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Perspective

p16INK4a as a Second Effector of the Telomere Damage Pathway

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ABSTRACT

Telomere damage resulting from telomere shortening can potentially suppress tumorigenesis by permanently arresting or eliminating incipient cancer cells. Dysfunctional telomeres activate the canonical DNA damage response pathway, resulting in a p53-mediated G_1/S arrest and senescence or apoptosis. Experimental induction of telomere damage through inhibition of the telomeric protein TRF2 recapitulates aspects of telomere attrition, including a p53-mediated cell cycle arrest. Using this system, we have shown that telomere damage can also elicit a G_1/S arrest through the RB-regulator p16INK4a, especially in cells lacking p53 function. Here we discuss the significance of p16INK4a as a second effector of the telomere damage response.

TELOMERE ATTRITION AS A TUMOR SUPPRESSOR PATHWAY

In the absence of telomerase human telomeres shorten at an average rate of 50–100 bp per population doubling (PD).[1] This process depletes the telomere reserve in 50–100 PD and results in senescence or apoptosis (Fig. 1). Inactivation of p53 and pRB can bypass this arrest and extend the life-span of the culture.[2] Eventually such cultures succumb to a crisis caused by the severe genomic instability associated with continued telomere shortening. Senescence and crisis can be averted through the activation of telomerase[3] or alternative mechanisms of telomere lengthening (ALT).[4] Because telomere attrition limits the proliferation of normal and transformed cells, it is thought to represent a tumor suppressor pathway. Telomere shortening is observed in early stages of human tumorigenesis[5] and modeling in the mouse has shown that short telomeres can diminish tumor incidence.[6,7] Furthermore, the fact that most human tumors express telomerase[8] (or have an active ALT pathway) suggests that telomere maintenance is an important aspect of the malignant phenotype.

Because cellular senescence was initially only observed in cultured cells, the idea that this response could repress tumorigenesis met with considerable skepticism. More recently, in vivo tumor models have confirmed that senescence can counteract tumorigenesis.[9-14] Thus, both the apoptotic and the senescent response to dysfunctional telomeres fit within the general frame-work of a telomere dependent tumor suppressor pathway.

THE CELLULAR RESPONSE TO TELOMERE SHORTENING

All available data indicates that shortening of telomeres eventually leads to the activation of the DNA damage response pathway. As cells approach replicative senescence, their telomeres become associated with DNA damage response factors and the ATM kinase pathway is activated.[15] The ATR kinase may also be activated by damaged telomeres, especially when ATM is absent.[16] How shortened telomeres activate these checkpoint kinases is not known.

Activation of the canonical DNA damage response explains how dysfunctional telomeres induce a cell cycle arrest (Fig. 2A). Most cells in a senescent culture have a 2N DNA content indicating a G_1 arrest. A G_1 arrest is consistent with p53 activation by ATM signaling and the concomitant induction of the p21 Cdk inhibitor. Inhibition of Cdk2/Cyclin E by p21 blocks E2F from mediating entry into S-phase (Fig. 2). Interestingly, $p21^{-/-}$ human fibroblasts lack the ability to enter senescence and continue to grow until they die of genome damage.[17]

The contribution of a second pRB regulator, $p16^{INK4a}$, to the telomere damage response has been controversial. As primary cells divide and telomeres shorten, $p16^{INK4a}$

levels progressively increase.[18-20] This $p16^{INK4a}$ accumulation is abolished when telomeres are stabilized through expression of hTERT, suggesting that $p16^{INK4a}$ is induced by telomere dysfunction.[3] Furthermore, $p16^{INK4a}$ can enforce a G_1/S arrest in response to DNA damage.[14,21-24] However, $p16^{INK4a}$ is also induced by telomere-independent signals, including the culture conditions routinely applied to primary human fibroblasts (reviewed in ref. 25). This culture stress response makes it difficult to assign changes in $p16^{INK4a}$ levels to telomere dysfunction, especially in the context of primary cells that are subjected to prolonged culturing to induce replicative senescence.

