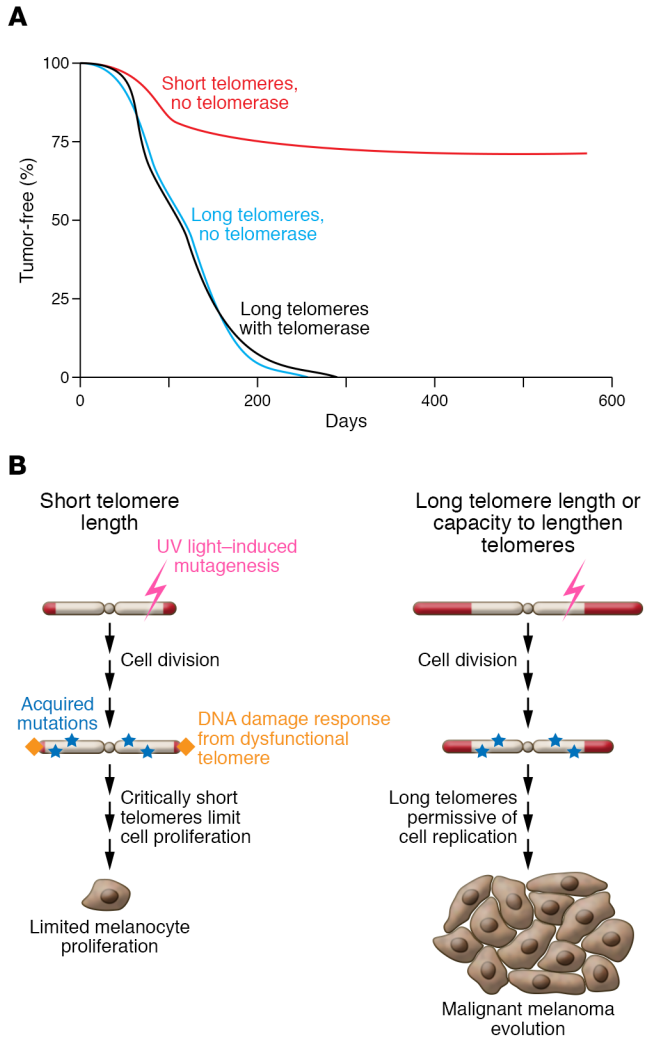

Figure 3. Long telomeres promote cancer-related mortality in mice and proposed mechanism for long-telomere melanomagenesis. (**A**) Survival curve summarizing data from mouse models examining the role of telomerase and telomere length in cancer-related survival. It shows a survival advantage for short-telomere mice in a model of Myc-induced lymphoma, according to Feldser et al. (adapted with permission from *Cancer Cell*; ref. 91). (**B**) Schematic model for how long-telomere melanoma cells prone to environmentally induced DNA damage may have an advantage in cancer progression.

but this model would not explain the fact that these cancers develop in phenotypically intact adults who show no evidence of genome instability during development. We propose that the single shared consequence of *TERT* promoter and shelterin mutations is a longer telomere and/or a telomere-lengthening capacity (Table 1). Several pieces of clinical data support this interpretation. The first is that *TERT* promoter and *POT1* mutation carriers have longer telomeres than their unaffected relatives (65, 70). The second is that families with *POT1* and *TPP1* mutations often show genetic anticipation, for both cancer onset and cancer mortality (13, 59, 60, 65). We have proposed before that this pattern of anticipation is likely because of successive telomere lengthening (13). In one *POT1* mutant family, telomere lengthening was observed across generations, although the telomere length measurements were not age-corrected and were performed by nonstandard methods (65). As discussed in detail below, there is an additional independent body of genetic epidemiology showing that long telomere length alone is associated with increased risk of the same cancers (i.e., melanoma, glioma, CLL) that are seen in cancer-prone families with *TERT* promoter and shelterin mutations.

Different *TPP1* mutations cause distinct disease phenotypes

The delicate regulation of telomere length is particularly highlighted in the case of *TPP1* mutations, where two distinct types of heterozygous, haploinsufficient mutations show opposing disease phenotypes. Mutations in the TEL patch, which is required for both TERT recruitment and processivity, cause an autosomal dominant short telomere syndrome phenotype (11, 71, 72). By contrast, mutations identified in cancer-prone families fall in the POT1-interacting domain and are predicted to interfere with POT1 binding to the telomere (10, 73). This would have the functional effect of removing the negative regulation on telomere elongation, making the telomere more accessible, and have a net effect of telomere lengthening. A schematic of these *TPP1* allele–specific mutations and their putative effects on telomere length is shown in Figure 1B.

Long telomere–associated SNPs increase cancer risk

Although the role of germline telomerase and shelterin mutations in familial cancer may at first appear limited to small subsets of cancer patients, there is epidemiologic evidence supporting long telomere length itself as being associated with cancer risk. This has been shown for melanoma and lung adenocarcinoma as well as other cancers (74–76). Larger genome-wide association studies (GWAS) further assert these associations (77). GWAS are designed to identify common variants that play a role in disease risk (78), and while the associated single nucleotide polymorphisms (SNPs) may not in themselves be pathogenic, they may be in *cis* with genes that are. GWAS for melanoma, glioma, and CLL risk have all identified SNPs near telomere maintenance genes, including *TERT*, *RTEL1*, *NAF1*, and *POT1* (79–82). In a meta-analysis of 372 GWAS data sets, SNPs near *TERT* were one of the most common recurrent findings in cancer studies (83). One important pattern emerges from examining these cancer GWAS. They show that the cancer-associated risk alleles are also the long-telomere alleles identified in telomere-length GWAS. A Danish study of more than 95,000 individuals found that long telomere–associated SNPs identified in GWAS were also associated with increased risk of cancers, especially melanoma and glioma (84). These data linking genetic variants with long telomere length, along with the data showing that long telomere length itself is cancer-associated, establish that genetically determined long telomere length is a risk factor for a subset of human cancers.

