

# Telomeres, aging and cancer: In search of a happy ending

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**Telomeres are distinctive structures, composed of a repetitive DNA sequence and associated proteins, that cap the ends of linear chromosomes. Telomeres are essential for maintaining the integrity and stability of eukaryotic genomes. In addition, under some circumstances, telomeres can influence cellular gene expression. In mammals, the length, structure, and function of telomeres have been proposed to contribute to cellular and organismal phenotypes associated with cancer and aging. Here, we discuss what is known about the basis for the links between telomeres, aging and cancer, and some of the known and proposed consequences of telomere dysfunction and maintenance for mammalian cells and organisms.**

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## Telomeres are essential genomic elements

Telomeres are distinctive DNA-protein structures that cap the ends of linear chromosomes. Telomeres enable cells to distinguish chromosome ends from double strand breaks (DSBs) in the genome. Uncapped chromosome ends are at great risk for degradation, recombination, or fusion by cellular DNA repair systems. Chromosome degradation causes loss of genetic information, and, if unchecked, cell death. Recombination can cause rearrangements or a decrease or increase telomere length, which can prematurely engage or delay the senescence checkpoint (discussed below). Telomere fusion results in dicentric chromosomes, which break during mitosis, creating additional DSBs, cycles of breakage and fusion, and genomic instability. Thus, without telomeres, genetic information can become lost, rearranged, or unstable (Shore, 1997; Blackburn, 2000; Gasser, 2000).

## Telomeres can affect cellular and organismal phenotypes

Telomeres can be lost or rendered dysfunctional by DNA damage, repeated cell division in the absence of

telomerase, or changes in telomere associated proteins (discussed below). In response to dysfunctional or damaged telomeres, cells can die, undergo a senescence arrest, or develop mutant phenotypes. These cellular phenotypes, of course, can have profound consequences for the organism, particularly for complex organisms that contain both mitotic and post-mitotic cells. There is now strong evidence that dysfunctional telomeres can promote the development of cancer (Blasco *et al.*, 1997; Chin *et al.*, 1999; Mitchell *et al.*, 1999; Artandi *et al.*, 2000), which arises from mitotic cells. As neoplastic tumors develop, however, cancer cells must acquire mechanisms to stabilize and restore telomere function in order to survive (Kim *et al.*, 1994; Hahn *et al.*, 1999a; Dunham *et al.*, 2000). On the other hand, there is increasing evidence that dysfunctional telomeres may contribute to the development of aging phenotypes, such as vascular disease, poor wound healing, and immunosenescence (Chang and Harley, 1995; Effros, 1998; Lee *et al.*, 1998; Herrera *et al.*, 1999; Rudolph *et al.*, 1999; Klapper *et al.*, 2001). Moreover, telomere stabilization may, at least under some circumstances, prevent or delay the development of aging phenotypes in certain tissues (Funk *et al.*, 2000; Gonzalez-Suarez *et al.*, 2001).

How can telomere dysfunction and stabilization play such apparently disparate roles in organisms? Although the answer to this question is still evolving, there are now sufficient data to reconcile apparently contradictory results, and to suggest plausible and testable hypotheses.

## Telomere composition and structure

### *Telomeric DNA*

Mammalian telomeres, like all vertebrate telomeres, are composed of the simple sequence 5'-TTAGGG-3', repeated hundreds to thousands of times at each chromosome end. Most of the telomeric tract is double stranded DNA, but each telomere ends with a short single-stranded 3' overhang. This overhang is the substrate for telomerase, the cellular reverse transcriptase that can add telomeric DNA to the chromosome ends. The telomeric structure, which is not yet completely understood, appears to protect the 3' overhang from degradation and, to some extent, from unregulated elongation by telomerase (Makarov *et al.*, 1997; Shore, 1997; Wellinger and Sen, 1997; Lingner

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and Cech, 1998; Griffith *et al.*, 1999; Blackburn, 2000; McEachern *et al.*, 2000; Dubrana *et al.*, 2001).

