[Cell Cycle 5:3, 260-265, 1 February 2006]; ©2006 Landes Bioscience

Extra View

p63

A New Link Between Senescence and Aging

William M. Keyes
Alea A. Mills*

Cold Spring Harbor Laboratory; Cold Spring Harbor, New York USA

*Correspondence to: Alea A. Mills; Cold Spring Harbor Laboratory; Cold Spring Harbor, New York 11724 USA; Tel.: 516.367.6910; Fax: 516.367.8874; Email: mills@cshl.edu

Received 12/13/05; Accepted 12/14/05

Previously published online as a Cell Cycle E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=2415

KEY WORDS

p63/p53, senescence, aging, epithelial development

ABSTRACT

Cellular senescence is a distinctive form of cell cycle arrest that has been suggested to modulate the processes of tumor suppression and aging. Though a detailed understanding of the cellular machinery regulating this process is emerging, a more thorough understanding of the key players linking senescence to organismal aging is needed. The recent discovery that loss of the p53-related protein p63 induces cellular senescence and causes features of accelerated aging provides further evidence that cellular senescence is intimately linked with organismal aging, and identifies p63 as a key regulator of both of these processes.

INTRODUCTION

Cellular senescence is a form of stable growth arrest in which cells remain metabolically active, yet acquire a distinctive morphology and gene expression pattern.[1-3] Replicative senescence was originally described as a process of terminal arrest in cultured human cells at the end of their proliferative capacity,[4] and was subsequently shown to be caused by telomere attrition.[5] A phenotypically similar process of premature or oncogene-induced senescence was described as a cellular response to a variety of stimuli, including activated oncogenes or excessive mitogenic signaling, DNA damage and stress signals, or in vivo in response to chemotherapy.[2,6-8] In both replicative- and oncogene-induced senescence, cells enter a state of irreversible arrest, displaying a characteristic morphology of an enlarged flattened vacuous cell with elongated processes. This phenotype is accompanied by a number of distinguishing features including reduced proliferation, enhanced senescence-associated β-galactosidase (SA-β-gal) activity, and an increase in expression of a number of key mediators, including p53, promyelocytic leukemia (PML) protein, $p16^{INK4a}$ and $p19^{Arf}$.[2,3,6] Though mostly studied in vitro, cellular senescence has been correlated with both processes of aging and tumor suppression.[9] Recent studies have now advanced our understanding of senescence regulation and it's in vivo roles. Collectively, results from a number of independent studies demonstrate that the growth arrest state can be bypassed by inactivation of key mediators of cellular senescence, allowing senescent cells to progress to malignancy.[10-13] Although these studies conclusively demonstrate that cellular senescence provides a tumor suppressive role in vivo, an equally convincing connection between cellular senescence and organismal aging has been lacking.

Recently, we described a previously unknown role for the p53 related protein p63 in cellular senescence and aging.[14] During the course of a spontaneous tumor study, we found that *p63* heterozygous mutant mice were not tumor prone, but that they had a decreased lifespan and developed features of accelerated aging. Induced ablation of p63 in primary keratinocytes (a cell type that expresses high levels of endogenous p63), caused an arrest in proliferation, a senescent morphology and expression of endogenous SA-β-gal activity, suggesting that cellular senescence is responsible for the age-related decline in mice with compromised p63. Indeed, conditional ablation of p63 specifically in keratin 5 (K5)-expressing stratified epithelia such as the skin (Fig. 1) supports this hypothesis. Activation of Cre during embryogenesis recapitulates a p63-deficient phenotype. Both germline- and somatically-induced p63 deficiency caused decreased proliferation and increased expression of the senescence markers SA-β-gal, $p16^{INK4a}$ and PML. Most interestingly, p63 ablation specifically within proliferating epithelia in adult mice caused a number of features of accelerated aging concurrent with senescence induction. Together, this data introduces new evidence that loss of p63 induces a senescence arrest, and strengthens the theory that senescence is a causative factor in the aging process.

p63: A COMPLEX GENE

Although p63 was initially expected to function similarly to p53 given the structural homology between these two proteins, subsequent studies ascribe very different and complex functions to p63. p63's unique role in epithelial morphogenesis was based on the striking phenotypes of p63-deficient mice.[15,16] Mice lacking p63 are born without skin and lack ectodermal derivatives, including glands, teeth and hair. Although *p63*$^{-/-}$ mice are born alive, they die within hours after birth, which prohibits using these mice to gain an understanding of p63's role in adult tissue. Abundant expression of p63 in proliferating keratinocytes of stratified epithelia (Fig. 1) and the fact that p63 is downregulated during differentiation also suggested a role for p63 in epithelial development.[17] Indeed, more recent evidence posits p63 as a key regulator of proliferation in epithelial cells, likely mediated by the specific transcriptional capabilities of its many isoforms. The structure of p63 is complex: two promoters generate both full-length transactivating (TA)- and amino-terminally truncated (ΔN) isoforms, with alternative splicing generating α, β and γ subtypes in each group, giving rise to a total of six known p63 proteins.[17,18] The predominant isoform in epithelia such as the skin is ΔNp63α, which can exert dominant-negative effects towards the TA isoforms and many other target genes.[17,19] Though the TA isoforms are not expressed as robustly as other classes of p63 proteins, they are increased and function in conditions of cell cycle withdrawal such as terminal differentiation, and in cell stress conditions such as apoptosis.[20,21] During epidermal development, it has been suggested that the TA isoforms are expressed first and dictate commitment to the epithelial lineage,[22] while in mature epithelia, it is the predominant ΔNp63α isoform and its interactions with the other isoforms that maintains the proliferative capacity of epidermal keratinocytes.[19,22,23] Together or individually, the different isoforms of this protein provide functional roles in many important cellular processes.

