[Cell Cycle 6:3, 305-311, 1 February 2007]; ©2007 Landes Bioscience

Spotlight on p63

p63, Cellular Senescence and Tumor Development

Xuecui Guo

Alea A. Mills*

Cold Spring Harbor Labratory; 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

Original manuscript submitted: 12/01/06
Manuscript accepted: 01/03/07

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=3794

KEY WORDS

p63, senescence, tumorigenesis, oncogene, tumor suppressor, animal model

ACKNOWLEDGEMENTS

We would like to thank Dr. Bill Keyes and Dr. Peter Adams for their helpful discussion and critical reading of this review.

ABSTRACT

Deficiency of p63, a p53-related protein, causes severe defects in epithelial morphogenesis. Studies of p63-compromised mouse models reveal that p63 deficiency induces cellular senescence both in cultured cells and in vivo, through regulation $p19^{Arf}$/p53 and $p16^{Ink4a}$/Rb pathways. An extensive tumor study of p63-compromised mice demonstrated that p63 deficiency does not predispose to, but rather protects from, tumor development. These findings further implicate p63 as a negative regulator of the tumor suppressive mechanism of cellular senescence.

INTRODUCTION

Senescence is an essentially irreversible form of cell cycle arrest. The term "replicative senescence" was first coined by Hayflick and colleagues to describe the limited replication capacity of human diploid cells in culture.[1] Subsequent studies revealed that senescence can be triggered in response to telomere attrition.[2] Stress stimuli such as DNA damage and oncogenic signals can induce a similar phenomenon referred to as premature senescence or oncogene-induced senescence.[3] The term "cellular senescence" is commonly used to encompass both replicative and premature senescence. Senescent cells remain metabolically active, undergo morphological changes, become flattened and vacuolar, and exhibit senescence-associated β-galactosidase (SA-β-gal) activity at acidic pH.[4] The tumor suppressors p53, $p16^{Ink4a}$ and promyelocytic leukemia protein (PML) are up-regulated in senescent cells.[3,5-7]

Recent work from our laboratory has determined that deficiency of p63 induces senescence.[8] p63 belongs to the p53 family of proteins that in addition to p53, also includes p73. p63 encodes at least six different proteins due to multiple promoter usage and alternative splicing.[9] Isoforms with an N-terminal transactivation domain homologous to that of p53 are referred to as the TA isoforms, whereas isoforms lacking this domain are referred to as the ΔN isoforms.[9] There is significant structural homology between p63 and p53 in the transactivation-, DNA binding- and oligomerization domains, suggesting that p63 shares functional similarities with p53.[9] Indeed, p63 binds to p53-responsive elements and transactivates a number of p53 target genes such as p21, Bax, Perp.[9-12] Like p53, p63 can induce apoptosis.[9,10,13,14] However, in contrast to the majority of p53 deficient mice that develop normally and are viable,[15] p63 deficient mice have severe developmental defects affecting the skin and limbs that cause them to die shortly after birth.[16,17] In addition, whereas p53 heterozygous mutant mice are highly tumor-prone,[15,18] mice heterozygous for inactivated p63 alleles are either tumor-resistant[19] or develop very few tumors.[20] Moreover, p63 heterozygotes have a shortened lifespan and exhibit features of accelerated aging.[8] To bypass the embryonic lethality imposed by complete absence of p63 throughout the embryo, a novel p63 conditional mouse model was developed, providing a system for studying the role of p63 at later stages.[21] Indeed, this p63 conditional model implicates p63 as a regulator of cellular senescence, thereby providing a link between cellular senescence and aging in vivo.[8] The finding that p63 deficiency induces cellular senescence suggests a potential mechanism for the decreased tumor incidence in p63-compromised mice. The validity of this hypothesis however, awaits further investigation.

p63 IS A MEDIATOR OF CELLULAR SENESCENCE AND AGING

The decreased life span and the striking age-related decline observed in p63 heterozygous mutant mice are consistent with the ability of p63 deficiency to induce senescence.[8]