A recent study attempted to circumvent these difficulties by examining individual cells for signs of telomere damage and correlating this with expression of $p16^{INK4a}$ and p21.[16] Telomere damage was assessed based on the occurrence of Telomere dysfunction Induced Foci (TIFs,[26]), cytological structures that represent telomeres associated with DNA damage response factors. Although cells with TIFs often had increased levels of p21, no such correlation was found for $p16^{INK4a}$, suggesting that $p16^{INK4a}$ does not respond to telomere damage in senescent primary human fibroblasts. However, confounding aspect in this study is that TIFs are formed rapidly (within hours) and are transient, probably because the damaged telomeres are repaired by non-homologous end-joining.[26,27] In comparison, $p16^{INK4a}$ induction is a relatively slow process. However, $p16^{INK4a}$ protein levels may not peak until after the telomere damage is repaired, explaining the occurrence of $p16^{INK4a}$ positive cells without TIFs.

EXPERIMENTAL DEPROTECTION OF TELOMERES: ADVANTAGES AND PITFALLS

Acute telomere damage can be created through inactivation of proteins that protect the chromosome end. Mammalian telomeres are protected by shelterin, a protein complex composed of six subunits: TRF1, TRF2, TIN2, TPP1, Rap1 and POT1[28] (Fig. 1A). A dominant-negative allele of TRF2 (TRF2-DN) has proven to be a versatile tool for the induction of telomere deprotection.[29] TRF2-DN can dimerize with the endogenous TRF2, rendering it incompetent for DNA binding (Fig. 1A). The advantage of TRF2-DN is that all cells experience telomere damage within a few days of its expression (Fig. 1B). Such synchronized deprotection can not be achieved through replicative telomere attrition. When a culture of primary cells progresses towards replicative senescence, the deprotection of telomeres is a stochastic process affected by the length of the individual telomeres and each cell will arrest at a different point in time.

The consequences of TRF2 inhibition include a DNA damage response at the deprotected telomeres and their fusion by non-homologous end-joining[26,30,31] (Fig. 1B). The ATM kinase pathway is activated, leading to phosphorylation of Chk2 and a rise in p53 levels (Fig. 2A). Induction of p21 is observed, pRB becomes hypo-phosphorylated, and the cells arrest with a 2N DNA content, showing the morphological hallmarks of senescent fibroblasts.[32] A small percentage of the cells have a 4N DNA content but rather than a G_2/M arrest, these cells appear to have undergone endoreduplication.[32] Collectively, the data suggests that TRF2-DN induces a cell cycle arrest that is indistinguishable from replicative senescence. A similar phenotype is observed in mouse cells from which TRF2 is removed by conditional deletion of the TRF2 gene,[30] confirming that TRF2-DN acts through inhibition of TRF2.

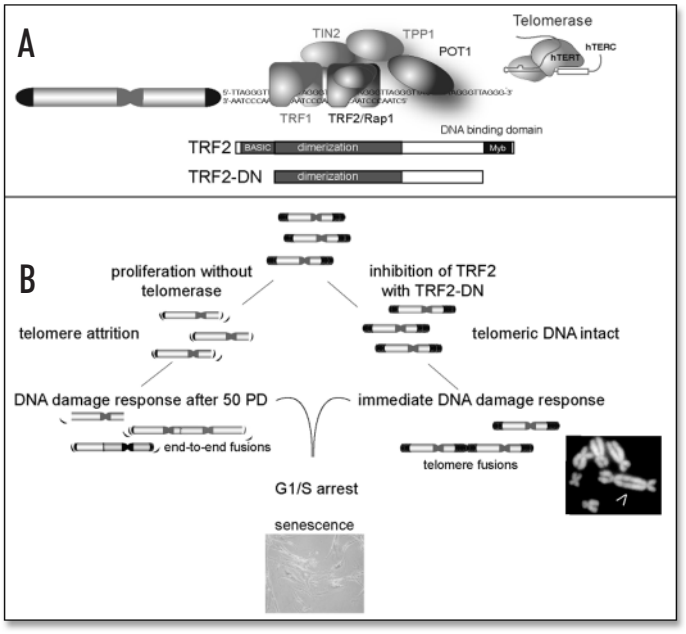

Figure 1. Telomeres and their effect on cell proliferation. (A) Top; Schematic of telomeric DNA, telomerase and the shelterin complex. Bottom; Domain structure of TRF2 and TRF-DN. (B) Comparison of replicative senescence due to telomere attrition and induction of senescence with TRF2-DN. Arrowhead points to a dicentric chromosome generated through telomere fusion.