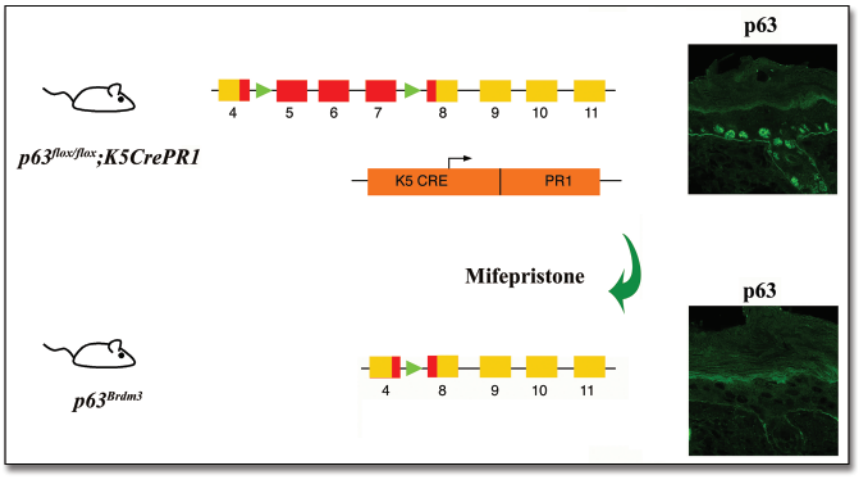

Figure 1. Conditional mouse model used for *p63* study. By intercrossing *p63*$^{flox/flox}$, in which *loxP* sites flank exons in the DNA-binding domain (red), to mice in which mifepristone-inducible Cre is driven by the keratin 5 (K5) promoter, mice with normal expression of p63 can be created. p63 protein can be seen in the basal layer of the epidermis, in the hair follicle and in the region of the epidermal stem cell niche. Treatment of these mice with inducer disrupts *p63* and ablates p63 protein in K5-expressing cells.

p63 AND SENESCENCE REGULATION

p63s involvement in maintaining proliferation of epithelial cells is in line with the findings that loss of such a key player results in activation of anti-proliferative process like senescence.[14] Irrespective of the initiating stimulus, the senescence pathway invariably involves activation of either or both the p19^{Arf}/p53 and p16^{INK4a}/Retinoblastoma (Rb) tumor suppressor pathways, with some differences between human and mouse cells (e.g., mouse cells do not undergo telomere-induced senescence).[2,24] Analysis of genetic models of p63 deficiency have begun to unravel the mechanism by which p63 affects other mediators of senescence.

The p53 and p16^{INK4a} connection. Central to most premature or oncogene-induced senescence is a p53-dependent response in which abrogation of p53 signaling delays or bypasses the arrest.[25,26] The connection between senescence and p53 and how modulation of p53 function affects organismal aging was significantly advanced by the generation of a number of mouse models with enhanced p53 function.[27,28] These mouse models express amino (N)-terminally truncated p53 isoforms and display features of accelerated aging. Several mouse models of enhanced p53 also exhibit increased tumor resistance, drawing parallels between mouse models with compromised p63 and those with enhanced p53. Given that p63 and p53 are structurally similar members of the same protein family and have been shown to interact in several cellular contexts, it is tempting to speculate that p63 and p53 functionally interact to regulate cellular senescence and aging. Indeed, many of the features of accelerated aging are manifested in epithelial tissues in both of these models.[14,27] As p53 directly binds and decreases levels of p63 by caspase-mediated degradation,[29] it is possible that senescence induced by enhanced p53 is in fact mediated by decreased levels of p63. However, the finding that senescence induced by p63 ablation in primary keratinocytes is prevented by shRNA-mediated knockdown of p53 suggests that p53 cooperates with p63 as a primary mediator of senescence in cultured cells. Given these results, it is clear that specific interactions between p53 and the individual p63 isoforms in this context will need further study. The accelerated aging phenotype caused by enhanced expression of the N-terminally truncated p53 isoform p44 (also called p53/p47; ΔNp53; p47, reviewed in ref. 30) involves the evolutionarily conserved insulin/insulin-like growth factor 1 [IGF1] -signaling pathway.[28] In many species ranging from yeast to nematodes to flies and mice, mutations that inhibit the insulin/IGF-1 axis extend lifespan significantly.[31,32] Conversely, enhanced expression of p44 increases the levels of IGF-1 and its receptor, contributing to accelerated aging.[28] The recent findings that p63 represses both the IGF-IR and insulin-like growth factor binding protein-3 (IGFBP-3) signaling, both mediators of the insulin/IGF-1 axis, further suggests a mechanism of how p63 deficiency leads to accelerated aging.[33,34]

