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Telomere dysfunction and tumour suppression: the senescence connection

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Abstract | Long-lived organisms such as humans have evolved several intrinsic tumour suppressor mechanisms to combat the slew of oncogenic somatic mutations that constantly arise in proliferating stem-cell compartments. One of these anticancer barriers is the telomere, a specialized nucleoprotein complex that caps the ends of eukaryotic chromosome. Impaired telomere function activates the canonical DNA damage response pathway that engages p53 to initiate apoptosis or replicative senescence. Here, we discuss how p53-dependent senescence induced by dysfunctional telomeres may be as potent as apoptosis in suppressing tumorigenesis *in vivo*.

More than 40 years ago, Hayflick and Moorhead discovered that normal human diploid fibroblasts (HDFs) cannot grow indefinitely in culture[1]. Rather, their proliferative capacities are intrinsically limited. After 60–80 population doublings in culture, HDFs stop dividing and adopt a phenotype characterized by large, flat cell size, a vacuolated morphology, inability to synthesize DNA and the presence of the senescence-associated β-galactosidase (SA-β-gal) marker[2]. We now know that the endpoint of this proliferative limit, termed replicative senescence, is due largely to erosion of telomeres, protective structures that cap the end of all eukaryotic chromosomes. Confirmation of this came from studies in which telomerase, the enzyme that maintains telomeres, was ectopically expressed in normal human somatic cells[3,4]. Activation of telomerase results in telomere elongation, abrogation of replicative senescence, a normal karyotype and cellular immortalization. These results clearly demonstrate that telomere length determines the proliferative lifespan of HDFs, and that upregulation of telomerase activity (and hence telomere length) restores proliferative capacity.

A large body of experimental evidence has demonstrated that telomere attrition contributes to tumorigenesis by promoting genome instability[5]. However, in the setting of a competent p53 pathway, mouse models have recently shown that telomere shortening is also tumour-suppressive by promoting replicative senescence to inhibit tumour formation. In this Review, we will discuss how telomeres shorten with cell replication and how this might initiate a DNA damage response (DDR) to induce replicative senescence (and cell death) and ultimately prevent tumorigenesis.

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Telomeres and telomerase

Telomeres are composed of TTAGGG repeats, oriented 5′-to-3′ towards the end of the chromosome, ending in an essential 3′ single-stranded G-rich overhang. They are maintained by telomerase, a specialized ribonucleoprotein complex that includes an RNA template (TERC) and a reverse transcriptase catalytic subunit (TERT) (FIG. 1a). The G-rich overhang is generated by post-replicative processing of the C-rich strand, and is the substrate for telomerase-mediated telomere elongation. Telomerase expression is low or absent in most human somatic tissues[6], whereas it is robust in early proliferative progenitor germ and stem cells[7,8]. In the absence of telomerase, each round of DNA replication is accompanied by telomere shortening owing to the failure of DNA polymerase to synthesize fully the extreme terminus of the lagging DNA strand. A total lifetime loss of ~2–4 kb in average telomere length has been observed in human cells[8–10]. Considering that human telomeres are only 8–12 kb in length or less at birth, this degree of attrition is significant over the natural lifespan of humans, and might be responsible for certain aspects of human ageing phenotypes[11].

Analysis of telomeres from a diverse array of organisms revealed that the telomere 3′ single-stranded overhang can invade the double-stranded telomeric tracts, displacing the homologous strand of the same telomere. Consequently, a telomere forms a lasso-like structure, termed the t loop, with a displacement (D) loop at the invasion site[12,13] (FIG. 1b).

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At a glance

- Telomeres are TTAGGG repetitive sequences that cap the ends of eukaryotic chromosomes.
- A core of telomere binding proteins, termed the shelterin complex, serve to protect telomeric ends.
- Critical telomere shortening or uncapping of telomere binding proteins results in telomere dysfunction.
- Dysfunctional telomeres activate a DNA damage response. In the setting of a competent p53 pathway, this initiates senescence and apoptotic programmes to inhibit tumorigenesis.
- In cells with mutant p53, dysfunctional telomeres promote genome instability and progression to cancer.
- Cellular senescence is as potent as apoptosis in suppressing spontaneous tumorigenesis in mouse models of telomere dysfunction.

It has been proposed that the t loop has a crucial role in sequestering the 3′ end of telomeric DNA, preventing recognition by the DNA damage machinery as DNA double-strand breaks (DSBs) and initiation of inappropriate activation of DNA damage checkpoints.

Cytogenetic analyses of breakage and fusion of maize chromosomes provided the first evidence that telomeres maintain genome stability[14]. Mammalian telomeres associate with the shelterin complex of six core proteins: telomeric repeat-binding factor 1 (TRF1, also known as TERF1), TRF2 (also known as TERF2), TERF1-interacting nuclear factor 2 (TIN2, also known as TINF2), protection of telomeres 1 (POT1), the POT1- and TINF2-interacting protein (TPP1, also known as ACD) and the transcriptional repressor/activator protein RAP1 (also known as TERF2-interacting protein (TERF2IP)) (REFS 15,16) (BOX 1; FIG. 1b). Telomeres that can no longer exert end-protective functions are said to be dysfunctional, and these telomeres could arise either from progressive telomere attrition, or when components of the shelterin complex are perturbed, resulting in inappropriate chromosomal end-to-end fusions through the non-homologous end joining (NHEJ) or homologous recombination (HR) DNA repair pathways[17]. One important cytogenetic distinction between these two repair processes is that the fusion points of chromosomes with naturally shortened telomeres do not normally possess telomeric DNA that is detectable by fluorescence *in situ* hybridization, whereas fused chromosomes resulting from loss of shelterin components frequently possess intense telomeric signals at the sites of fusion. NHEJ and HR pathways account for the vast majority of chromosomal fusions, but other pathways of repair such as microhomology-mediated telomere–telomere recombination may also be involved, especially when the classic repair pathways are compromised[18–20].