How loss of TRF2 or telomere attrition induces telomere damage is not clear. In the case of TRF2 inhibition, loss of telomeric DNA does not occur, indicating that DNA processing is not required for a telomere damage response. Clearly, the DNA damage surveillance has the ability to detect some other aspect of telomere structure as defective. Given the uncertainty on the telomere damage signal, it is possible that the telomere dysfunction induced by TRF2 inhibition is qualitatively different from that in cells with shortened telomeres. Arguing against such a qualitative difference, overexpression of TRF2 in cells close to replicative senescence delays their arrest and protects the telomeres from fusion.[33] Thus, additional TRF2 loading on telomeres shortened through replicative attrition appears to stabilize them, suggesting that the phenotype arising from telomere attrition is primarily due to insufficient TRF2 on shortened telomeres. A second potential pitfall of TRF2-DN as a surrogate for telomere attrition is that there may be a quantitative difference in the telomere damage. TRF2-DN creates only a modest level of telomere damage and has a much less severe phenotype than complete loss of TRF2 through gene deletion.[30] Yet, many TRF2-DN expressing cells usually have ~10 dysfunctional telomeres.[26] How many telomeres are deprotected due to replicative senescence is not yet clear but the number may be low.[34] Finally, the studies with TRF2-DN require that this protein fragment is overexpressed 10–30 fold, which has the potential of creating misleading phenotypes. However, since TRF2-DN closely mimics the TRF2 null phenotype, its overexpression may not be a concern.

p53 AND p16INK4a AS EFFECTORS OF THE TELOMERE DAMAGE RESPONSE

Upon expression of TRF2-DN, $p16^{INK4a}$ protein levels rise.[26,32,35] The induction of $p16^{INK4a}$ can be detected in immunoblots as well