The second key mediator of senescence is a Rb-mediated pathway involving activation of the cyclin-dependent kinase inhibitor p16^{INK4a}, which functions as a senescence regulator particularly in p53-independent cellular contexts.[2,9] Interestingly, an increase in p16^{INK4a} levels in vivo was noted in p63-compromised tissues, suggesting that p63 is upstream of Rb in the senescent process.[14] Thus, as in many cases of senescence, it appears that p63 deficiency-induced senescence is a context-dependent process. In cultured primary

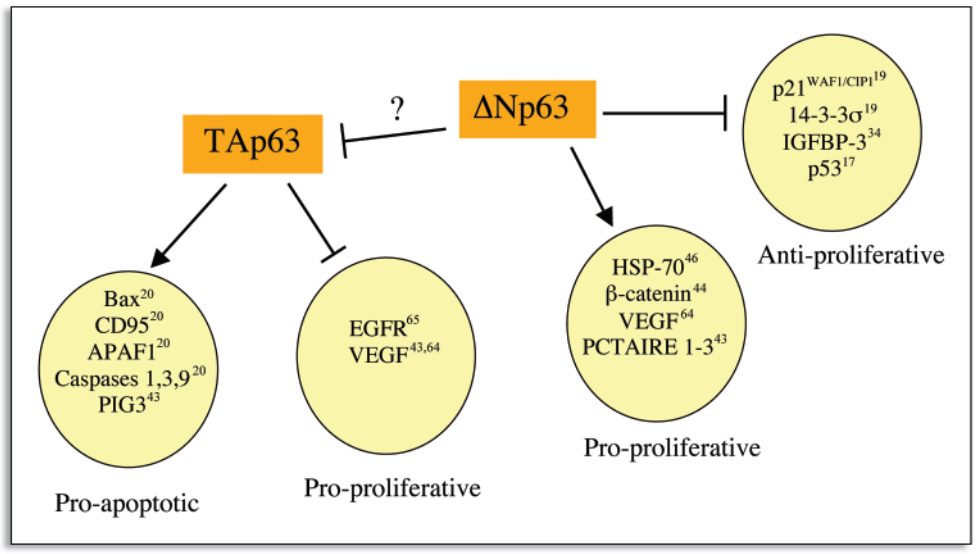

Figure 2. p63-mediated pathways. ΔNp63 can repress many inhibitors of cellular proliferation such as $p21^{WAF1/CIP1}$, 14-3-3σ,[19] insulin-like growth factor binding protein-3 (IGFBP-3)[34] and p53.[17] The ΔNp63 isoforms have also been shown to activate pro-proliferative factors including heat-shock protein-70 (HSP-70),[46] β-catenin,[44] vascular endothelial growth factor (VEGF)[64] and PCTAIRE protein kinases 1-3.[43] ΔNp63 has also been suggested to repress the TAp63 isoforms[17] which can affect cellular proliferation both positively and negatively. TAp63 has been implicated as both an inducer and regulator of apoptosis, affecting mediators such as Bcl-2 associated X-protein (Bax), CD95, apoptotic protease activating factor-1 (APAF1),[20] PIG3[43] as well as caspases.[20] Repression by the TA isoforms repress expression of growth factors and receptors such as epidermal growth factor receptor (EGFR)[65] and VEGF.[43,64]

keratinocytes p63 deficiency-induced senescence is p53-dependent, whereas in the developing embryo this process occurs in a p53-independent p16-dependent manner. An important difference between primary keratinocytes and intact embryos that may influence the effect of p53 deficiency is that keratinocytes were isolated from the mature skin and were then rendered deficient for both p63 and p53, whereas in the embryo, the cells never have p63 or p53, and are thus a fundamentally different model. However, further work will be necessary to determine exactly how p63 fits into this picture, and how p53 deficiency alters the cellular phenotype in certain contexts.