These fusions are the basis of genomic instability associated with telomere dysfunction as they lead to anaphase bridging and subsequent breakage, which requires further repair. Repetition of this breakage–fusion–bridge cycle leads to aneuploidy and further fusions and, depending on how such DNA damage is resolved, it has been hypothesized that loss of heterozygosity or gene amplification could result in tumorigenesis[5,21].

Breakage–fusion–bridge cycle
Chromosomal ends with critically shortened telomeres are highly recombinogenic, and undergo repeated cycles of end-to-end fusions, followed by random breakage, and then subsequent fusions to generate loss of heterozygosity or amplification of certain chromosomal loci.

Telomere dysfunction initiates a DDR

Biochemical analyses revealed that HDFs that entered replicative senescence display molecular markers characteristic of cells bearing DNA DSBs, suggesting that dysfunctional telomeres elicit a potent DDR[22]. These markers of the DDR include phosphorylated γ-H2AX, p53-binding protein 1 (TP53BP1), NBS1 (also known as nibrin), MDC1 and CHK2. Many of these proteins localize directly to dysfunctional telomeres to form dysfunctional telomere-induced foci, and their inactivation in senescent cells restores cell cycle progression into S-phase[22,23]. These results suggest that dysfunctional telomeres are recognized as DSBs that impinge on p53 and/or RB tumour suppressor pathways to induce expression of their regulators p21 (encoded by *CDKN1A*) and INK4A (also known as p16 and encoded by *CDKN2A*) to initiate replicative senescence[24–26].

Dysfunctional telomeres activate upstream checkpoint phosphatidylinositol 3-kinase-like kinases (PIKKs), such as ATM (ataxia-telangiectasia mutated) or ATR (ataxia telangiectasia- and Rad3-related)[22,27–30] (FIG. 2). Once activated, these kinases phosphorylate downstream factors, including CHK1 and CHK2, that in turn phosphorylate p53 (REF. 29). Phosphorylation of p53 results in the displacement of the murine double minute 2 (MDM2) protein, liberating p53 from degradation, and stimulation of the expression of the cyclin-dependent kinase inhibitor p21. p21 inhibits cell cycle progression by inhibiting cyclin-dependent kinases that phosphorylate and inactivate RB. In addition to promoting cell cycle arrest, dysfunctional telomeres can also activate p53-dependent apoptosis, as mice bearing dysfunctional telomeres show increased apoptosis in proliferative cells[31–33]. In the setting of a competent p53 pathway, dysfunctional telomeres appear to function as potent tumour suppressors by engaging cellular pathways that activate replicative senescence and/or apoptosis to inhibit tumour formation. Therefore, it is not surprising that p53 loss results in a permissive environment that favours proliferation and survival of genomically damaged cells and the eventual progression to cancer. The role of the INK4A–RB pathway in mediating the telomere DDR is less clear (BOX 2).

Inactivation of p53 and RB by antisense oligonucleotides[34] or by viral oncoproteins[35] can extend replicative potential in HDFs, driving further telomere erosion and culminating in a period of massive cell death and rampant genomic instability that is termed crisis. Crisis serves as a second, p53-independent checkpoint in tumour suppression and is characterized by multiple chromosomal fusions[36]. Virally transformed human cells that eliminate p53 and/or RB function can escape crisis at low frequencies, and invariably adopt telomere-maintenance programmes: 80–90% of human tumours have some telomerase activity, and the remainder maintain telomeres by a recombination-mediated process termed ALT (alternative lengthening of telomeres)[37–40]. Together, these observations support the view that replicative senescence and crisis provide potent barriers to tumour development and, by extension, that proper telomere maintenance is an essential aspect of full malignant transformation.