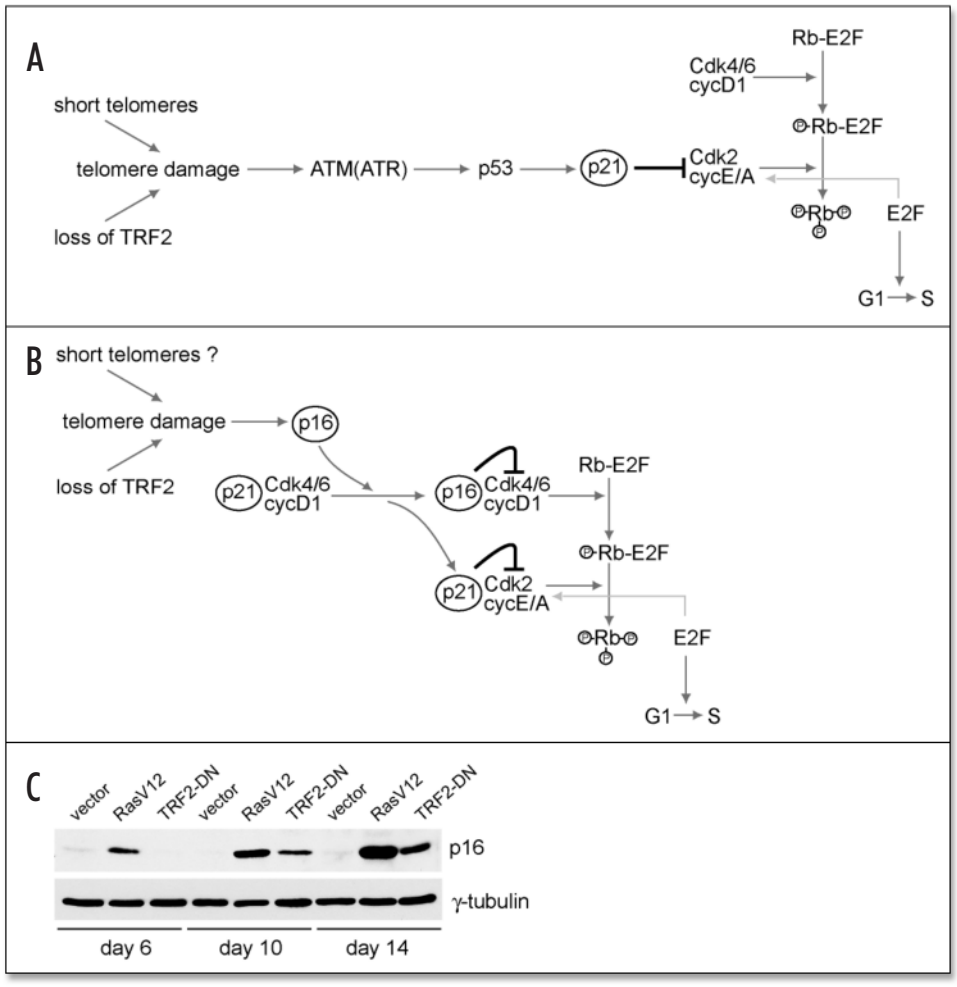

Figure 2. Proposed role for p16^{INK4a} in the telomere damage response. (A) The p53-dependent induction of G_1/S arrest. This is the major pathway by which cells respond to telomere damage. (B) The p16^{INK4a}-dependent induction of G_1/S arrest. This pathway is active in p53 deficient cells expressing TRF2-DN. It has not been established whether this pathway responds to telomere attrition. (C) Immunoblot of the induction of p16^{INK4a} by oncogenic ras and telomere damage. BJ1 primary human fibroblasts were infected with the indicated retroviruses and analyzed on the indicated days after drug selection. Note the earlier response in cells infected with a retrovirus expressing RasV12 and the absence of p16^{INK4a} in vector control cells.

as by immunohistochemistry (IHC), but the kinetics are slow (Fig. 2C). While p53 and p21 are induced in a few days, p16^{INK4a} changes only become detectable after eight to ten days. The increase in p16^{INK4a} is not the result of culture stress since parallel cultures lacking TRF2-DN do not show this response (Fig. 2C). In addition to human IMR90 fibroblasts, p16INK4a induction upon TRF2-DN expression occurs in WI38, BJ1, hTERT-immortalized BJ1 fibroblasts and fibroblasts from A-T patients (refs. 26, 32 and 35; Jacobs J, de Lange T, unpublished), (Fig. 2C).

The contribution of p53 and p16^{INK4a} to the G_1/S arrest in cells with telomere damage is best measured using BrdU incorporation to assess changes in the S-phase index.[32,35] An alternative is to determine changes in proliferation of the cells but this can be misleading because the dicentric chromosomes formed by telomere fusion can slow progression through mitosis even in cells with a deficient G_1/S checkpoint. In response to TRF2-DN, the S-phase index of primary fibroblasts gradually drops to ~30% relative to control cells. When cells lack normal p53 function (due to expression of a dominant negative allele of p53 or knockdown with shRNA), the S-phase index still drops in response to TRF2-DN but the reduction is less, now showing 60% of the controls.[35] The residual drop can be abrogated when p16^{INK4a} is inhibited, resulting in TRF2-DN expressing cells with an S-phase index of 90% of the control (cells with inhibited p53 and p16^{INK4a} but not expressing TRF2-DN).

The ability of p16^{INK4a} to mediate a telomere damage response in p53 deficient cells is also obvious from microscopic inspection of the cells.[35] Within 2 weeks of infection with TRF2-DN, cells that express the dominant negative allele of p53 show a senescent morphology and stain with SA-β-gal. This response is abrogated in cells that also have been infected with a p16^{INK4a} shRNA retrovirus. The effects of p16^{INK4a} shRNA inhibition were also observed when the p16^{INK4a} levels were repressed with Bmi1. It is unlikely that the reduction in p16^{INK4a} abrogates the arrest by somehow improving the protection of the telomeres, because chromosome end fusions still occur. Collectively the data suggest that p16^{INK4a} can respond to telomere damage and contributes to telomere-directed senescence in cells that lack normal p53 function. However, p16^{INK4a} inhibition alone does not have a significant effect on the growth or S-phase index of cells expressing TRF2- DN. Therefore, p53 constitutes the dominant effector pathway of the telomere damage response.

The binding of p16^{INK4a} to Cdk4/6-cycD prevents the inactivation of RB directly by inhibiting Cdk4/6-cycD activity and can also act indirectly by binding to Cdk4/6-cycD complexes thereby releasing p21 for inhibition of Cdk2-cycE/A[36-38] (Fig. 2B). The latter effect of p16^{INK4a} could explain why targeted disruption of p21 is sufficient to abolish replicative senescence,[17] whereas in our studies p53 inactivation alone is not sufficient to fully abrogate telomeredirected senescence, but combined inactivation of p53 and p16^{INK4a} is.