PML. The PML tumor suppressor is another key senescence regulator of both the p53- and Rb-mediated pathways. PML is a component of nuclear bodies (NB's) and is involved in multiple cellular processes, including senescence, apoptosis and proliferation.[35] PML protein is upregulated in response to senescence stimuli, and when over expressed is a potent inducer of senescence that facilitates the transcriptional activity of p53. PML and p53 colocalize in the NB's of senescent cells,[36-38] and PML also interacts with and regulates both p53 and p73 by inhibiting their ubiquitin-mediated proteasomal degradation.[39,40] Interestingly, p63 deficiency induced in either the germline or in somatic cells resulted in a dramatic increase in both the size and number of PML-NB's, suggesting that p63 acts both upstream and possibly as a repressor of PML.[14] Recent work, however, demonstrates that p63 colocalizes in the NB's and that TAp63 is a transcriptional target of PML.[41] The observation that p63 transcriptional activity is increased by its interactions with PML places p63 genetically downstream of PML signaling.[41] However, similar findings for p53 and its interactions with PML have been described previously, initially placing PML upstream of p53, while a more recent study demonstrates that p53 can also transactivate PML directly.[42] It remains to be seen whether p63 and PML are involved in a feedback loop as has been described for PML and p53, and which p63 isoforms regulate this process. One possible scenario would likely involve individual isoforms such as ΔNp63α repressing PML, which, when activated could subsequently activate the TAp63 isoforms,[41] though more needs to be done to elucidate this process.

p63 targets. Central to p63's role in maintaining the proliferative state is an ongoing balance of interactions between the individual isoforms and their downstream targets (Fig. 2). One of ΔNp63α's key functions is as a transcriptional repressor, having previously been shown to inhibit a number of key cell cycle mediators. ΔNp63 acts as a repressor of cell cycle inhibitors such as $p21^{Waf1/Cip1}$ and 14-3-3σ,[19] which are key mediators in cell cycle withdrawal. However, p63 can also repress IGFBP-3, a negative regulator of proliferation.[34] Whether p63 directly inhibits key senescence mediators such as $p16^{INK4a}$ or $p19^{Arf}$ remains to be shown. In vitro transactivation studies show that ΔNp63α can also repress activation of the TAp63 isoforms, which can both suppress pro-proliferative, and activate pro-apoptotic pathways.[17] It has been proposed that a balance between ΔNp63 and TAp63 isoforms is responsible for maintaining the proliferation of undifferentiated epithelia.[22] In expression studies, TAp63α activates a number of apoptotic mediators in both the death receptor and mitochondrial mediated pathways, including Bax, CD95, Apaf1 and caspases 1, 3, and 9,[20] while microarray studies identified pro-apoptotic PIG3 as a transcriptional target of TAp63α.[43] In a similar mode, ΔNp63 is also capable of inhibiting p53 activity.[17] As such, maintained expression of the dominant ΔNp63α isoform promotes a proliferative state with suggested oncogenic capacity,[44] predominantly by repressing anti-proliferative mediators. Elevated expression of ΔNp63α in head and neck squamous cell carcinoma was also shown to favor a positive clinical response to platinum-based chemotherapy, presumably with drug treatment inducing decreased levels of ΔNp63α and inhibiting proliferation.[45] However, the ΔNp63α isoforms (which are lacking the transactivating domains that are present in TAp63 isoforms), also possess transactivation functions, and some of ΔNp63s targets also promote the cell cycle and favor proliferation. The proliferation mediator of the Wnt pathway, β-catenin is activated by ΔNp63α,[44] while microarray analysis revealed that a number of cell cycle regulatory genes are induced by p63, including the protein kinases PCTAIRE1-3, which play positive roles in cell proliferation and *Hsp70*, a key mediator of cell survival in response to stress.[43,46] Of course there are going to be exceptions and cell-specific activities for p63 also. For example, ΔNp63α can also activate the cyclin-dependent kinase inhibitor $p57^{Kip2}$, which in other cell types is repressed by TAp63,[47] further supporting the balance of p63 isoforms in regulating proliferation. However, given ΔNp63's key role in promoting proliferation, expression of the ΔNp63α isoform alone was insufficient to prevent senescence in response to total p63

ablation,[14] further suggesting an intricate interplay of p63 isoforms in regulation of proliferation. Taken together, it is now clear that p63 is a key mediator of epithelial cell proliferation, and that loss of this protein induces cellular senescence.