In support of the hypothesis that p63 modulates organismal aging, high levels of endogenous SA-β-gal activity, a marker of senescent cells, was detected in p63 deficient embryos. This augmented SA-β-gal activity was also observed when p63 was ablated somatically. Indeed, Cre-mediated p63 disruption specifically in proliferative cells of stratified epithelia such as the skin during midgestation induced cellular senescence. Immunofluorescence analysis indicated that the expression of additional senescence markers p16 and PML increased significantly in embryos rendered p63 deficient either in the germline or in somatic tissues. Markers of cellular senescence were also induced when p63 was ablated in proliferating keratinocytes of adult mice at 8 months of age. Interestingly, induced p63 deficiency caused features of accelerated aging, a phenotype that is somewhat similar to that caused by elevated p53 activity.[22,23] These studies suggest a causative link between cellular senescence and aging in vivo. Senescence induced in response to Cre-mediated p63 ablation was also confirmed in primary keratinocytes in culture. Thus, p63 deficiency can evoke cellular senescence both in vivo and in cultured cells.

A separate study also implicates p63 as a regulator of aging. The Sidransky group reported that transgenic mice that express ΔNp63α under the control of keratin14 (K14) promoter displayed an aging phenotype in vivo.[24] The authors observed an inverse correlation between expression of the K14-ΔNp63α transgene and expression of SIRT1, a histone deacetylase that can inhibit PML/p53-mediated cellular senescence.[25] It is surprising that the seemingly opposing approaches of loss- and gain of p63 function both led to the conclusion that p63 is a modulator of the aging process. It would be interesting to understand the mechanism whereby exogenous expression of a single p63 isoform causes a similar effect as loss of all the p63 isoforms.

p63-REGULATED MOLECULAR PATHWAYS IN THE SENESCENT PROCESS

Cellular senescence can be induced by a variety of stimuli such as DNA damage, shortening of telomeres, over-expression of oncogenes, and chromatin modification.[26] Irrespective of the diverse stimuli that converge to regulate cellular senescence, this program mainly involves $p19^{Arf}$/p53 and $p16^{Ink4a}$/Rb, the two major tumor suppressor pathways. These pathways play a key role during the induction and the maintenance of senescence.[26,27] Molecules that regulate these pathways may also regulate the senescent pathway.

p63 and the Arf/p53 pathway. p53 is a key regulator of the senescence response to DNA damage, oncogenic signals and many other stimuli, and its downstream target gene p21 has been proposed to encode a major regulator of cell cycle arrest during the senescent process.[26,27] The functional interaction between p53 and p63 has been well documented. For example, p63 interferes with p53's transcriptional activity.[9] p63 can inhibit the expression of a number of p53 target genes, and thus p63 may counteract p53 activity, at least partly by competing for p53 consensus DNA binding sites.[9] On the other hand, p53 interacts directly with ΔNp63α to mediate its proteasomal degradation.[28] Induction of apoptosis in primary keratinocytes in response to ultraviolet (UV) light simultaneously stabilizes p53 and decreases ΔNp63α levels, the predominant isoform in proliferating epidermal keratinocytes.[29] Forced expression of ΔNp63α in the epidermis can inhibit UV-induced apoptosis.[29] Given these close physical and functional interactions between p53 and p63, it is tempting to speculate that both senescence and aging induced by enhanced p53 activity involves a compromise in p63 activity. Indeed, p63 deficiency evokes cellular senescence in vivo, and this phenotype is retained in a p53 deficient background, suggesting that p63 functions either downstream or in parallel with p53.[8,30] In cultured primary keratinocytes, RNAi-mediated knockdown of p53 bypasses senescence induced by p63 ablation, indicating that p63 functions upstream of p53 during the senescent process. It will be interesting to determine whether these findings reflect a negative feedback loop between p63 and p53.