The length of the TTAGGG tracts (measured in the germ line) varies substantially among species (Greider, 1996; Coviello-McLaughlin and Prowse, 1997; Kakuo *et al.*, 1999; Campisi, 2001). Thus, human telomeres are 10–15 kb, but laboratory mice (*Mus musculus*) have telomeres that are much longer (>30 kb) and more heterogeneous. On the other hand, the telomeres of a related mouse species (*Mus spretus*) are slightly shorter than those of humans. Telomere lengths also vary, albeit to a lesser extent, among somatic cells within a species. In this case, genotype, cell type, and cellular replicative history appear to be important variables (Allsopp *et al.*, 1995; Chang and Harley, 1995; Prowse and Greider, 1995; Zhu *et al.*, 1998; Campisi, 2001).

How important is overall length for telomere function? The interspecies comparisons suggest that, beyond an as yet undefined minimum, there is little effect of telomere length *per se* on cellular or organismal phenotypes. Thus, *Homo sapiens* and *Mus spretus* have similar telomere lengths (Allsopp *et al.*, 1995; Prowse and Greider, 1995), but differ greatly in the rates at which they develop cancer and age. In contrast, many laboratory strains of *Mus musculus* have much longer telomeres than those of wild *Mus musculus* strains or closely the related species *Mus spretus* (Prowse and Greider, 1995; Hemann and Greider, 2000), yet these animals have similar life spans. As discussed below and proposed by Blackburn (2000), telomere function is more likely to depend on structure, rather than length alone. However, the ability to maintain a functional telomere may be jeopardized by many external and intracellular events, including the acquisition of telomeres that are near or shorter than the minimum length.

#### Telomere associated proteins

Several proteins have been identified that associate with mammalian telomeres. Most of these regulate one or more aspect of telomere structure or telomere length. Some associate exclusively with telomeres, whereas others localize to additional subnuclear or subcellular sites. A comprehensive review of telomere-associated proteins is beyond the scope of this article. However, a brief description of the major players and their functions are discussed below and illustrated by Figure 1.

Two mammalian proteins, TRF1 and TRF2, bind directly and specifically to double stranded telomeric DNA (Chong *et al.*, 1995; Broccoli *et al.*, 1997), and a third protein, POT1, binds specifically to the single stranded 3' overhang (Baumann and Cech, 2001). TRF2 and POT1 appear to be particularly important for stabilizing the telomeric structure, and protecting chromosome ends from degradation and fusion (van Steensel *et al.*, 1998; Karlseder *et al.*, 1999; Baumann and Cech, 2001; Bailey *et al.*, 2001). Two additional mammalian telomere-associated proteins, TIN2 and hRAP1, also localize specifically to telomeres, but do

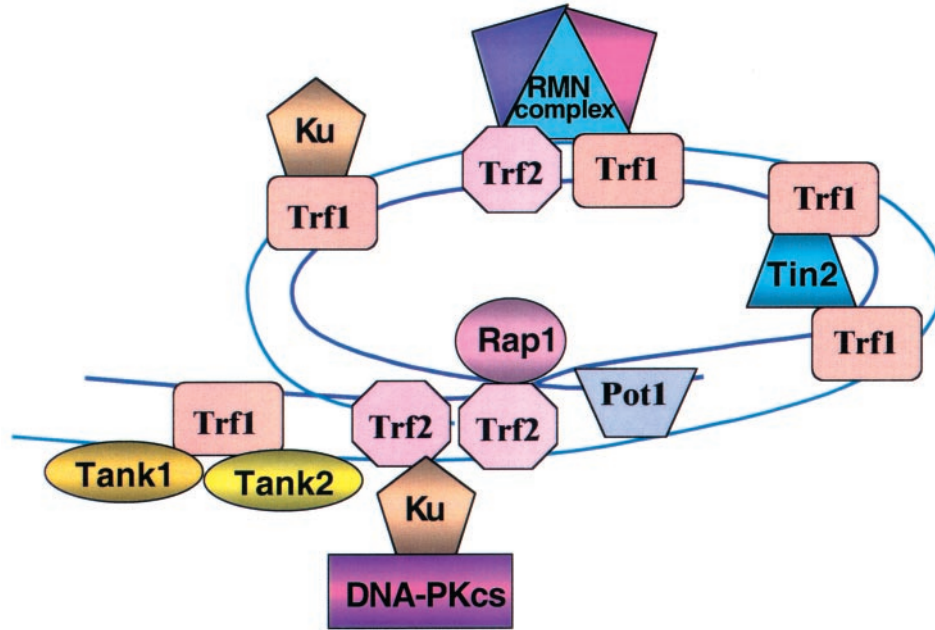
so indirectly by binding TRF1 (Kim *et al.*, 1999) and TRF2 (Li *et al.*, 2000), respectively. These proteins, together with TRF1, appear to be particularly important for regulating telomere length. They do not appear to act on telomerase, but rather appear to regulate the telomeric structure and hence the ability of telomerase to access the 3' overhang. TRF1, RAP1 and TIN2 very likely also help stabilize telomere structure.