p63 AND AGING

This raises the question as to how induction of senescence triggered by p63 deficiency leads to an accelerated aging phenotype in the animal. It has been proposed that induction of senescence hinders the replicative capacity of cells that are necessary for tissue renewal and homeostasis, thus leading to age-related decline. At present, there are two possible scenarios for how this may occur: senescence acts in either a cell-autonomous or non-cell autonomous manner.[3] Cell autonomous mechanisms would directly impair the regeneration of a tissue, through induction of senescence in proliferating stem/progenitor cell populations, which ultimately compromises the renewal capacity of the tissue. Cell non-autonomous senescence likely influences neighboring or distant environments through induction of senescence in support cells. In such a case, senescence may disrupt direct cell-cell paracrine signaling, or affect hormone and cytokine release which can translate a localized response to distant sites or tissues. Alternatively, senescent cells release secreted proteins that may alter the local tissue environment and thereby decrease tissue function.[9,48] Intriguingly, the expression of senescence markers observed in p63-deficient tissues in vivo are localized in areas of the skin that are rich in proliferating and stem/progenitor cell populations.[14] Indeed, epithelial specific ablation of p63 in adult mice caused enhanced expression of the senescence markers SA-β-gal and $p16^{INK4a}$ in the hair follicles and hyperplastic sebaceous glands—the location of the stem cell niche of the skin. Although further work will be required to conclusively demonstrate that these senescent cells are truly in the stem cell population, the correlative evidence that induction of senescence in the skin corresponds with the development of many aging features, lends support to the theory that cellular senescence plays a causal role in aging in vivo. Much of the basis for this theory has come from previous reports that show that SA-β-gal and $p16^{INK4a}$ are among the true markers of senescent cells, and that both have been shown to accumulate during aging. Indeed, in other settings, repression of the INK4a/Arf pathway is a key mediator of stem cell longevity.[49,50] A recent report has taken this a step further by suggesting that p16 is in fact a biomarker and an effector of aging and can be manipulated by caloric restriction—a key mediator of lifespan in lower organisms and mice.[51] Together, the data suggests that senescence is a causative factor in the aging process, and now that p63 is a key factor regulating both these processes in vivo.

THE p63 LINEAGE VS. STEM CELL QUESTION

The hypothesis that p63 plays an important role in the regulation of stem cell function has been previously suggested.[52] Since the initial studies describing the striking developmental phenotype of p63 deficient mice, there has been debate about the true role of p63, particularly during development.[15,16] One group interpreted the phenotype as being defective in the commitment ability of the primitive ectoderm to the epithelial lineage,[15] while the other favored a necessary role for p63 in stem cell maintenance.[16,52] The finding that p63 deficiency induces cellular senescence and accelerated aging will undoubtedly contribute in part to the debate, but supports a role for maintenance of both stem and non-stem cells.

Lineage commitment. The role of p63 in commitment to the epithelial lineage has been addressed using a *p63* conditional mouse model. Using this system, p63 is normally expressed during embryogenesis and is later disrupted by expressing Cre in a tissue-specific and inducible manner[14] (Fig. 1). Consistent with excision of the DNA-binding domain (a region common to all p63 isoforms), p63 ablated mice failed to express markers of epidermal proliferation or differentiation when p63 was ablated prior to stratification (E8.5–E10.5), further demonstrating p63's essential role in the commitment of ectodermal cells to the epithelial lineage. This suggested role of p63 in lineage commitment is supported by other recent findings. Specifically in the skin, the TAp63α isoform is the molecular switch for epithelial development,[22] while in the zebrafish, absence of p63 favors repression of epithelial development.[53,54] Also, p63 has been shown as a key commitment factor necessary for prostate development and lineage commitment,[55] as well as an identity switch necessary for uterine/vaginal epithelial cell fate.[56,57] Together, these results highlight the role played by p63 in regulating cell fate decisions and commitment to the epithelial lineage. However, the location of senescence markers in the adult skin in response to p63 ablation and the striking phenotype that develops is further suggestive that p63 is necessary for maintenance of the stem cell population, although this remains to be definitively shown.

However, p63 is expressed in both stem and non-stem cell compartments of the epidermis (see Fig. 1), and it is very likely that different isoforms have distinct roles in each cell type, as is evident from the increasing number of target genes and functions that is emerging. The fact that induction of differentiation results in downregulation of p63 in both murine and human keratinocytes[19,58] and that ΔNp63α expression inhibits calcium-induced differentiation of primary keratinocytes[58] points to a key role for this protein in maintaining proliferative potential of epidermal keratinocytes. In human keratinocytes, ΔNp63α can bind and repress both $p21^{Waf1/Cip1}$ and 14-3-3σ promoters, thereby maintaining the undifferentiated proliferative state,[19] while the notch ligand jagged*1*, which is also required for keratinocyte differentiation is directly activated by p63γ.[59] Recent data also highlights that *Perp* is a direct p63-regulated gene necessary for maintenance of epithelial integrity, acting as a critical component of desmosomes.[60] Thus, many independent studies support a necessary role for p63 in regulating commitment of precursors to specific lineages, and maintaining proliferation of the committed population.

Stem cell renewal. The data pointing to a role for p63 in stem cell function is equally convincing. Clonal analysis of cells from the limbal epithelium of the cornea suggest that stem cells of this area express higher levels of the protein, and that p63 is a marker of these stem cells.[61] Recent findings also show that p63 can transactivate the *ITGA3* gene, which encodes for the integrin α3 subunit of the laminin receptor and serves to anchor the stem cell in the niche.[62] The ΔNp63α isoform of p63 also functions to activate β-catenin nuclear accumulation and signaling,[44] which in turn is responsible for directing stem cells toward the hair follicle instead of the interfollicular epidermal cell fate.[63] It is thus possible that individual p63 isoforms are involved in dictating cell fate as they exit from the stem cell niche, as p63 and its isoforms dictate cell fate in other regions. It is clear that p63 is expressed in both stem and nonstem cell populations, and given the complexity of this gene, it may have many roles that are dependant on its cellular location.