Recently, the insulin and insulin-like growth factor 1 (IGF-1)-signaling pathway has been linked to organismal aging. Mutations that inhibit this signaling pathway significantly extend the life span of many species.[31,32] This evolutionarily conserved pathway is induced by the N-terminally truncated p53 isoform p44. The enhanced IGF-1 signaling sustains p21 expression through the Ras-MAP kinase pathway, thus contributing to the decreased proliferation, increased cellular senescence and accelerated aging phenotypes.[23] Interestingly, p63 inhibits the expression of both the IGF-1 receptor and the IGF binding protein IGFBP3,[33,34] two important components of the insulin/IGF-1 axis. Thus, an enhanced insulin/IGF-1 signaling caused by loss of p63 might mediate senescence. Furthermore, previous work demonstrates that TAp63 up-regulates[9,35-38] while ΔNp63α represses,[39] transcription of p21. Future studies should determine whether p21 is induced in senescent tissues of p63 deficient mice.

Another mechanism by which p63 might regulate cellular senescence is through regulating the activity of Arf ($p14^{Arf}$ in human, $p19^{Arf}$ in mouse), an upstream negative regulator of Mdm2. Mdm2 is a negative regulator of p53, therefore $p19^{Arf}$ facilitates p53 function. It was shown that Arf interacts with p63 and inhibits its transcriptional activity, indicating that these two proteins are physically and functionally connected.[40] However, the authors also observed that overexpression of TAp63 isoforms (but not ΔNp63 isoforms) can relocalize Arf from nucleoli to nucleoplasm.[40] Thus, TAp63 may interfere with the normal function of this nucleolar protein, suggesting that p63 has a negative effect on Arf and that reduced p63 expression enhances Arf/p53 function.

p63 and the p16/Rb pathway. The second critical mediator of senescence induction and maintenance is the $p16^{Ink4a}$/Rb pathway.[26,27] The requirement of $p16^{Ink4a}$/Rb pathway for the formation of senescence-associated heterochromatic foci (SAHF), which may keep pro-proliferative genes in a repressed state, provides a molecular mechanism underlying $p16^{Ink4a}$/Rb regulation of senescence.[7] In our study,[8] p16 was dramatically upregulated in p63-ablated tissues in vivo, suggesting that p63 functions upstream of the $p16^{Ink4a}$/Rb pathway in the senescent process. Interestingly, crosstalk between the $p19^{Arf}$/p53 and $p16^{Ink4a}$/Rb pathways has been suggested through p21. This involves the hypophosphorylation and activation of Rb through p21-mediated inhibition of Cyclin E-Cdk2.[26,41] As mentioned above, the TAp63- and ΔNp63 isoforms regulate p21 expression in a positive and negative manner, respectively. Thus, it is likely that p63 regulates the $p16^{Ink4a}$/Rb senescence pathway through regulation of $p16^{Ink4a}$ and p21 expression (Fig. 1).

p63 and PML. PML was originally discovered based on its role in the pathogenesis of acute promyelocytic leukemia, and later was found to play an essential role in cellular senescence and tumor suppression.[42-44] Keyes et al. found an increase in PML expression in p63-deficient cells, and that both the size and number of PML nuclear bodies was enhanced by p63 loss.[8] Functionally, knockdown of PML in primary keratinocytes by PML-specific short hairpins abolished the induction of cellular senescence, implicating PML in p63-deficiency induced cellular senescence. These data also

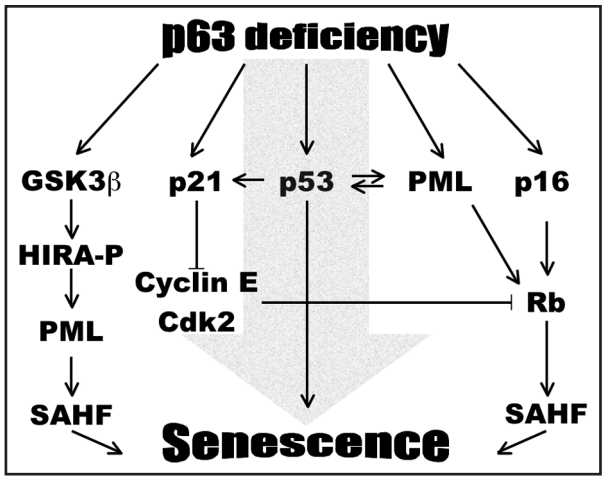

Figure 1. p63 deficiency induces cellular senescence. p63 deficiency induces the expression $p16^{Ink4a}$ and PML.[8] The enhanced $p16^{Ink4a}$ and PML promote SAHF formation and senescence. Given the finding that ΔNp63α inhibits GSK3β activity,[55] p63 deficiency potentially enhances GSK3β activity. The increased activity of GSK3β can regulate p53 and the chromatin regulator HIRA, thus regulating senescence. On the other hand, p63 can inhibit the transactivation of cell cycle inhibitors p21.[39] Thus, p63 deficiency can cause upregulation of p21. p21 has been proposed to be a key effector of p53-mediated senescence by inhibiting Cyclin E-Cdk2.[26]