Two non-classical poly-ADP ribose polymerases (PARPs), enzymes commonly associated with DNA repair and maintenance of chromosome stability (d'Amours *et al.*, 1999; d'Adda di Fagagna *et al.*, 1999), also interact with TRF1 (Smith *et al.*, 1998; Kaminker *et al.*, 2001). However, these proteins, TANK1 and TANK2, are most abundant at the nuclear periphery, in Golgi vesicles, and, like classical PARPs, localize to centrosomes during mitosis (Smith and deLange, 1999; Chi and Lodish, 2000; Kaminker *et al.*, 2001). Given their largely extranuclear localization, the TANKs may function in non-telomeric cellular processes. On the other hand, their location at the nuclear periphery and PARP activity raises the possibility that TANKs participate in repairing, or signaling the occurrence of, dysfunctional telomeres.

Several proteins known to participate in DNA repair were recently found at telomeres. One example is Ku, the DNA end-binding component of DNA-dependent protein kinase (DNA-PK), which is essential for DSB repair by non-homologous end joining (Smith and Jackson, 1999). The 70 kD Ku subunit binds TRF1 and TRF2 in cells, and a significant fraction of Ku associates with mammalian telomeres (Hsu *et al.*, 1999, 2000; Song *et al.*, 2000). Cells from mice deficient in either Ku subunit, or the DNA-PK catalytic subunit, are genomically unstable owing to frequent telomere fusions (Bailey *et al.*, 1999; Samper *et al.*, 2000). Thus, DNA-PK, in addition to its role in DNA repair, may also play a role in telomere maintenance. This idea is supported by recent data showing that DNA-PK, together with TRF2, is required for strand-specific processing of the telomeres after they are replicated in S phase (Bailey *et al.*, 2001). Likewise, RAD50, MRE11 and NBS1 (RMN), another important DNA repair complex, may function at telomeres. This complex associates with mammalian telomeres, at least during S phase, very likely owing to an interaction between NBS1 and TRF1 (Wu *et al.*, 2000) and TRF2 (Zhu *et al.*, 2000).

#### Telomere structure

Recently, telomeres were shown to end in a large tailed loop resembling a lasso, termed the t loop. Telomeric t loops have been isolated from mice, humans and protozoa, and may be an evolutionarily conserved structure (Griffith *et al.*, 1999; Munoz-Jordan *et al.*, 2001). In mice and humans, the size of the t loop circles correlates with telomere length, ranging from ~3 kb in normal human lymphocytes to 18 kb in mouse liver. Protozoa, by contrast, have smaller t loops (~1 kb), despite telomere lengths of 10–20 kb.



**Figure 1** Telomere-associated proteins. Mammalian telomeres are thought to end in a large t-loop whose structure and stability may depend on a number of proteins, such as TRF2, TRF1, POT1, TIN2 and RAP1. Non-classical PARPs, such as TANK1 and TANK2, may also associate with the telomeres through their interaction with TRF1. In addition, TRF1 and TRF2 recruit to the telomere a number of proteins known to participate in DNA repair, most notably the RAD50-MRE11-NBS1 (RMN) complex and the DNA-dependent protein kinase, which is composed of a catalytic subunit (DNA-PKcs) and a DNA end binding subunit (Ku)

These findings suggest that the size of the t loop can be regulated. Moreover, t loops should be able to form on relatively short (~1 kb) telomeres, but presumably there is a minimum size below which they cannot form.