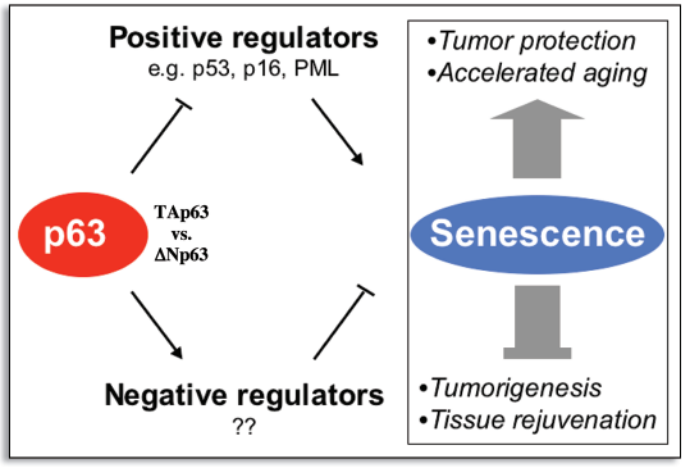

Figure 3. Proposed model for the mechanism of p63 in cellualar senescence. p63 may repress factors that normally activate the senescence program, such as p53, $p16^{INK4a}$ and PML. p63 may also activate as yet unknown negative regulators of senescence. These interactions are likely to be mediated by a balance of different p63 isoforms. The effect on senescence is then translated to effects on the processes of tumor suppression and aging, or alternatively to tumorigenesis and tissue renewal.

CLOSING REMARKS

Together, these studies highlight the many separate roles that p63 proteins have at different stages of growth and development. Prior to epidermal stratification during embryonic development, p63 facilitates commitment of the ectoderm to the epidermal lineage, possibly via effects of the TA isoforms on an uncommitted precursor cell population. However, once initiated, p63 assumes multiple roles in maintenance of proliferation of stem- and non-stem cell populations in the skin (and other stratified epithelial tissue). This is likely accompanied by differential expression of p63 isoforms and their interactions with specific targets. Also, in conditions of aberrant growth such as tumorigenesis, p63 has been shown to be intimately involved, though its exact contribution remains at the centre of some debate. The processes of senescence and apoptosis are two of the primary mechanisms activated to curtail such deregulated growth, and the accumulating evidence now highlights p63s involvement in both processes. However, our current model with regards to senescence (Fig. 3) posits p63 as a positive regulator of epithelial proliferation, mediated predominantly by the ΔN isoforms, but also by their interactions with the TA isoforms and downstream targets. This is likely through repression of positive regulators of senescence such as p53 and PML, and by promoting as yet unknown negative regulators of the process, such that in the absence of p63 expression, an overall anti-proliferative program is effected. Together, these findings have implications that merit investigation of p63 activity in specific cellular scenarios and that have broad implications for further understanding p63s role in modulating important processes such as aging, stem cell maintenance and cancer.