suggest that p63 is an upstream suppressor of PML. The molecular mechanism for p63-mediated regulation of PML remains unknown. In contrast to these studies that place PML downstream of p63, Bernassola et al[11] reported that PML interacts with p63, potentially through p63's DNA binding domain, and that over-expression of PML increases p63 accumulation at least partially by inhibiting ubiquitination of p63. In addition, PML regulates TAp63 transcriptional activity, suggesting a regulatory feedback loop between p63 and PML. PML interacts with and enhances p53's transactivation activity[42] and p53 is also a direct transcriptional activator of the PML promoter.[45] Based on the high sequence homology between p53 and p63, it is very likely that p63 can directly bind to PML and also inhibit expression of PML at the transcript level. Given the fact that there are at least six isoforms of p63, and each has potentially different, even opposing functions, further studies will be required to elucidate the reciprocal regulation between p63 and PML.

PML can be upregulated by oncogenic Ras. It directly interacts with the DNA binding domain of p53 and regulates the acetylation of p53 through acetyltransferase CBP/p300 in the PML-NBs, thereby inducing cellular senescence.[42,43] PML was also shown to enhance the stability of p53 by inhibiting Mdm2 function.[46] Moreover, PML can interact with Rb, thereby promoting Rb's transcriptional repression activity.[47] Given the role of PML in tumor suppression[44] and the reciprocal regulation between PML and p63, it is very likely that p63 interferes with oncogene-induced cellular senescence by regulating PML. Notably, a recent study implies a critical role of PML bodies in SAHF formation.[48] Given the report that p63 colocalizes with PML in PML bodies,[11] it would be interesting to determine the role of p63 in SAHF formation during senescence.

THE PRO-PROLIFERATION OR PRO-SURVIVAL PROPERTY OF p63

Consistent with the induction of senescence that is mediated by deficiency of p63, an extensive body of evidence demonstrates p63's striking pro-proliferative or pro-survival function. These studies suggest a number of potential pathways that p63 regulates to modulate these processes. It was shown that overexpression of ΔNp63α in Rat 1a cells enhances growth in soft agar and increases tumor volume in nude mice.[49] A DNA microarray gene expression profile in Saos-2 cells showed that ΔNp63, but not TAp63, up-regulates Hsp70, a gene that is actively involved in inhibition of apoptosis and enhancement of survival.[50] Furthermore, Wu et al. found that ΔNp63α transactivates Hsp70 expression by direct interaction with the CCAAT binding factor.[51] On the other hand, ΔNp63α can also bind to the regulatory regions of genes encoding cell cycle inhibitors p21 and 14-3-3σ to repress their transcription.[39] The expression of ΔNp63α is induced by epidermal growth factor activation through phosphoinositide 3-kinase (PI3K) pathway,[52] which is a potent pro-survival and pro-proliferation factor in mammalian cells.[53] The report that ΔNp63α is a downstream target of PI3K implies that ΔNp63α is an important player in cell proliferation and survival. Recently, over-expression of ΔNp63α in squamous cell carcinoma cells was shown to promote cell survival in a p53-independent manner by interfering TAp73β-regulated transactivation of the proapoptotic genes Puma and Noxa.[54]