The formation of telomeric t loops is critically dependent on TRF2, one of the telomere-associated proteins that binds double stranded telomeric DNA (Griffith *et al.*, 1999). In addition, the formation and/or maintenance of telomeric t loops may be facilitated or stabilized by other telomere-associated proteins, particularly TRF1 and TIN2 (Griffith *et al.*, 1999; Kim *et al.*, 1999). How might the t loop protect telomeres from degradation or fusion and regulate telomere length? Indirect evidence suggests the 3' overhang is buried in the telomeric duplex DNA at the junction between the t loop circle and tail (Griffith *et al.*, 1999). Thus, the t loop may provide a structure that is devoid of recognizable DNA 'ends' and hence does not resemble a DSB. It also can protect the 3' overhang from degradation, and limit the ability of telomerase to access its substrate. Disruption of the t loop is thought to signal a cellular response that, in at least some regards, resembles the cellular response to a DSB in the genome.

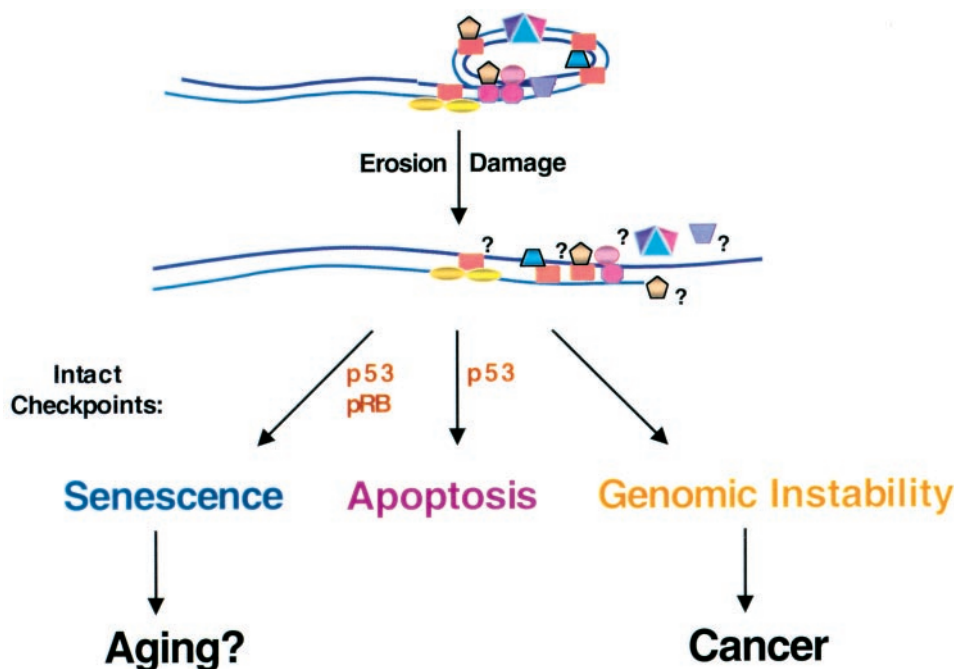
### Cellular consequences of telomere disruption

Telomere states can have profound consequences for cell phenotype and viability. Cells may attempt to repair dysfunctional telomeres, particularly if they express telomerase. However, most cells do not express

telomerase (reviewed in Chiu and Harley, 1997; Shay and Wright, 2001), and respond to telomere dysfunction by undergoing a senescence growth arrest (discussed below). The senescence response requires cell cycle and DNA damage checkpoints controlled by the pRB and p53 tumor suppressors. If only the p53 checkpoint is intact, cells with dysfunctional telomeres may die; if neither checkpoint is intact, cells may survive with genomic rearrangement and, frequently, instability. Thus, telomere dysfunction can cause cell senescence, death, or genomic instability, depending on the cell context. These possible outcomes, illustrated in Figure 2, are undoubtedly oversimplified. For example, some cell types, or some cells under certain physiological conditions, may die rather than senesce in response to telomere disruption. It is likely that interactions among several factors, only some of which are known, dictate how a cell will respond to a dysfunctional telomere.