Differential effects of short telomere– and long telomere–associated alleles

Another set of analyses illustrates how differential effects of common alleles affect disease risk. An initial review of the data may show that hits from GWAS for leukocyte telomere length, IPF, and lung cancer converge on hits near telomere-related genes (Figure 2A). To better illustrate this, we will focus on SNPs near *TERT*, *RTEL1*, and *NAF1*, which were identified in studies on telomere length, IPF,

and lung adenocarcinoma. For the *TERT* SNP rs2736100, which is likely the most commonly recurrent hit in cancer GWAS (83, 85), the A short telomere allele, which has a frequency of 0.5, is associated with IPF risk (77, 86), consistent with the known link between short telomeres and IPF risk (41). By contrast, the C allele, which is associated with long telomere length, is a recurrent hit in lung adenocarcinoma (77, 85, 87). SNPs near *RTEL1* and *NAF1* (rs755017 and rs7675998, respectively) follow similar differential effects, with the short telomere allele associated with IPF and the long telomere allele with lung adenocarcinoma (refs. 86, 87, and Figure 2B).

A recent meta-analysis further illustrates the importance of telomere length extremes in disease risk. The study pooled 83 GWAS and collectively included data from 400,000 cases and 1 million controls (88). Among these various phenotypes, the disease that had the strongest association with short telomere SNPs was IPF. The additive effect of these common short telomere SNPs translated to an odds ratio of 10. This finding also underscores the existing literature linking a major subset of IPF risk to short telomere length (14, 15, 41, 89, 90). In contrast, long telomere SNPs were associated with cancers that we have considered here and elsewhere to be part of the long telomere syndrome spectrum, including melanoma and glioma (ref. 88 and Figure 2B).

Limited survival of cancer-prone mice with long telomeres

The evidence that long telomere length is cancer-predisposing has been well documented in vertebrate animal models. When the tumorigenic potential of oncogenes, such as overexpressed *Myc* or $Kras^{G12D}$, was compared in short- and long-telomere mice, long-telomere mice invariably had a worse outcome, developing more aggressive tumors and showing decreased survival (refs. 91, 92, and Figure 3A). These adverse outcomes were seen in both telomerase–wild-type and -null long-telomere mice (Figure 3A). Similar patterns have also been seen in cancer-prone models in which cancers are inducible by loss of a tumor suppressor such as Apc^{Min} or *Ink4a* (93, 94). These models contrast with the exception of $Tp53^{+/-}$ mice, which developed more tumors on the short telomere background (95). Since most humans are germline *TP53*-intact, and in light of the emerging observations that humans with short telomere syndrome have a relatively low risk of cancer, we believe the current models that are informed primarily on the basis of in vitro data may be overestimating the impact of short telomeres as a driver of genome instability and cancer in humans.

Longer telomeres in melanocytes may promote a cancer-prone state

The tight association between melanoma risk and long telomere length raises the question of whether there may be some tissue specificity in melanocytes. Melanocytes are highly vulnerable to ultraviolet-induced genotoxic damage. In this context, short telomeres may limit the proliferative potential of mutation-bearing melanocytes, while longer telomere length may be permissive for increased replicative potential. This in turn would allow the acquisition of additional genetic or epigenetic changes that would allow melanomagenesis. This model is clinically supported by the recent observations showing a high penetrance of cutaneous nevi in some *POT1* mutation carriers (69). It would also explain the absence of any melanoma cases in patients with short telomere syndrome phenotypes.

Human genetic studies contextualize laboratory-based discoveries

Initial paradigms of the role of telomeres in cancer benefitted from foundational studies in simple organisms and cell-based models. Now, with the advent of new human genetic observations, there is an opportunity to integrate new data to refine the current understanding of the role of telomeres in cancer. Our synthesis of the recent body of work indicates that the risk of cancer susceptibility associated with long telomeres is greater than that associated with genetically determined short telomere length in humans. This observation is also supported by a large body of existing animal model data. The collective overview thus raises the question as to whether current models may be overestimating the role of short telomeres as a driver of human carcinogenesis. The opportunity to study the role of telomere length in human cancer is a prime example of how cancer biology is enriched and challenged by clinical observations. One final note regarding the role of long telomere length in cancer susceptibility relates to the commercial advertising of products that claim to lengthen telomeres for purposes of reversing or preventing aging. This discourse has limitations and does not have a rigorous scientific basis (96). The human genetic observations we reviewed here support the idea that excessively long telomeres do not equate with youth but rather with a capacity for cancer cells to grow unchecked with fewer brakes.

Acknowledgments

We are grateful to Carol Greider, Alexandra Pike, Dustin Gable, and other members of the Armanios laboratory for helpful comments and discussions. Work in the Armanios group is supported by NIH grants CA225027 and HL119476, the Maryland Cigarette Restitution Fund, the Commonwealth Foundation, the Gary Williams Foundation, and an S&R Foundation Kuno Award (to MA). We also acknowledge a gift in the name of P. Godrej. PJL received support from NIH T32CA009071.

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