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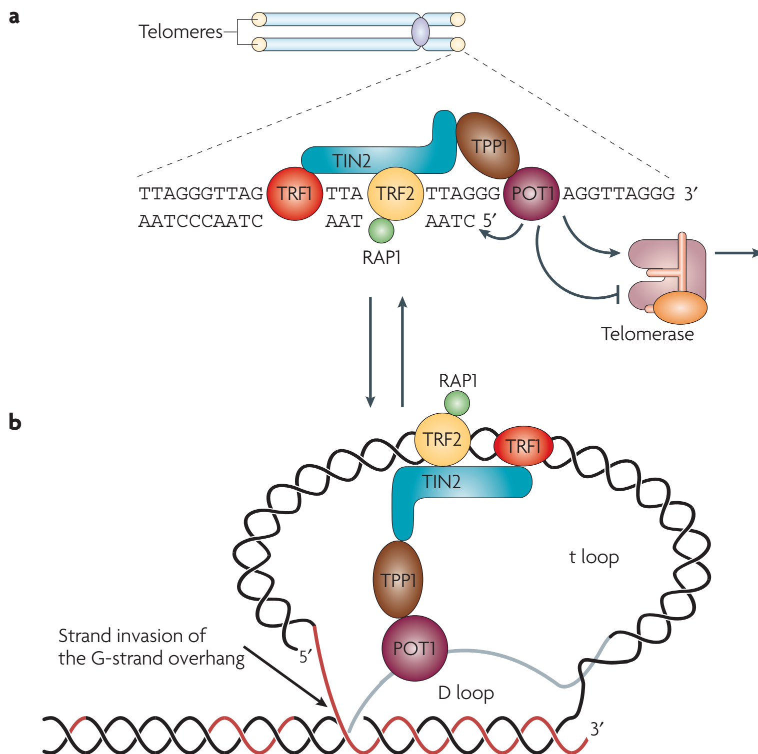

Figure 1 | **Telomere structure. a** | Telomeres cap mammalian chromosomes and are composed of TTAGGG repetitive sequences that terminate in a 3′ single-stranded (ss) overhang. Telomeric DNA is complexed by the six-protein shelterin complex, composed of telomeric-repeat binding factor 1 (TRF1), TRF2, RAP1, TERF1-interacting nuclear factor 2 (TIN2), TPP1 and POT1. The TPP1–POT1 heterodimer regulates telomerase access to the telomeric substrate. **b** | The ss overhang can invade the double-stranded region of the telomere to form a protective telomere (t) loop with a ss displacement (D) loop at the invasion site. Mammalian telomeres also transiently interact with a host of other factors, many of which are involved in the DNA damage response.

Dysfunctional telomeres, p53 and tumorigenesis

Telomere dysfunction has been proposed to fuel tumorigenesis, as, depending on how dicentric chromosomes are resolved in the breakage–fusion–bridge cycle, loss of heterozygosity of tumour suppressors and/or amplification of oncogenes could result in a pro-cancer genotype[5]. The cloning of the gene encoding *Terc* enabled the construction of the telomerase-knockout mouse to formally test this hypothesis *in vivo*[41,42]. The telomerase-deficient mouse lacks the crucial RNA subunit *Terc*, is viable and fertile, and has no significant morphological abnormalities[42]. To drive telomeres to shorter and potentially dysfunctional lengths in later generations, successive *Terc*$^{-/-}$ intercrosses were instituted. By the sixth generation (G6), increased apoptosis and compromised function were observed in highly proliferative tissues, including skin, haematopoietic tissue and reproductive systems[31,42–44]. Loss of haematopoietic function probably stems from accumulation of damaged DNA as a result of dysfunctional telomeres[45]. Importantly, despite the presence of critically short telomeres, removal of p53 function rescued many of the cellular defects, including growth arrest in cell culture, testicular atrophy and intestinal and germ-cell apoptosis[46]. Cell culture transformation assays showed that *Trp53*-null cells with critically shortened telomeres exhibited increased susceptibility to transformation by *Myc* and *Hras*. Similar findings were observed *in vivo*: the progressive decline in telomere function correlated with increased tumour incidence and decreased survival. Therefore, in the absence of *Trp53*, telomere dysfunction and the resultant genomic instability promote tumorigenesis. As a further demonstration of the effect of p53 status on dysfunctional telomere-induced tumour growth, late-generation *Terc*$^{-/-}$;*Trp53*$^{+/-}$ mice emerged with a tumour range that strikingly resembled those of aged humans: carcinomas of the skin, breast and intestine with cytogenetic hallmarks of telomere dysfunction emerged as the largest group of tumours[47]. Together, these findings demonstrate that telomere dysfunction promotes the development of cancer in the setting of p53 deficiency, and further reinforce the importance of an intact p53 pathway in tumour inhibition in the setting of telomere dysfunction.

Dicentric chromosome
A chromosome that has two centromeres, formed by breakage and reunion of two chromosomes.

To further examine how dysfunctional telomeres cooperate with p53 activation to limit neoplastic growth *in vivo*, mouse models bearing deletions of various tumour suppressor genes were mated with telomerase-knockout mice (TABLE 1). For example, the *CDKN2A* locus encodes the INK4A and ARF tumour suppressors. Both genes share a common second exon that is deleted in the *Cdkn2a*$^{-/-}$ mouse[48]. These mice often develop lymphomas and sarcomas, and importantly, the pathway resulting in activation of p53 through DNA damage is intact in these animals[25]. Treatment of early-generation *Terc*$^{-/-}$;*Cdkn2a*$^{-/-}$ mice with DMBA and ultraviolet B revealed that these mice are cancer-prone. However, similar treatment of G6 *Terc*$^{-/-}$;*Cdkn2a*$^{-/-}$ mice that have short dysfunctional telomeres yielded a marked reduction in tumour incidence (from 64% to 31%) and much longer survival[49]. Primary fibroblasts isolated from these embryos also exhibited resistance to transformation by *Myc* and *Hras*. A similar finding was observed in a chemical carcinogenesis skin cancer model, in which late-generation *Terc*$^{-/-}$ mice produced 20-fold fewer skin tumours than wild-type controls with long telomeres[50]. In addition, stablization of p53 was detected in late-generation *Terc*$^{-/-}$ papillomas. Taken together, these results suggest that dysfunctional telomeres inhibit tumour initiation *in vivo* in the setting of an intact DNA damage-induced p53 signalling pathway, either by activating p53-dependent apoptosis or replicative senescence.