### Cellular senescence

In contrast to the mammalian germline and early embryonic cells, most somatic cells do not express telomerase (Prowse and Greider, 1995; Holt *et al.*, 1997; Kim *et al.*, 1994). This poses a problem for dividing cells (Levy *et al.*, 1992). Because DNA replication is bidirectional, DNA polymerases are unidirectional, and the polymerases require a primer, supplied as a short labile RNA tract, 50–200 bp of 3' telomeric DNA remain unreplicated at the end of each S phase. Thus, in the absence of telomerase, telomeres shorten with each



**Figure 2** Cellular consequences of telomere dysfunction. Telomere structure can be disrupted by direct damage, critical shortening or defects in telomere-associated proteins. In general, normal cells, with intact p53 and pRB checkpoints, respond to a dysfunctional telomere by undergoing cellular senescence, which may contribute to certain aging phenotypes. If only the p53 checkpoint is intact, telomere dysfunction generally promotes p53-mediated cell death. If neither the p53 nor pRB pathway is intact, cells may survive with genomic rearrangements and instability, which can lead to cancer

cell division. When telomeres reach a critically short length, normal cells irreversibly arrest proliferation and acquire a characteristic enlarged morphology and a variety of altered functions. This response has been termed replicative or cellular senescence.

Some adult somatic cells express telomerase, although this is not widespread, particularly among human cells. For example, telomerase is transiently expressed by activated human T cells (Buchkovich and Greider, 1996; Weng *et al.*, 1996), and telomerase-positive stem cells are thought to populate human skin (Harlebachor and Boukamp, 1996). Despite telomerase activity, human T cells lose telomeric DNA with each division and eventually senesce (Effros, 1998). Thus, in some cases, telomerase is insufficient to prevent the telomere erosion and senescence that occurs as a consequence of DNA replication. On the other hand, ectopic expression of telomerase can prevent telomere erosion and replicative senescence in some human cells, including fibroblasts, retinal epithelial cells and endothelial cells (Bodnar *et al.*, 1998; Vaziri and Benchimol, 1998; Yang *et al.*, 1999).

Several lines of evidence suggest that the senescence response evolved to suppress tumorigenesis, acting as a failsafe mechanism to prevent the proliferation of cells at risk for neoplastic transformation (reviewed in Campisi, 2000, 2001). Consistent with this idea, normal cells undergo a senescence arrest when faced with a variety of stimuli, all of which have the potential to induce or promote neoplastic transformation. These stimuli include dysfunctional telomeres, as well as

certain types and levels of DNA damage, perturbations of chromatin structure, and certain mitogen signal transducing oncogenes, such as mutant RAS. Moreover, telomerase does not prevent human fibroblasts from senescing in response to mutant RAS (Wei *et al.*, 1999) or DNA damage (Rubio and Campisi, unpublished), indicating that cells can undergo a senescence response despite functional telomeres. Thus, dysfunctional telomeres trigger a fundamental cellular response, which is also triggered by many potentially oncogenic stimuli, including DNA damage and certain oncogenes.

Cellular senescence causes cells to irreversibly arrest growth with a G1 DNA content owing to repression of genes required for cell cycle progression (e.g., *c-fos*), and upregulation of growth inhibitory genes (e.g., p21 and p16). The senescence response also causes changes in differentiated functions – for example, adoption of a matrix-degrading phenotype by senescent fibroblasts or secretion of an altered profile of steroid hormones by senescent adrenal cortical epithelial cells (reviewed in Campisi *et al.*, 1996; Campisi, 1999, 2000). The senescence growth arrest and functional changes are tightly linked, and together define the senescent phenotype.

#### *Differences between mice and humans*

Progressive telomere shortening, culminating in cellular senescence, is readily seen in human cells in culture (Allsopp *et al.*, 1995; Yang *et al.*, 2001). In addition,

short telomeres and cells with senescent characteristics (Allsopp *et al.*, 1995; Dimri *et al.*, 1995; Paradis *et al.*, 2001; Yang *et al.*, 2001) are increasingly apparent in tissues from aged human donors. In contrast, telomere shortening is not readily seen in mouse cells, whether in culture or *in vivo* (Prowse and Greider, 1995; Hemann and Greider, 2000; Sherr and DePinho, 2000; Wright and Shay, 2000). This difference between mice and humans has engendered several debates. Are mice poor models for telomere function in humans? Do telomeres play important roles in human aging and cancer, given the apparent lack of telomere involvement in these processes in mice?