ΔNp63α has also been linked to glycogen synthase kinase 3β (GSK3β) and the β-catenin pathway.[55] ΔNp63 binds to GSK3β and the regulatory subunit of protein phosphotase PP2A, leading to the inhibition of GSK3β reactivation, and subsequently decreased phosphorylation and nuclear accumulation of β-catenin.[55] Increased nuclear β-catenin has been heavily implicated in human cancers. The ability of p63 to mediate β-catenin signaling provides a molecular basis for the oncogenic function of p63. p63's regulation of GSK3β activity may provide an important clue as to a mechanism whereby p63 deficiency causes cellular senescence. Given the evidence that GSK3β accumulates in the nucleus of senescent human fibroblasts,[56] and the fact that p53 activity can be regulated through serine phosphorylation by GSK3β,[57] it is possible that p63 loss increases GSK3β activity and subsequently enhances p53 activity. Most interestingly, recent work from the Adams group showed that GSK3β interacts with and phosphorylates chromatin regulator HIRA, which leads to the translocation of the phosphorylated HIRA to PML bodies and an increase in SAHF formation (Personal communication, Peter Adams). It would be interesting to determine whether p63 deficiency enhances GSK3β activity, thus increasing HIRA phosphorylation, induction of SAHF formation and senescence (Fig. 1).

The tumor-related pro-proliferative function of ΔNp63 has also been reported for TAp63 isoforms. Upregulation of TAp63 isoforms at the transcript level was recently reported in human squamous cell carcinomas.[58] Koster et al. found that targeted overexpression of TAp63α in the basal layer of the epidermis caused widespread hyperproliferation and severe hyperplasia at the cost of epidermal differentiation.[59] A report from the same laboratory recently demonstrated that TAp63α expression is frequently enhanced in the majority of human well-differentiated head and neck squamous cell carcinomas (HNSCC).[60] Furthermore, over-expression of TAp63α in the epidermis promoted chemical tumorigenesis and malignant progression. A correlation between TAp63 expression and epithelial-mesenchymal transition (EMT) and EMT regulators Twist and N-cadherin was also shown. However, p63's involvement in EMT has not been reported.

Recently, Sbisa et al. reported that both TAp63α and ΔNp63α promote cellular proliferation by direct transactivation of adenosine deaminase (ADA) and that TAp63α is the p63 isoform that has the most profound effect in this process.[61] These findings suggest that p63 can promote tumor development not only by counteracting p53 activity, but also by directly controlling the expression of proliferative genes. Interestingly, enhanced expression of TAp63 isoforms was not

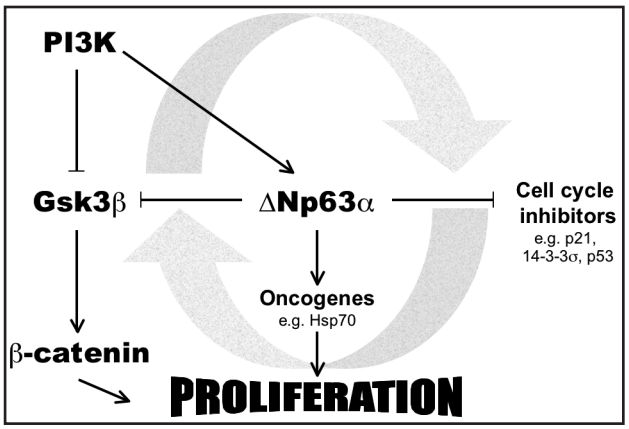

Figure 2. ΔNp63α promotes cellular proliferation. ΔNp63α can inhibit GSK3β activity through interacting with and inhibiting the protein phosphotase PP2A. The decreased GSK3β activity leads to β-catenin nuclear accumulation, thus its pro-proliferative function.[55] ΔNp63α is induced by EGF through the PI3K pathway.[52] ΔNp63α can promote cellular proliferation by inducing oncogenic proteins such as Hsp70.[51] On the other hand, ΔNp63α can inhibit the activity of p53[9] and the transactivation of cell cycle inhibitors such as p21 and14-3-3σ,[39] thus enhancing cellular proliferation.

only seen in epithelial tissues, but was also observed in non-epithelial tumors such as lymphomas.[62-66] The role of enhanced TAp63 activity in lymphomagenesis remains to be investigated.