References

1. Campisi J. Cellular senescence as a tumor-suppressor mechanism. Trends Cell Biol 2001; 11:S27-31.
2. Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ and Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003; 113:703-16.
3. Sharpless NE and DePinho RA. Telomeres, stem cells, senescence, and cancer. J Clin Invest 2004: 113:160-8.
4. Hayflick L. The Limited in Vitro Lifetime of Human Diploid Cell Strains. Exp Cell Res 1965; 37:614-36.
5. Kim Sh SH, Kaminker P and Campisi J. Telomeres, aging and cancer: in search of a happy ending. Oncogene 2002; 21:503-11.
6. Serrano M, Lin AW, McCurrach ME, Beach D and Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 1997; 88:593-602.
7. Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM and Lowe SW. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 2002; 109:335-46.
8. Ben-Porath I and Weinberg RA. When cells get stressed: an integrative view of cellular senescence. J Clin Invest 2004; 113:8-13.
9. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 2005; 120:513-22.
10. Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dorken B, Jenuwein T and Schmitt CA. Oncogene-induced senescence as an initial barrier in lymphoma development. Nature 2005; 436:660-5.
11. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C and Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature 2005; 436:725-30.
12. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ and Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 2005; 436:720-4.
13. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D and Serrano M. Tumour biology: senescence in premalignant tumours. Nature 2005; 436:642.
14. Keyes WM, Wu Y, Vogel H, Guo X, Lowe SW and Mills AA. p63 deficiency activates a program of cellular senescence and leads to accelerated aging. Genes Dev 2005; 19:1986-1999.
15. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR and Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999; 398:708-13.
16. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, Tabin C, Sharpe A, Caput D, Crum C and McKeon F. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999; 398:714-8.
17. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dotsch V, Andrews NC, Caput D and McKeon F. p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol Cell 1998; 2:305-16.
18. Blandino G and Dobbelstein M. p73 and p63: why do we still need them? Cell Cycle 2004; 3:886-94.
19. Westfall MD, Mays DJ, Sniezek JC and Pietenpol JA. The Delta Np63 alpha phosphoprotein binds the p21 and 14-3-3 sigma promoters in vivo and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutations. Mol Cell Biol 2003; 23:2264-76.
20. Gressner O, Schilling T, Lorenz K, Schulze Schleithoff E, Koch A, Schulze-Bergkamen H, Maria Lena A, Candi E, Terrinoni A, Valeria Catani M, Oren M, Melino G, Krammer PH, Stremmel W and Muller M. TAp63alpha induces apoptosis by activating signaling via death receptors and mitochondria. Embo J 2005; 24:2458-71.
21. Petitjean A, Cavard C, Shi H, Tribollet V, Hainaut P and Caron de Fromentel C. The expression of TA and DeltaNp63 are regulated by different mechanisms in liver cells. Oncogene 2005; 24:512-9.
22. Koster MI, Kim S, Mills AA, DeMayo FJ and Roop DR. p63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev 2004; 18:126-31.
23. Koster MI and Roop DR. Transgenic mouse models provide new insights into the role of p63 in epidermal development. Cell Cycle 2004; 3:411-3.
24. Dimri GP. What has senescence got to do with cancer? Cancer Cell 2005; 7:505-12.
25. Ferbeyre G, de Stanchina E, Lin AW, Querido E, McCurrach ME, Hannon GJ and Lowe SW. Oncogenic ras and p53 cooperate to induce cellular senescence. Mol Cell Biol 2002; 22:3497-508.
26. Itahana K, Dimri G and Campisi J. Regulation of cellular senescence by p53. Eur J Biochem 2001; 268:2784-91.
27. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A and Donehower LA. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002; 415:45-53.
28. Maier B, Gluba W, Bernier B, Turner T, Mohammad K, Guise T, Sutherland A, Thorner M and Scrable H. Modulation of mammalian life span by the short isoform of p53. Genes Dev 2004; 18:306-19.
29. Ratovitski EA, Patturajan M, Hibi K, Trink B, Yamaguchi K and Sidransky D. p53 associates with and targets Delta Np63 into a protein degradation pathway. Proc Natl Acad Sci USA 2001; 98:1817-22.