Some answers to these questions have come from mice in which the telomerase RNA component was inactivated in the germline. Such mice do not express telomerase, and are relatively asymptomatic for the first few generations, during which time the telomeres shorten progressively. After 4–5 generations, however, when telomeres have eroded to near-human lengths, telomerase null mice develop cancer, including epithelial cancers common in humans, and certain other pathologies associated with human aging (Blasco *et al.*, 1997; Lee *et al.*, 1998; Herrera *et al.*, 1999; Rudolph *et al.*, 1999; Artandi *et al.*, 2000). These findings suggest that late generation telomerase-null mice are a better model for studies of human cancer and aging than wild-type mice. They also indicate that telomeres can play a role in the development of cancer and at least some pathologies associated with mammalian aging.

Despite long telomeres and, frequently, expression of telomerase, mouse cells senesce after many fewer doublings in culture than human cells. It has been suggested that cultured mouse cells senesce because they acquire telomere-independent damage inflicted by the culture conditions (Sherr and DePinho, 2000; Wright and Shay, 2000), possibly the high oxygen in which most mammalian cells are cultured (Campisi, 2001). Whatever the case, inactivation of the p53 and pRB tumor suppressors prevents cultured human and mouse cells from undergoing senescence in response to multiple stimuli, including repeated cell division (Shay *et al.*, 1991), DNA damage (Chen *et al.*, 1998), and oncogenic RAS (Serrano *et al.*, 1997). These findings suggest that oxidative damage may arrest the proliferation of mouse cells in culture before the telomeres can shorten sufficiently to do so. However, the checkpoints that mediate the senescence response are similar in mice and humans.

### Cell death

p53 and pRB regulate interacting pathways that lead to cell cycle arrest or apoptosis (Kohn, 1999). Mutations that affect components of either pathway, which is often the case in tumor cells, partially abrogate the senescence response to short telomeres, as well as other stimuli that induce cellular senescence (Zhang *et al.*, 1999; Lundberg *et al.*, 2000) (Figure 2).

If the pRB pathway is inactive, but the p53 pathway is intact, cells are more prone to die when they acquire

a critically short or dysfunctional telomere (Karlseder *et al.*, 1999; Dimri, Itahana and Campisi, unpublished), compared to normal cells or cells that lack an intact p53 checkpoint. For example, disruption of the telomere structure (by expressing a dominant negative TRF2 mutant) causes immortal human tumor cells to die (Karlseder *et al.*, 1999) in a p53-dependent manner.

When both p53 and pRb are inactive, most human cells proliferate until the telomeres become extremely short, whereupon they enter an unstable state termed crisis (reviewed in Campisi *et al.*, 1996; Wright and Shay, 1996). Cells in crisis attempt to proliferate, but because telomere erosion and chromosome instability are so severe, they frequently die. A few cells, however, acquire a mutation or epigenetic event that enables them to stabilize their telomeres, most commonly by reactivation of telomerase (Kim *et al.*, 1994). Such cells can then proliferate indefinitely and resist senescence inducing signals, but are at a greatly increased risk for malignant transformation.

### Genomic instability

Chromosomes that lack a protective telomeric structure are highly unstable, being subject to degradation, rearrangement and/or fusion. If such cells fail to senesce and lack p53 and pRB function, they may survive despite genomic rearrangements and instability (Figure 2).

Genomically unstable cells must stabilize their telomeres in order to survive. However, because genomic instability greatly increases the frequency of mutations, it favors the chances of acquiring a mutation or epigenetic change that permits telomere stabilization. The most common means by which such cells stabilize their telomeres is by expressing telomerase, specifically the catalytic component, TERT (Kim *et al.*, 1994; Chiu and Harley, 1997; Shay and Wright, 2001). In addition, telomerase-independent mechanisms can maintain telomeres (Bryan *et al.*, 1997), most likely by homologous recombination (Dunham *et al.*, 2000). The genomic instability that occurs when telomeres malfunction in the absence of the normal senescence response is one of the most striking hallmark of cancer cells (Cahill *et al.*, 1999; Gray and Collins, 2000).