These studies suggest a potential oncogenic role for p63 when it is over-expressed (Fig. 2). The consequences of loss of such a potent pro-proliferation or pro-survival gene in normal or cancerous cells could be either apoptosis or cellular senescence, depending on the specific cellular context. Indeed, senescence was induced in primary keratinocytes both in cultured cells and in vivo when p63 was ablated.[8] Consistent with senescence induction induced by p63 loss, Sbisa and colleagues observed that knockdown of p63 using siRNAs reduced ADA expression concomitant with decreased cellular proliferation.[61] In human squamous cell carcinoma cells, apoptosis is the main consequence of shRNA-mediated knockdown of p63.[67] Although the molecular mechanism underlying a cell's choice between apoptosis and senescence is not thoroughly understood, regulation of these tumor suppressive responses is likely to have important implications for anticancer therapies.

p63 AND TUMOR DEVELOPMENT

Although p63 shares significant sequence similarity and protein domain conservation with p53, there is strong evidence supporting an oncogenic role for p63 in tumorigenesis, whereas p53 is a classic tumor suppressor. In contrast to p53 that is highly mutated in the majority of human tumors,[68] p63 is rarely mutated.[69] A thorough analysis of p63 expression in primary esophageal tumors conducted by Cui et al[58] demonstrated that p63 is often upregulated at the transcript level in human tumors with a predominance of enhanced ΔNp63α expression at the protein level. The p63 locus is frequently amplified in squamous cell carcinoma of the lung and head and neck.[49,70] Even though p63 expression appears to be most pronounced in epithelial tissues, enhanced expression of p63 is also found in human hematopoietic malignances such as diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL).

p53 heterozygous- or homozygous mutant mice are highly tumor-prone.[15,18] The homology between p53 and p63 originally suggested a tumor suppressive function for p63. To assess the impact of p63 deficiency on tumorigenesis in an intact organism, we performed an extensive spontaneous and chemical-induced tumor study on a large cohort of p63 heterozygous mutant mice.[19] We found that $p63^{+/-}$ mice are not prone to spontaneous tumor development, but often develop non-malignant pathology. Moreover, $p53^{+/-}$ and $p63^{+/-}$ compound mice had fewer tumors than $p53^{+/-}$ mice. Surprisingly, p63-comprised mice are not susceptible to chemically-induced tumorigenesis, even though p63 plays an essential role in the development and homeostasis of the epidemis. These observations indicate that p63 does not function as a tumor suppressor in a manner similar to p53, rather, that decreased p63 expression could be tumor protective.[19,71] Using the same p63 deficient model, Perez-Losada et al. demonstrated that p63 did not show tumor suppressive function in irradiation-induced lymphomagenesis.[72] Consistent with the Keyes study,[19] this report demonstrated that combined p53 and p63 deficiency did not enhance irradiation-induced lymphomagenesis. These findings are consistent with reports in the literature demonstrating that p63 is upregulated in human tumors, strongly supporting an oncogenic, rather than a tumor suppressive, role for p63.

An independent study of spontaneous tumors in $p63^{+/-}$ mice using a different p63 mouse model reported a tumor predisposition, suggesting a tumor suppressor function for p63.[20] The different conclusions drawn from the Flores and the Keyes studies could be due to the distinct p63 alleles that were generated in the two different p63 mouse models.[16,17,19,20] Notably however, there is some consistency in the tumor incidence from the two different p63 models.[19,20] Both p63 deficient models displayed shortened life span, features of aging and a high incidence of premalignant hyperplasia. When compared to p53 heterozygous mutant mice, both studies observed a decreased tumor incidence of lymphomas and sarcomas in $p63^{+/-}$ and $p63^{+/-}$;$p53^{+/-}$ compound mutant mice. These observations suggest that at least in these types of tumors, haploid levels of p63 does not promote tumor development, but rather is potentially protective.

Senescence has long been proposed to interfere with tumor development.[3] However, only recently has the senescence program been shown to prevent tumor progression in premalignant lesions in vivo.[73-77] With regards to the findings that p63 deficiency causes cellular senescence, it is intriguing to speculate that loss of p63 decreases tumor incidence by activating the senescence program. Given the high incidence of hyperplasia in p63 compromised mice, it would be interesting to determine whether senescence induction occurs in premalignant lesions such as in lung adenomas and squamous cell hyperplasia. The ability of cellular senescence to decrease tumor incidence in p63 compromised mice and the isoform-specific effects of p63 on induction and maintenance of senescence remain to be elucidated.