30. Mills AA. p53: link to the past, bridge to the future. Genes Dev 2005; 19:2091-9.
31. Tatar M, Bartke A and Antebi A. The endocrine regulation of aging by insulin-like signals. Science 2003; 299:1346-51.
32. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP and Kuro-o M. Suppression of aging in mice by the hormone Klotho. Science 2005; 309:1829-33.
33. Nahor I, Abramovitch S, Engeland K and Werner H. The p53-family members p63 and p73 inhibit insulin-like growth factor-I receptor gene expression in colon cancer cells. Growth Horm IGF Res 2005.
34. Barbieri CE, Perez CA, Johnson KN, Ely KA, Billheimer D and Pietenpol JA. IGFBP-3 is a direct target of transcriptional regulation by DeltaNp63alpha in squamous epithelium. Cancer Res 2005; 65:2314-20.
35. Salomoni P and Pandolfi PP. The role of PML in tumor suppression. Cell 2002; 108:165-70.
36. Le XF, Vallian S, Mu ZM, Hung MC and Chang KS. Recombinant PML adenovirus suppresses growth and tumorigenicity of human breast cancer cells by inducing G1 cell cycle arrest and apoptosis. Oncogene 1998; 16:1839-49.
37. Ferbeyre G, de Stanchina E, Querido E, Baptiste N, Prives C and Lowe SW. PML is induced by oncogenic ras and promotes premature senescence. Genes Dev 2000; 14:2015-27.
38. Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP and Pelicci PG. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature 2000; 406:207-10.
39. Bernardi R, Scaglioni PP, Bergmann S, Horn HF, Vousden KH and Pandolfi PP. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. Nat Cell Biol 2004; 6:665-72.
40. Bernassola F, Salomoni P, Oberst A, Di Como CJ, Pagano M, Melino G and Pandolfi PP. Ubiquitin-dependent degradation of p73 is inhibited by PML. J Exp Med 2004; 199:1545-57.
41. Bernassola F, Oberst A, Melino G and Pandolfi PP. The promyelocytic leukaemia protein tumour suppressor functions as a transcriptional regulator of p63. Oncogene 2005; 24:6982-6.
42. de Stanchina E, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G and Lowe S. W. PML is a direct p53 target that modulates p53 effector functions. Mol Cell 2004; 13:523-35.
43. Wu G, Nomoto S, Hoque MO, Dracheva T, Osada M, Lee CC, Dong SM, Guo Z, Benoit N, Cohen Y, Rechthand P, Califano J, Moon CS, Ratovitski E, Jen J, Sidransky D and Trink B. DeltaNp63alpha and TAp63alpha regulate transcription of genes with distinct biological functions in cancer and development. Cancer Res 2003; 63:2351-7.
44. Patturajan M, Nomoto S, Sommer M, Fomenkov A, Hibi K, Zangen R, Poliak N, Califano J, Trink B, Ratovitski E and Sidransky D. DeltaNp63 induces beta-catenin nuclear accumulation and signaling. Cancer Cell 2002; 1:369-79.
45. Zangen R, Ratovitski E and Sidransky D. Delta-Np63-alpha levels correlate with clinical response to cisplatin. Cell Cycle 2005; 4.
46. Wu G, Osada M, Guo Z, Fomenkov A, Begum S, Zhao M, Upadhyay S, Xing M, Wu F, Moon C, Westra WH, Koch WM, Mantovani R, Califano JA, Ratovitski E, Sidransky D and Trink B. DeltaNp63alpha up-regulates the Hsp70 gene in human cancer. Cancer Res 2005; 65:758-66.
47. Beretta C, Chiarelli A, Testoni B, Mantovani R, and Guerrini L. Regulation of the cyclin-dependent kinase inhibitor p57Kip2 expression by p63. Cell Cycle 2005; 4:1625-31.
48. Parrinello S, Coppe JP, Krtolica A and Campisi J. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J Cell Sci 2005; 118:485-96.
49. Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ and Clarke MF. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 2003; 423:302-5.
50. Park IK, Morrison SJ and Clarke MF. Bmi1, stem cells, and senescence regulation. J Clin Invest 2004; 113:175-9.
51. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L and Sharpless NE. Ink4a/Arf expression is a biomarker of aging. J Clin Invest 2004; 114:1299-307.
52. McKeon F. p63 and the epithelial stem cell: more than status quo? Genes Dev 2004; 18:465-9.
53. Lee H and Kimelman D. A dominant-negative form of p63 is required for epidermal proliferation in zebrafish. Dev Cell 2002; 2:607-16.
54. Bakkers J, Hild M, Kramer C, Furutani-Seiki M and Hammerschmidt M. Zebrafish DeltaNp63 is a direct target of Bmp signaling and encodes a transcriptional repressor blocking neural specification in the ventral ectoderm. Dev Cell 2002; 2:617-27.
55. Signoretti S, Pires MM, Lindauer M, Horner JW, Grisanzio C, Dhar S, Majumder P, McKeon F, Kantoff PW, Sellers WR and Loda M. p63 regulates commitment to the prostate cell lineage. Proc Natl Acad Sci USA 2005; 102:11355-60.
56. Kurita T, Mills AA and Cunha GR. Roles of p63 in the diethylstilbestrol-induced cervicovaginal adenosis. Development 2004; 131:1639-49.
57. Kurita T and Cunha GR. Roles of p63 in differentiation of Mullerian duct epithelial cells. Ann N Y Acad Sci 2001; 948:9-12.
58. King KE, Ponnamperuma RM, Yamashita T, Tokino T, Lee LA, Young MF and Weinberg WC. deltaNp63alpha functions as both a positive and a negative transcriptional regulator and blocks in vitro differentiation of murine keratinocytes. Oncogene 2003; 22:3635-44.
59. Sasaki Y, Ishida S, Morimoto I, Yamashita T, Kojima T, Kihara C, Tanaka T, Imai K, Nakamura Y and Tokino T. The p53 family member genes are involved in the Notch signal pathway. J Biol Chem 2002; 277:719-24.
60. Ihrie RA, Marques MR, Nguyen BT, Horner JS, Papazoglu C, Bronson RT, Mills AA and Attardi LD. Perp is a p63-regulated gene essential for epithelial integrity. Cell 2005; 120:843-56.
61. Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, Ponzin D, McKeon F and De Luca M. p63 identifies keratinocyte stem cells. Proc Natl Acad Sci USA 2001; 98:3156-61.
62. Kurata S, Okuyama T, Osada M, Watanabe T, Tomimori Y, Sato S, Iwai A, Tsuji T, Ikawa Y and Katoh I. p51/p63 Controls subunit alpha3 of the major epidermis integrin anchoring the stem cells to the niche. J Biol Chem 2004; 279:50069-77.
63. Huelsken J, Vogel R, Erdmann B, Cotsarelis G and Birchmeier W. beta-Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 2001; 105:533-45.
64. Senoo M, Matsumura Y and Habu S. TAp63gamma (p51A) and dNp63alpha (p73L) two major isoforms of the p63 gene, exert opposite effects on the vascular endothelial growth factor (VEGF) gene expression. Oncogene 2002; 21:2455-65.
65. Nishi H, Senoo M, Nishi KH, Murphy B, Rikiyama T, Matsumura Y, Habu S and Johnson AC. p53 Homologue p63 represses epidermal growth factor receptor expression. J Biol Chem 2001; 276:41717-24.