MECHANISM OF p16INK4a ACTIVATION BY TELOMERE DAMAGE

The p16INK4a gene can be activated by Ets1, Ets2, JunB and MITF and is repressed by Bmi1, CBX7, Id1 and Tal1/Scl or promoter methylation.[13,38-43] The p38-stress-activated kinase pathway has been implicated in p16^{INK4a} induction, and the RNA binding protein AUF1 appears to negatively control p16^{INK4a} mRNA stability.[44,45] In addition, in human cells p16^{INK4a} may be subject to negative feedback regulation by RB.[46] Which (if any) of these pathways is mediating the p16^{INK4a} response to telomere damage is not clear.

A puzzling feature of the p16^{INK4a} response to telomere damage is that, in comparison to stress-induced p16^{INK4a} upregulation and

the p53/p21 response to telomere damage, it appears to be rather slow. Figure 2 (panel C) shows a comparison of $p16^{INK4a}$ levels in primary human BJ1 fibroblasts infected in parallel with retroviruses encoding oncogenic Ras or the dominant-negative TRF2 allele. Whereas $p16^{INK4a}$ is already elevated six days after infection with RasV12 retrovirus. For the TRF2-DN expressing cells induction of $p16^{INK4a}$ is seen first after 10 days, with a further increase at day 14. These slow kinetics are similar to the kinetics of $p16^{INK4a}$ induction after global DNA damage induced by UV or γ-irradiation (refs. 21-24; Jacobs J, de Lange T, unpublished). This suggests that the mechanisms by which telomere damage and oncogenic stress induce $p16^{INK4a}$ are different. Perhaps the upregulation of $p16^{INK4a}$ upon telomere damage (and global DNA damage) is slow because it requires extensive chromatin remodeling or because the inducing signal is weak. Another possibility is that the induction of $p16^{INK4a}$ is counteracted by activation of RB (as part of a negative feedback loop) which occurs rapidly when cells undergoing telomere damage activate p53.

RELEVANCE TO HUMAN CANCER

The effectiveness of the telomere damage response determines the fate of cells experiencing dysfunctional telomeres. Cells will cause no harm if they cease proliferation or die, but if the telomere damage response is not effective, the genome instability associated with telomere dysfunction will increase the risk of a malignant conversion. Thus, the telomere damage response determines whether telomere dysfunction will have tumor suppressive or oncogenic consequences. From this perspective, the finding of $p16^{INK4a}$ as a second effector of the telomere damage response is of interest. The rapid cell cycle arrest enforced by p53 may allow cells to repair the telomere damage. However, if this arrest fails, $p16^{INK4a}$ could ensure that potentially malignant cells do not arise by arresting the defective cells. This view is supported by a recent study on the reversibility of human cellular senescence, in which it was found that p53 inactivation only resulted in the reversal of senescence in cells with low $p16^{INK4a}$ levels.[48] The ability of $p16^{INK4a}$ to enforce irreversible senescence may be related to the role of the $p16^{INK4a}$/Rb pathway in the formation of Senescence-associated heterochromatin foci (SAHFs).[49] Furthermore, recent data has suggested that early in tumorigenesis, before the telomere reserve has been depleted, replication stress selects for p53.[50,51] If this scenario is correct, p53 function is lost before telomere directed senescence occurs, leaving $p16^{INK4a}$ as the main barrier in the telomere tumor suppressor pathway.

One hurdle in the analysis of telomere dynamics and tumorigenesis is that mouse models do not fully recapitulate the human setting (reviewed in ref. 52). Because mouse telomeres are very long and telomerase is constitutive, the telomere reserve has to be depleted experimentally using telomerase-deficient mice (reviewed in ref. 53). A second concern is that the balance between the contribution of p53 and $p16^{INK4a}$ to the telomere damage response appears to be different in mouse cells.[32] Although MEFs infected with the TRF2-DN allele show induction of $p16^{INK4a}$ protein, this pathway does not appear to contribute to the telomere damage induced G_1/S arrest. MEFs that lack p53 are completely refractory to TRF2-DN and even continue to enter S-phase when TRF2 is deleted (Celli G, de Lange T, unpublished). The unresponsiveness of MEFs to $p16^{INK4a}$ -mediated growth inhibition in a p53-deficient setting is not unique to telomere-directed senescence, but is also observed for stress-induced senescence,[54] pointing to a different wiring or balance between the p53/p21 and p16INK4a/RB pathways in mouse and man.