The role of p63 in tumorigenesis so far manifests high complexity. In addition to the evidence supporting an oncogenic role for p63, a tumor suppressive function of p63 has also been proposed. Indeed, both p53 and p63 can be induced following DNA damage. Functionally, p63 can exhibit p53-like activity in both binding and transactivation of certain p53 target genes, such as Bax, Perp and Noxa, Pig3 and p21—genes encoding proteins involved in the execution of apoptosis or cell cycle arrest. Over-expression of TAp63α or TA63γ was shown to be able to induce apoptosis in certain types of cells.[9,10,14] p63, together with p73, is required for p53 to bind and activate certain apoptosis-related genes, and p53 mutants derived from human cancers interact with p63α and reduce p63α-mediated growth inhibition in Saos-2 cells.[78] There is also evidence showing that loss of p63 occurs in certain types of human cancers. Whether

this decreased expression is a consequence of a switch to a mesenchymal cell type (e.g. during the process of EMT that frequently occurs in tumors derived from epithelial tissues), rather than from p63 loss, per se, awaits further investigation. These studies suggest that p63 is involved in apoptosis and cell cycle arrest, and that some isoforms may function as tumor suppressors. Thus, whether p63 functions as tumor suppressive gene or oncogene in tumor development is still controversial.[71]

AN ISOFORM- AND CELLULAR CONTEXT-SPECIFIC ISSUE

TAp63 and ΔNp63 are not only likely to be regulated differentially in response to certain stimuli,[79,80] but these different isoform classes probably also regulate sets of genes that have completely distinct biological functions.[50] It is very likely that there are cell-type specific functions for different isoforms of p63. For example, TAp63α has been shown to induce apoptosis in Hep3B and Saos2 cells through affecting both the intrinsic and extrinsic apoptosis pathways.[10] However, this effect of TAp63α was not observed in BHK cells.[9] Although p63 was shown to be required for p53 to induce apoptosis in E1A-expressing mouse embryonic fibroblasts (mefs),[13] loss of p63 in primary thymocytes does not interfere with either p53-dependent or p53-independent apoptosis.[81] Also, as specifically demonstrated by Jacobs et al,[14] developing neurons express only full length TAp63 isoforms, and TAp63γ promotes apoptosis during neural development, consistent with the observation by Flores and colleagues that loss of p63 confers partial resistance to irradiation-induced apoptosis in the developing nervous system.[13] In murine keratinocytes, where ΔNp63α is the dominant isoform, ablation of p63 induces cellular senescence.[8] In contrast, shRNA-mediated inhibition of p63 induces apoptosis in HNSCC cell lines.[54] Although over-expression of ΔNp63α promotes survival in HNSCC,[54] an apoptosis-inducing activity of ΔNp63α was demonstrated in non-small cell lung carcinoma cell line H1299.[38,82] Another example for cellular context issue is TAp63α: it promotes proliferation in mouse epidermis,[60] while it induces apoptosis in Hep3B cells.[10] We found that exogenous expression of either isoform of p63 did not induce apoptosis in wild type mefs or immortalized NIH 3T3 cells (Guo and Mills, unpublished data). Thus, the tissue specific consequence of p63 isoform expression is evident.

The interplay among the p53 family members and among the p63 isoforms further complicates the net effect of p63 in specific cellular circumstances. Interactions have been found between p53 family members.[9,54,78] For example, p53 not only negatively regulates p63 expression,[83,84] but also interacts with and targets p63 for proteasomal degradation.[28] On the other hand, p63 interacts with the p53 response elements and interferes with p53's transactivation activity.[9] Recently, ΔNp63α was found to promote tumor cell survival by inhibiting TAp73β-dependent transactivation of proapoptotic genes in a p53-independent manner.[54] The interplay between p63 isoforms was also demonstrated. As shown by Yang et al., ΔNp63 not only interferes with p53, but also with TAp63 isoforms to inhibit their transactivation function.[9] On the other hand, TAp63 isoforms can regulate the expression of ΔNp63 isoforms.[67,85] Two recent studies reported the presence of p63 binding sites in the p63 gene itself,[83,84] suggesting that ΔNp63 is transcriptionally regulated by TAp63. Recently, a keratinocyte-specific enhancer element was found in the fourth intron of the p63 gene.[86] This enhancer requires p63 but not p53 for appropriate regulation of p63, thus creating an autoregulatory loop. Interestingly, ΔNp63α and ΔNp63γ exert opposing effects via this cis acting control element, with ΔNp63γ enhancing and ΔNp63α inhibiting the expression of endogenous p63.[86]