In summary, most normal cells respond to dysfunctional telomeres by mounting a senescence response. This response requires the functions of both pRb and p53. If, however, the senescence checkpoint is compromised – for example, by mutations or epigenetic events – p53 ensures that such cells die. In the absence of p53, genomically unstable cells – cells that attempt to repair the damaged telomere by fusing it to another telomere or broken DNA end – may survive (Figure 2). Such cells are at great risk for developing increasingly malignant phenotypes.

### Implications for cancer

Several lines of evidence suggest that telomeres contribute to the initiation and progression of

malignant tumors in several ways. As discussed above, telomere dysfunction – whether caused by replication-mediated shortening, direct damage, or defective telomere-associated proteins – can have three cellular outcomes: senescence, death, or genomic instability. Genomic instability clearly predisposes cells to neoplastic transformation. Cellular senescence and death, then, can be considered tumor suppressive responses that prevent the proliferation or survival of cells at risk for developing genomic instability. Cellular senescence may be a double-edged sword in this regard, but this idea will be discussed below.

The first defense against telomere dysfunction, used by many normal cells, may be the senescence response. Cellular senescence prevents cell proliferation, thereby preventing tumorigenesis. However, somatic mutations, some of which can inactivate genes required for the senescence response, accumulate throughout life (Dollé *et al.*, 1997, 2000). In addition, loss of heterozygosity and mutations in tumor suppressors, such as p53, and oncogenes, such as *RAS*, occur even in apparently normal tissue (Deng *et al.*, 1996; Jonason *et al.*, 1996; Cha *et al.*, 1994). Thus, the second defense, which requires only an intact p53 pathway, causes cells with dysfunctional telomeres to die. Eventually, however, cells accumulate mutations in p53 or components of the p53 pathway. Such cells develop genomic instability. These cells then are at enormous risk for neoplastic transformation, providing they can acquire a mutation or epigenetic event that stabilizes their telomeres.

There is strong evidence for this scenario. Mice that lack telomerase gradually lose telomere length, but, because they have such long telomeres, it takes 4–6 generations before the telomeres shorten to lengths typical of human cells. Late generation telomerase-deficient mice are cancer-prone, particularly in tissues that are exposed to environmental damage or undergo high cell turnover (Blasco *et al.*, 1997; Lee *et al.*, 1998; Rudolph *et al.*, 1999; Artandi *et al.*, 2000). Likewise, humans with dyskeratosis congenita, a hereditary disease caused by defective processing of the telomerase RNA component, are partially telomerase-deficient and cancer prone (Mitchell *et al.*, 1999). However, late generation telomerase deficient mice, as well as humans with dyskeratosis congenita, suffer from additional pathologies, some of which are associated with aging. These include immune senescence, loss and graying of hair, and impaired wound healing (Rudolph *et al.*, 1999; Herrera *et al.*, 1999, 2000). Moreover, late generation telomerase-deficient mice can be resistant to cancer in some tissues and some genetic backgrounds (Gonzalez-Suarez *et al.*, 2000; Greenberg *et al.*, 1999). However, when telomerase-deficient mice are crossed with p53-null mice, cancer development and progression are markedly accelerated (Chin *et al.*, 1999). Moreover, p53 and telomerase deficiency favor the development of epithelial tumors, the most common age-associated cancers that occur in humans (Artandi *et al.*, 2000; reviewed in DePinho, 2000). Thus, cancer is a prominent consequence of the telomere erosion that results from the deficiency in

telomerase. In the tumors that develop in telomerase deficient mice, telomere stabilization occurs by a telomerase-independent pathway. In most other tumors, however, telomere stabilization occurs by reactivation of telomerase.

Repression of telomerase very likely ensures that cells senesce when telomeres become dysfunctional. On the other hand, telomerase may act preferentially on short telomeres (Ouellette *et al.*, 2000), thereby reducing their risk for acquiring a dysfunctional structure (Blackburn, 2000). Thus, under some circumstances telomerase can be protective, preventing telomere dysfunction. Because telomerase also prevents cellular senescence, under some circumstances it might retard or prevent age-related pathology. For example, a TERT transgene targeted to basal keratinocytes in mice, increased telomerase expression in the skin and increased epidermal wound healing (Gonzalez-Suarez *et al.*, 2001).