The link between telomere shortening and tumour suppression is further highlighted in the *Terc*$^{-/-}$;*Apc*^{Min} mouse[51]. *Apc*^{Min} mice develop benign intestinal microadenomas and late-stage macroadenomas after loss of the wild-type *Apc* allele[52]. In early-generation *Terc*$^{-/-}$;*Apc*^{Min} mice with competent telomeres, early-stage adenomas predominated, and many of these progressed into the more aggressive macroadenomas. By contrast, only microadenomas were present in G6 *Terc*$^{-/-}$;*Apc*^{Min} animals. These results suggest that progression from microadenomas to macroadenomas was inhibited in G6 *Terc*$^{-/-}$;*Apc*^{Min} mice, presumably owing to the activation of p53-dependent tumour-suppressive pathways by

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Box 1 | The shelterin complex

The shelterin complex[15] is composed of telomeric-repeat binding factor 1 (TRF1), TRF2, TERF1-interacting protein 2 (TIN2), protection of telomeres 1 (POT1), the POT1- and TINF2-interacting protein TPP1 and the transcriptional repressor/activator protein RAP1. Proteins that directly bind the double-stranded telomeric repeats include TRF1 and TRF2. TRF1 is a negative regulator of telomere length whereas TRF2 has important roles in preventing a DNA damage response (DDR) at telomeres. POT1 belongs to a family of evolutionarily conserved oligosaccharide/oligonucleotide-binding (OB) fold-containing proteins and specifically recognizes the single-stranded G-overhang[78–81]. Shelterin components that do not bind telomeric DNA directly include TIN2, which associates with TRF1 and TRF2, and TPP1, which forms a heterodimer with POT1 (REFS 82,83). RAP1 is recruited to telomeres by TRF2 and negatively regulates telomere length. Depletion of endogenous TRF2 levels, either by overexpression of dominant-negative TRF2 (TRF2-DN) or through genetic knockout approaches in mouse cells with long telomeres, results in massive chromosomal fusions with telomeric sequence at the sites of fusions. Mouse models in which shelterin components have been deleted experience telomere dysfunction, a DDR, and chromosomal fusions resembling those observed in telomerase-null mice[79,81,84–88]. These results suggest that both critically short telomeres and direct disruption of the shelterin structure can initiate telomere dysfunction and trigger a DDR and chromosomal fusions.

dysfunctional telomeres. Similar results were observed in p53-competent mouse models of hepatocellular carcinoma, in which dysfunctional telomeres served to initiate tumour growth, but also limited the size of these tumours (that is, their progression) by activating apoptosis[53].

Short telomeres and p53-dependent senescence

The mouse models discussed above all express wild-type p53, which is capable of inducing both apoptosis and/or senescence, so it remained unclear how dysfunctional telomeres limit neoplastic growth *in vivo*. Although apoptosis clearly has a tumour-suppressive role *in vivo*, until recently it was not clear whether p53-dependent replicative senescence had a role in tumour suppression *in vivo*. Several reasons account for why replicative senescence was not initially recognized as a tumour suppressor mechanism *in vivo*. First, as laboratory mice normally have long telomeres, it was not clear whether a replicative senescence mechanism existed in mice. Second, compared with apoptosis, it is relatively difficult to reliably detect the presence of senescent cells *in vivo*. Until recently, only the SA-β-gal assay was available to mark senescent cells. However, there are now several promising markers to detect both senescent mouse and human cells *in vivo*[54–56].

Two recent studies specifically examined the role of telomere-induced replicative senescence as a mechanism of tumour suppression in the telomerase-knockout mice (FIG. 3). The first study looked at lymphoma development in the context of telomere dysfunction using the established *Eμ-Myc* transgenic model for Burkitt lymphoma[57]. Tumorigenesis was markedly reduced in late-generation *Terc*$^{-/-}$;*Eμ-Myc* cohort; wild-type mice and early-generation *Terc*$^{-/-}$;*Eμ-Myc* mice developed B-cell lymphoma by 200 days, and only 25% of *Terc*$^{-/-}$;*Eμ-Myc* mice with dysfunctional telomeres developed this cancer. Examination of tumours from late-generation mice revealed increased end-to-end chromosomal fusion and non-reciprocal translocations, hallmarks of genomic instability due to telomere dysfunction. To examine the role of p53-dependent apoptosis in mediating tumour suppression, the anti-apoptotic gene *Bcl2* was overexpressed in haematopoietic stem cells harvested from *Terc*$^{-/-}$ mice with dysfunctional telomeres. If p53-dependent apoptosis is the main driver of tumour suppression in the setting of telomere dysfunction, then its elimination by BCL2 overexpression should result in rapid development of lymphoma when transplanted into lethally irradiated recipients. Indeed, reconstitution of *Bcl2*-expressing stem cells derived from wild-type and G1 *Terc*$^{-/-}$;*Eμ-Myc* bone marrows resulted in palpable tumours within 6 weeks of transplantation. However, transplantation of G5/6 *Terc*$^{-/-}$;*Eμ-Myc Bcl2* bone marrow failed to produce any tumours 100 days after transplantation. Examination of lymph nodes from these mice revealed the presence of small encapsulated tumours with a threefold decrease in mitotic index and positive staining for senescent markers SA-β-Gal, INK4A and p15 (also known as INK4B and encoded by *Cdkn2b*). These results suggest that dysfunctional telomeres can activate a cellular senescence pathway to suppress tumorigenesis in the absence of apoptosis, and this pathway is not elicited in the tumours with competent telomeres.