With regard to the $p16^{INK4a}$ response to telomere damage, there are several challenges for the future. First, we need to know whether the findings with TRF2-DN recapitulate telomere attrition. In order to determine whether shortened telomeres induce $p16^{INK4a}$ it will be necessary to study replicative senescence under conditions that do not create culture stress so that the basal level of $p16^{INK4a}$ remains low. Preferably, this should be done in p53 deficient cells so that the induction of a G_1/S arrest by $p16^{INK4a}$ can be assessed. The second important issue is the pathway by which $p16^{INK4a}$ is induced and the mechanism of its action. Is p21 indeed downstream of both p53 and $p16^{INK4a}$? Finally, it will be important to understand the difference between human and mouse cells better, in hopes of generating a mouse model that recapitulates the telomere tumor suppressor pathway more closely.

References

1. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990; 345:458-60.
2. Shay JW, Wright WE, Brasiskyte D, Van der Haegen BA. E6 of human papillomavirus type 16 can overcome the M1 stage of immortalization in human mammary epithelial cells but not in human fibroblasts. Oncogene 1993; 8:1407-13.
3. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal human cells. Science 1998; 279:349-52.
4. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med 1997; 3:1271-4.
5. Chin K, De Solorzano CO, Knowles D, Jones A, Chou W, Rodriguez EG, Kuo WL, Ljung BM, Chew K, Myambo K, Miranda M, Krig S, Garbe J, Stampfer M, Yaswen P, Gray JW, Lockett SJ. In situ analyses of genome instability in breast cancer. Nat Genet 2004; 36:984-8.
6. Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, Greider CW, DePinho RA. Short dysfunctional telomeres impair tumorigenesis in the INK4a(delta2/3) cancer-prone mouse. Cell 1999; 97:515-25.
7. Gonzalez-Suarez E, Samper E, Flores JM, Blasco MA. Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. Nat Genet 2000; 26:114-7.
8. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997; 33:787-91.
9. Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dorken B, Jenuwein T, Schmitt CA. Oncogene-induced senescence as an initial barrier in lymphoma development. Nature 2005; 436:660-5.
10. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C, Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005; 436:725-30.
11. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D, Serrano M. Tumour biology: Senescence in premalignant tumours. Nature 2005; 436:642.
12. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 2005; 436:720-4.
13. Passegue E, Wagner EF. JunB suppresses cell proliferation by transcriptional activation of p16(INK4a) expression. EMBO J 2000; 19:2969-79.
14. Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, Lowe SW. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 2002; 109:335-46.
15. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomereinitiated senescence. Nature 2003; 426:194-8.
16. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell 2004; 14:501-13.
17. Brown JP, Wei W, Sedivy JM. Bypass of senescence after disruption of *p21CIP1/WAF1* gene in normal diploid human fibroblasts. Science 1997; 277:831-4.
18. Hara E, Smith R, Parry D, Tahara H, Stone S, Peters G. Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. Mol Cell Biol 1996; 16:859-67.
19. Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC. Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc Natl Acad Sci USA 1996; 93:13742-7.
20. Palmero I, McConnell B, Parry D, Brookes S, Hara E, Bates S, Jat P, Peters G. Accumulation of p16INK4a in mouse fibroblasts as a function of replicative senescence and not of retinoblastoma gene status. Oncogene 1997; 15:495-503.