Two lines of evidence suggest that telomerase may be more likely to promote cancer than prevent it. First, somatic expression of telomerase is more prevalent in the tissues of mice than humans (Wright and Shay, 2000). However, even when normalized for differences in cell number, mice are more cancer-prone than humans (Miller, 1991). Second, although telomerase expression *per se* does not cause neoplastic transformation (Jiang *et al.*, 1999; Morales *et al.*, 1999; Vaziri *et al.*, 1999), telomerase cooperates with potentially oncogenic genetic changes to promote tumorigenesis. This was true for genetically modified human cells, in which telomerase was essential for their ability to form tumors in immunocompromised mice (Hahn *et al.*, 1999b). It was also true for the transgenic mice that constitutively expressed telomerase in basal keratinocytes. Although epidermal wound healing was improved in these mice, the TERT transgene also promoted skin carcinogenesis (Gonzalez-Suarez *et al.*, 2001).

### Implications for aging

As noted earlier, the senescence response entails not only an irreversible growth arrest, but also selected changes in differentiated functions, including the secretion of factors that can alter the integrity, function and proliferative homeostasis of tissues (reviewed in Campisi *et al.*, 1996; Campisi, 2000). This senescence-associated secretory phenotype is particularly striking in fibroblasts, a major component and regulator of the stroma. Senescent fibroblasts secrete extracellular matrix components, matrix-degrading enzymes, inflammatory cytokines, and growth factors. Cellular senescence, particularly factors secreted by senescent stromal cells, may contribute to the decline in tissue function and integrity that is a hallmark of aging. Telomere dysfunction, then, may contribute to aging by virtue of its ability to induce cellular senescence.

As noted above, cellular senescence very likely evolved to protect mammalian organisms from cancer. If,

however, cellular senescence also contributes to aging, it very likely is an example of evolutionary antagonistic pleiotropy. This theory predicts that the some traits that were selected to optimize fitness in young adult organisms can have unselected deleterious effects in aged organisms (reviewed in Kirkwood and Austad, 2000). The growth arrest associated with cellular senescence may be the selected trait, which suppresses tumorigenesis in young organisms. By contrast, the altered functions of senescent cells may be unselected traits that can have deleterious effects. Presumably, these deleterious effects are negligible in young tissues, where senescent cells are rare. However, as organisms age, senescent cells accumulate (Dimri *et al.*, 1995; Mishima *et al.*, 1999; Pendergrass *et al.*, 1999; Paradis *et al.*, 2001). It is possible, then, that as senescent cells accumulate, their altered functions, particularly their secretory phenotype, compromise the physiology and integrity of tissues (Campisi *et al.*, 1996).

This idea can be extended to understand the relationship between aging and cancer. We and others have proposed that senescent cells may also contribute to the exponential rise in cancer that occurs with age in many mammalian species (Campisi, 1997, 2000; Rinehart and Torti, 1997; DePinho, 2000). The secretory phenotype of senescent cells can disrupt the tissue microenvironment, which is crucial for suppressing the growth and progression of cells with oncogenic mutations (Park *et al.*, 2000). Thus, damage, telomere

dysfunction, or errors in mitogenic signaling may cause senescent cells to accumulate, but their influence may become significant and deleterious only later in life when they reach sufficient numbers. Simultaneously, mutations accumulate with age, and thus the probability that senescent cells and cells with oncogenic mutations occur in close proximity may also increase with age. When this occurs, senescent cells may create a microenvironment that promotes the proliferation and neoplastic progression of the mutant cells. Our recent results indicate that senescent human fibroblasts can indeed stimulate the growth and tumorigenic progression of preneoplastic, but not normal, epithelial cells (Krtolica *et al.*, 2001). Moreover, much of this growth promoting activity could be attributed to the secretory phenotype of the senescent fibroblasts. Thus, despite protecting from cancer in young adults, cellular senescence – driven in part by telomere dysfunction – may promote cancer progression in aged organisms.

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