A second approach to examining the role of cellular senescence in tumorigenesis used a knock-in mouse with a single amino acid mutation (Arg172Pro) within the p53 protein[58]. Cells harbouring this mutation (*Trp53*$^{P/P}$) are incapable of activating p53-dependent apoptosis[59], but the p53-dependent cellular senescence pathway is intact as induction of telomere dysfunction through overexpression of a dominant-negative TRF2 in these cells led to dramatic reduction of cellular proliferation and the appearance of senescent cells that stained positive for SA-β-Gal[58]. To genetically dissect the contribution of p53-dependent apoptosis versus cellular senescence to tumour suppression in the setting of telomere dysfunction *in vivo*, an intergenerational (iG) mating scheme was used to generate four cohorts of mice: *Terc*$^{+/-}$;*Trp53*$^{P/+}$ and *Terc*$^{+/-}$;*Trp53*$^{P/P}$ mice with intact telomere function and *iG1 Terc*$^{-/-}$;*Trp53*$^{P/+}$ and *iG1 Terc*$^{-/-}$;*Trp53*$^{P/P}$ mice with dysfunctional telomeres. Metaphase spreads of primary bone marrow and splenocyte cultures derived from telomerase-competent *Trp53*$^{P/+}$ or *Trp53*$^{P/P}$ mice showed minimal structural chromosome abnormalities. By contrast, *iG1 Terc*$^{-/-}$;*p53*$^{P/+}$ and *iG1 Terc*$^{-/-}$;*Trp53*$^{P/P}$ cells showed a 6–8-fold increase in chromosomal p–p arm fusions and a threefold increase in the formation of anaphase bridges, both hallmarks of telomere dysfunction. To determine whether p53-dependent apoptosis is required to suppress spontaneous tumorigenesis, tumour development was monitored in all four mouse cohorts over a 28-month period. Whereas *Terc*$^{+/-}$;*Trp53*$^{P/+}$ and *Terc*$^{+/-}$;*Trp53*$^{P/P}$ cohorts readily developed lymphomas, the presence of dysfunctional telomeres was associated with a near complete suppression of tumour formation in both iG1 *Terc*$^{-/-}$;*Trp53*$^{P/+}$ (0/23 mice with tumours) and iG1 *Terc*$^{-/-}$;*Trp53*$^{P/P}$ mice (1/9 mice with tumours). Immunohistochemical staining with antibodies against p21 and p53 revealed abundant immunopositive cells in intestinal epithelium from iG1 *Terc*$^{-/-}$;*Trp53*$^{P/+}$ and

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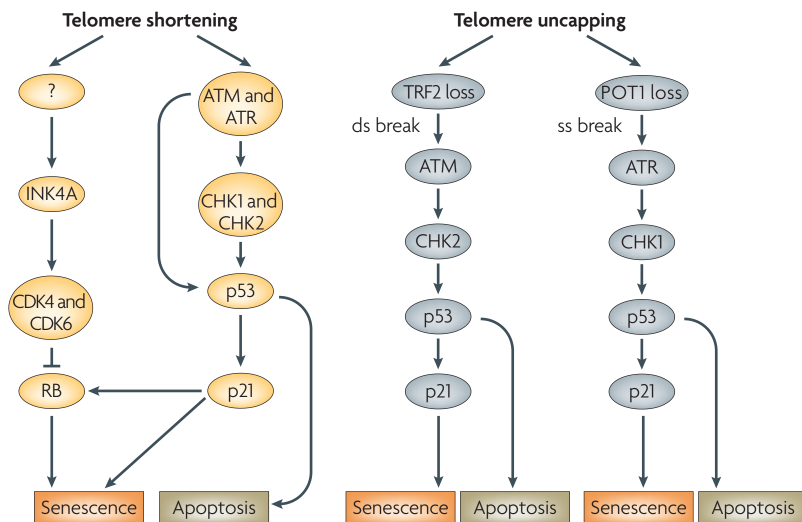

Figure 2 | **Telomere dysfunction activates the p53 and RB pathways.** Progressive telomere shortening or uncapped telomeres initiate a DNA damage response, resulting in the activation of ataxia telangiectasia mutated (ATM) and ataxia telangiectasia- and Rad3-related (ATR), and downstream kinases CHK1 and CHK2, and phosphorylation of p53. Phosphorylated p53 transcriptionally upregulates genes that mediate cellular senescence and/or apoptosis to inhibit tumorigenesis. Depending on how telomeres are uncapped, removal of telomeric-repeat binding factor 2 (TRF2) preferentially engages an ATM-dependent checkpoint, whereas removal of POT1 preferentially engages ATR. Although less well-understood, telomere dysfunction could also activate the INK4A–RB pathway and inhibit cellular proliferation. CDK, cyclin-dependant kinase; ds, double strand; ss, single strand.