21. Robles SJ, Adami GR. Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. Oncogene 1998; 16:1113-23.
22. Shapiro GI, Edwards CD, Ewen ME, Rollins BJ. p16INK4A participates in a G_1 arrest checkpoint in response to DNA damage. Mol Cell Biol 1998; 18:378-87.
23. Piepkorn M. The expression of p16(INK4a), the product of a tumor suppressor gene for melanoma, is upregulated in human melanocytes by UVB irradiation. J Am Acad Dermatol 2000; 42:741-5.
24. te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP. DNA damage is able to induce senescence in tumor cells in vitro and in vivo. Cancer Res 2002; 62:1876-83.
25. Drayton S, Peters G. Immortalisation and transformation revisited. Curr Opin Genet Dev 2002; 12:98-104.
26. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. Curr Biol 2003; 13:1549-56.
27. Bakkenist CJ, Drissi R, Wu J, Kastan MB, Dome JS. Disappearance of the telomere dysfunction-induced stress response in fully senescent cells. Cancer Res 2004; 64:3748-52.
28. de Lange T. Shelterin, the protein complex that shapes and safeguards human telomeres. Genes & Development 2005; 18:In press.
29. de Lange T. Protection of mammalian telomeres. Oncogene 2002; 21:532-40.
30. Celli G, de Lange T. DNA processing not required for ATM-mediated telomere damage response after TRF2 deletion. Nat Cell Biol 2005; 7:712-8.
31. Smogorzewska A, Karlseder J, Holtgreve-Grez H, Jauch A, de Lange T. DNA ligase IV-dependent NHEJ of deprotected mammalian telomeres in G_1 and G_2. Curr Biol 2002; 12:1635.
32. Smogorzewska A, de Lange T. Different telomere damage signaling pathways in human and mouse cells. Embo J 2002; 21:4338-48.
33. Karlseder J, Smogorzewska A, de Lange T. Senescence induced by altered telomere state, not telomere loss. Science 2002; 295:2446-9.
34. Zou Y, Sfeir A, Gryaznov SM, Shay JW, Wright WE. Does a sentinel or a subset of short telomeres determine replicative senescence? Mol Biol Cell 2004; 15:3709-18.
35. Jacobs JJ, de Lange T. Significant Role for p16(INK4a) in p53-independent telomere-directed senescence. Curr Biol 2004; 14:2302-8.
36. Cheng M, Olivier P, Diehl JA, Fero M, Roussel MF, Roberts JM, Sherr CJ. The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. Embo J 1999; 18:1571-83.
37. Polyak K, Kato JY, Solomon MJ, Sherr CJ, Massague J, Roberts JM, Koff A. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. Genes Dev 1994; 8:9-22.
38. Sherr CJ, Roberts JM. CDK inhibitors: Positive and negative regulators of G_1-phase progression. Genes Dev 1999; 13:1501-12.
39. Gil J, Bernard D, Martinez D, Beach D. Polycomb CBX7 has a unifying role in cellular lifespan. Nat Cell Biol 2004; 6:67-72.
40. Hansson A, Manetopoulos C, Jonsson JI, Axelson H. The basic helix-loop-helix transcription factor TAL1/SCL inhibits the expression of the *p16INK4A* and *pTalpha* genes. Biochem Biophys Res Commun 2003; 312:1073-81.
41. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene *bmi-1* regulates cell proliferation and senescence through the ink4a locus. Nature 1999; 397:164-8.
42. Loercher AE, Tank EM, Delston RB, Harbour JW. MITF links differentiation with cell cycle arrest in melanocytes by transcriptional activation of INK4A. J Cell Biol 2005; 168:35-40.
43. Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, Baylin SB, Sidransky D. 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. Nat Med 1995; 1:686-92.
44. Ohtani N, Zebedee Z, Huot TJ, Stinson JA, Sugimoto M, Ohashi Y, Sharrocks AD, Peters G, Hara E. Opposing effects of Ets and Id proteins on p16INK4a expression during cellular senescence. Nature 2001; 409:1067-70.
45. Naka K, Tachibana A, Ikeda K, Motoyama N. Stress-induced premature senescence in hTERT-expressing ataxia telangiectasia fibroblasts. J Biol Chem 2004; 279:2030-7.
46. Wang W, Martindale JL, Yang X, Chrest FJ, Gorospe M. Increased stability of the p16 mRNA with replicative senescence. EMBO Rep 2005; 6:158-64.
47. Li Y, Nichols MA, Shay JW, Xiong Y. Transcriptional repression of the D-type cyclin-dependent kinase inhibitor p16 by the retinoblastoma susceptibility gene product pRb. Cancer Res 1994; 54:6078-82.
48. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: Roles of the p53 and p16 pathways. Embo J 2003; 22:4212-22.
49. Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of *E2F* target genes during cellular senescence. Cell 2003; 113:703-16.
50. Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, Ditullio RAJ, Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C and Halazonetis TD. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005; 434:907-13.
51. Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K, Guldberg P, Sehested M, Nesland JM, Lukas C, Orntoft T, Lukas J and Bartek J. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005; 434:864-70.
52. Rangarajan A, Weinberg RA. Opinion: Comparative biology of mouse versus human cells: modelling human cancer in mice. Nat Rev Cancer 2003; 3:952-9.
53. Maser RS, DePinho RA. Connecting chromosomes, crisis, and cancer. Science 2002; 297:565-9.
54. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997; 88:593-602.