iG1 *Terc*$^{-/-}$;*Trp53*$^{P/P}$ mice, whereas minimal staining was observed for *Terc*$^{+/-}$;*Trp53*$^{P/+}$ and *Terc*$^{+/-}$;*Trp53*$^{P/P}$ intestines. Robust SA-β-gal staining was also detected in multiple organs from iG1 *Terc*$^{-/-}$;*Trp53*$^{P/P}$ mice. Taken together, both studies suggest that p53-dependent apoptosis is dispensable for mediating telomere-dependent spontaneous tumour suppression *in vivo*. Instead, the p53–p21-dependent cellular senescence pathway is potently activated in mice bearing dysfunctional telomeres, and might be responsible for the tumour suppression observed in these animals.

One surprising result is the failure of the iG1 *Terc*$^{-/-}$;*Trp53*$^{P/P}$ mouse to suppress tumorigenesis in a DMBA skin carcinogenesis model[58]. This is in sharp contrast to the strong tumour suppression observed in late-generation *Terc*$^{-/-}$-null mice with intact p53 function[50]. One explanation is that although telomere-induced cellular senescence is capable of suppressing spontaneous tumorigenesis from mesenchymal tumours, it is insufficient to suppress cancer formation of epithelial tissues such as the skin. This notion that p53 is able to activate different checkpoint programmes in different tissues is supported by the observation that restoration of endogenous p53 function in p53-null mice activates primarily an apoptotic response to inhibit T-cell lymphomas, whereas inhibition of sarcomas requires activation of a cellular senescence programme[60].

From the results presented above, one would hypothesize that telomere dysfunction in the absence of p21 would abrogate the senescence response *in vivo*, resulting in accelerated tumour formation. Surprisingly, this was not the case: late-generation *Terc*$^{-/-}$;*Cdkn1a*$^{-/-}$ mice do not show increased chromosomal instability nor do they succumb to increased tumorigenesis[61]. Instead, loss of p21 extended the lifespan of the mice and rescued cellular proliferative defects due to dysfunctional telomeres, presumably owing to reduced entry of proliferative cells into cellular senescence. The fact that tumorigenesis is not increased in the context of p21 deficiency indicates that, in this mouse model, p53-mediated apoptosis functions as a redundant anti-tumour barrier in response to short telomeres. Genetic abrogation of p21 in *Terc*-null;p53^{R172P} mice will test this possibility, as loss of p21 would inhibit p53-mediated cellular senescence, whereas the presence of the p53^{R172P} allele would abrogate p53-dependent apoptosis. This mouse should therefore resemble *Trp53*$^{-/-}$ mice and be tumour prone. However, the age-dependent increases in apoptosis in certain tissues were not altered in *Terc*$^{-/-}$;*Cdkn1a*$^{-/-}$ mice, suggesting it is possible that p53-dependent but senescence- and apoptosis-independent effects of dysfunctional telomeres might account for some of the observed tumour

Box 2 | **INK4A and a telomere-induced DNA damage response**

The role of the INK4A–RB pathway in mediating the telomere DNA damage response (DDR) is not clear. INK4A is a cyclin-dependent kinase inhibitor that is markedly increased in senescent cells and results in RB hypophosphorylation[89]. Expression of telomerase reverse transcriptase (TERT) in human diploid fibroblasts (HDFs) prevents INK4A induction, suggesting that TERT prevents formation of dysfunctional telomeres that would otherwise activate INK4A. Support for this hypothesis comes from experiments in which treatment of HDF with dominant-negative telomeric-repeat binding factor 2 (TRF2-DN) induces INK4A protein levels and entry into senescence, suggesting that, in addition to p53, INK4A could be a second effector of the telomere DDR[90]. However, compared with the DDR-mediated p53 checkpoint, the kinetics of telomere DDR-mediated INK4A are slow. As telomere-induced focus formation occurs relatively transiently, this slow induction of an INK4A damage response could explain why telomere-induced foci were not observed in senescent HDFs with increased INK4A levels[91]. Interestingly, INK4A does not appear to be an important effector of the telomere DDR in mouse embryo fibroblasts (MEFs), as MEFs lacking p53 are completely refractory to the effects of TRF2-DN irrespective of INK4A levels[92]. However, it is not clear whether this is a unique property of MEFs or a general property of DDR signal transduction wiring differences between mouse and man. Mouse models of p21 and p53 deficiency rescue some of the degenerative phenotypes of late-generation telomerase-null mice[46,61]; INK4A deficiency does not[93]. However, the *Cdkn2a*-knockout mouse compromises the function of both INK4A and ARF, resulting in impingement of both the RB and p53 pathways. A mouse model that examines how telomere-initiated DDR is perturbed in the setting of INK4A deficiency only *in vivo* is required.

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Table 1 | **Dysfunctional telomeres (*Terc*$^{-/-}$) reduce tumour incidence in mouse models of cancer**

Genotype or treatment	Tumour phenotypes	Effect of dysfunctional telomeres (phenotype in *Terc*$^{-/-}$ background)	Ref
No mutations	Few tumours normally seen	Compared with wild-type and early-generation telomerase-null mice, ageing late-generation mice show an increase in the incidence of cancer	32,42
DMBA/TPA treatment	Treatment with these carcinogens allow for the monitoring of tumour initiation (papillomas) and progression to SCCs of the skin	Loss of telomerase (G1) resulted in decreased growth rate and size of papillomas, with a slight decrease in numbers. G5 mice with dysfunctional telomeres were almost completely resistant to papilloma formation	50
Cdkn2a	Deletion of this locus results in loss of both INK4A and ARF. The resulting mice develop lymphomas and sarcomas	Late-generation double knockouts show decreased incidence of spontaneous and carcinogen-induced tumours, and increased tumour latency	49,93
Apc^{min}	100% of mice with this mutation develop multiple intestinal neoplasias that progress from microadenomas to macroadenomas	Short telomeres led to increased tumour initiation (microadenomas) but decreased size and number of macroscopic adenomas	51
Alb–uPA transgene or CCl_4 or DEN treatment	This transgenic mouse and the carcinogenic treatment are both effective ways of modelling HCC	Successive breeding of *Alb–uPA* onto a late-generation telomerase-null background or treatment of G3/G4 mice with CCl_4 or DEN resulted in decreased number and size of liver nodules	53
Pms2	Deficiency of this mismatch repair gene leads to increased susceptibility for lymphomas, sarcomas and colon carcinomas	Progressively shortening telomeres reduced the incidence of all three tumour types	94
Eμ-Myc	Transgenic expression of *Myc* in B cells leads to potent formation of lymphoma in this model for Burkitt lymphoma	Formation of lymphoma was almost completely suppressed for 2 years in mice with dysfunctional telomeres (G5/G6), unlike wild-type and G1 mice that developed cancer within 6 months	57
Atm	Thymic lymphoma	Delayed onset and decreased incidence of thymic lymphomas	95,96
Trp53	Loss of this important tumour suppressor leads to rapid development of mainly lymphomas and soft tissue sarcomas	Combined homozygous loss of p53 and dysfunctional telomeres led to increased incidence of lymphomas. In addition, late-generation *Terc*$^{-/-}$ combined with heterozygous loss of p53 showed a shift in tumour range from lymphomas to epithelial cancers (breast, GI and SCC) with non-reciprocal translocations	47
p53^{R172P}	This point mutation commonly found in human tumours abolishes the ability of p53 to induce apoptosis and delays tumour formation for about 6 months.	Mice from an intergenerational cross with *Terc*$^{-/-}$ animals (iG1) have dysfunctional telomeres and show almost complete suppression of tumorigenesis	58
Atm and *Trp53*	T-cell lymphoma	Loss of p53 accelerated onset of lymphomas in *Terc*$^{-/-}$ ATM$^{-/-}$ mice	97
K5-Trf2	Specific overexpression of TRF2 in the skin of these mice leads to development of spontaneous SCC on the skin	Increasing generations of telomerase-deficient mice showed accelerated onset of spontaneous and UV-induced skin neoplasms	98

Apc^{min}, adenomatous polyposis coli min (multiple intestinal neoplasia) allele; ATM, ataxia telangiectasia mutated; DEN, diethylnitrosamine; G, generation; GI, gastrointestinal; HCC, hepatocellular carcinoma; K5, keratin 5; PMS2, postmeiotic segregation increased 2; *Terc*, telomerase RNA component; SCC, squamous cell carcinoma; TRF2, telomeric repeat-binding factor 2; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UV, ultraviolet.

suppression *in vivo*. It would be interesting to test the hypothesis that p53-mediated autophagy contributes to tumour suppression in the setting of telomere dysfunction[62].

Taken together, the above studies suggest that activation of either an apoptosis or a senescence pathway is sufficient to block tumorigenesis in most tissues. It also appears that when one anticancer pathway is selectively eliminated, the other one can serve as a back-up. In carcinomas, perhaps activation of both senescence and apoptotic pathways are required to enforce tumour suppression. How cells are ushered to undergo either apoptosis or/and cellular senescence in response to telomere dysfunction remains an important question to address in the future.

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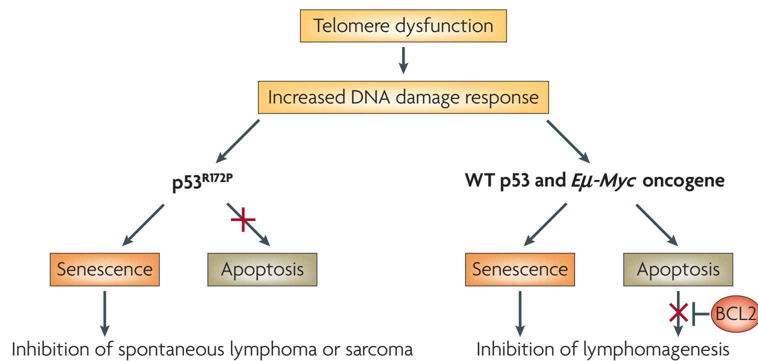

Figure 3 | **Activation of cellular senescence suppresses tumorigenesis *in vivo*.** Dysfunctional telomeres are sensed as DNA damage signals that impinge on the p53 pathway to initiate either apoptosis or cellular senescence to suppress cancer. Specific elimination of the apoptotic pathway, either through use of a knock-in allele of *Trp53* that is defective in promoting apoptosis ($p53^{R172P}$) or by overexpressing the BCL2 oncogene, eliminates apoptosis but cellular senescence remains intact. In the absence of apoptosis, cellular senescence is able to potently inhibit both spontaneous and oncogene-mediated tumour formation *in vivo*. WT, wild-type.

Therapeutic strategies that target telomeres

The demonstration that dysfunctional telomeres could engage a senescence program to suppress tumorigenesis *in vivo* suggests possible future therapeutic applications[63]. The upregulation of telomerase in most human cancers and its requirement for proliferation make anti-telomerase compounds a potential means to induce telomere shortening and initiation of a senescence programme in tumour cells[64]. For example, treatment of HT1080 fibrosarcoma cell lines with the telomerase inhibitor BIBR1532 results in progressive telomere shortening, induction of a senescence phenotype and inhibition of tumour growth when transplanted into recipient mice[65]. GRN163L is a modified oligonucleotide complementary to TERC and a potent and specific telomerase antagonist[66]. GRN163L effectively inhibits the telomerase activity of various human cancer cell lines[67–70], resulting in progressive telomere shortening and induction of cellular senescence to suppress tumour cell growth *in vitro*. Furthermore, administration of GRN163L is effective in preventing lung metastases in breast cancer xenograft animal models[68].

In addition to telomerase, targeting components of the shelterin complex such as TPP1 or POT1 to induce a DDR might also induce the onset of cellular senescence. The G-rich strand of telomeric DNA can fold into a four-stranded G-quadruplex (G4), stabilization of which perturbs telomere function. The G4-inducing ligand RHPS4 triggers a potent DDR at telomeres specifically in transformed human fibroblasts and melanoma cells[71]. Interestingly, telomere-induced focus formation correlated with delocalization of POT1 and was antagonized by overexpression of POT1 or TRF2. In mice, RHPS4 exerted its anti-tumour effect on xenografts of diverse human tumour cell lines. These data provide encouraging evidence that telomere dysfunction initiates a DDR in malignant cells to suppress tumorigenesis.

Conclusions

Understanding the molecular mechanisms that limit neoplastic growth could provide insights into novel anti-cancer therapies. Telomere-induced cellular senescence has long been hypothesized to contribute to tumour suppression[72]. However, this process is typically studied in cultured cells, and how it contributes to tumour suppression *in vivo* has been poorly defined. The mouse studies outlined above provide the first direct evidence that dysfunctional telomeres initiate p53-dependent cellular senescence to suppress spontaneous tumorigenesis *in vivo*. Surprisingly, p53-dependent apoptosis appears largely dispensable for spontaneous tumour suppression when the senescence pathway is activated, thereby placing cellular senescence on an equal footing with apoptosis in mediating tumour suppression.

Dysfunctional telomere-induced senescence was accompanied by increases in senescence markers, including p53, p21, p15 and SA-β-gal activity, suggesting that a DDR is activated by dysfunctional telomeres *in vivo*. Recent observations indicate that both telomere dysfunction[73,74] and the DDR[75,76] are activated at the earliest stages in many human carcinomas. The results presented would predict that activation of an intact DDR pathway by dysfunctional telomeres in premalignant lesions would engage cellular senescence or apoptotic pathways, suppressing further tumour progression. However, the increased genome instability in nascent tumour cells that stochastically inactivate components of the DDR pathways would promote tumour progression.

Although there is still much to learn about whether anti-telomerase therapy would be efficacious against human cancers, it is encouraging to see promising new drugs enter clinical trials. However, several limitations for this class of drugs remain. For anti-telomerase drugs to engage p53-dependent apoptotic and/or senescence pathways to suppress tumorigenesis, an intact p53 pathway is required to effectively inhibit cancer cell growth, a daunting scenario considering that p53 is mutated in approximately 50% of all human cancers. One must also be concerned about the possibility that tumour cells rendered senescent would yield secretory products that might themselves be tumour-promoting[77]. Finally, this therapy might not work in ALT tumours, which lack functional telomerase. Further progress in our understanding of the basic biology of telomeres should enable us to circumvent these problems.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ACD | ATM | ATR | *Bcl2* | *CDKN1A* | *CDKN2A* | *Cdkn2b* | CHK1 | CHK2 | H2AX | *Hras* | MDC1 | MDM2 | nibrin | p53 | POT1 | RB | *Pms2* | TERC | TERF1 | TERF2 | TERF2IP | TERT | TINF2 | TP53BP1
National Cancer Institute: http://www.cancer.gov/
breast carcinoma | HCC | skin carcinoma | SCC
National Cancer Institute Drug Dictionary:
http://www.cancer.gov/drugdictionary/
GRN163L

FURTHER INFORMATION

S. Chang's homepage: http://www.mdanderson.org/departments/cancergen/display.cfm?id=233ed916-9c38-492b-adc524eebbce099e&method=displayfull&pn=e5ec13fd-1810-4e9e-88ee7a73269a632b

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