Thus, different isoforms of p63 can be involved in different biological events; a given isoform can function differently in a specific cell type and in a specific cellular context. The net effects of p63 are probably determined by dominant expression of a specific isoform and the balance between the different isoforms of p63 and amongst additional members of the p53 family as well. The precise function of each p63 isoform in primary wild-type cells including epithelial cells and non-epithelial cells and in tumorigenesis needs to be more fully characterized using genetic approaches. The p63 deficient mouse models generated to date presumably interfere with the expression of all the isoforms,[16,17,21] so that it is not possible to dissect the specific functions for each isoform using these models. Thus, isoform-specific knockout or conditional models will certainly be crucial for addressing this issue in an in vivo setting. In order to make isoform-specific knockout models, exons specific for the TAp63 isoforms can be deleted, generating a TAp63 deficient mouse model. Similarly, an exon that is specific for ΔNp63 isoforms can be deleted, generating the ΔNp63 deficient mouse model. With the generation of isoforms-specific models, the role of TA- or ΔNp63 in the tumor development and aging will certainly be clarified in the context of the whole organism.

In summary, p63 regulates a number of pathways to form a complex molecular network, and the number of isoforms adds even further complexity to this system. p63 could serve as an oncogene during tumorigenesis, either by facilitating the expression of pro-proliferative genes such as Hsp70 or by the inhibition of expression of proliferation regulatory genes such as p21 and GSK3β kinase (Fig. 2). On the other hand, p63 deficiency can induce cell cycle arrest and/or senescence by regulating p16, PML, p21 and GSK3β. The senescence program activated by loss of oncogenic p63 is a potential barrier for tumor development that may be able to be exploited to design more effective anticancer therapies.

References

1. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 1965; 37:614-36.
2. Chiu CP, Harley CB. Replicative senescence and cell immortality: The role of telomeres and telomerase. Proc Soc Exp Biol Med 1997; 214:99-106.
3. Campisi J. Cellular senescence as a tumor-suppressor mechanism. Trends Cell Biol 2001; 11:S27-31.
4. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 1995; 92:9363-7.
5. 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.
6. Itahana K, Dimri G, Campisi J. Regulation of cellular senescence by p53. Eur J Biochem 2001; 268:2784-91.
7. 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.
8. Keyes WM, Wu Y, Vogel H, Guo X, Lowe SW, Mills AA. p63 deficiency activates a program of cellular senescence and leads to accelerated aging. Genes Dev 2005; 19:1986-99.
9. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dotsch V, Andrews NC, Caput D, 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.
10. 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, Muller M. TAp63α induces apoptosis by activating signaling via death receptors and mitochondria. Embo J 2005; 24:2458-71.
11. Bernassola F, Oberst A, Melino G, Pandolfi PP. The promyelocytic leukaemia protein tumour suppressor functions as a transcriptional regulator of p63. Oncogene 2005; 24:6982-6.
12. Ihrie RA, Marques MR, Nguyen BT, Horner JS, Papazoglu C, Bronson RT, Mills AA, Attardi LD. Perp is a p63-regulated gene essential for epithelial integrity. Cell 2005; 120:843-56.

13. Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, Jacks T. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 2002; 416:560-4.
14. Jacobs WB, Govoni G, Ho D, Atwal JK, Barnabe-Heider F, Keyes WM, Mills AA, Miller FD, Kaplan DR. p63 is an essential proapoptotic protein during neural development. Neuron 2005; 48:743-56.
15. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA, Jr., Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992; 356:215-21.
16. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999; 398:708-13.
17. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, Tabin C, Sharpe A, Caput D, Crum C, McKeon F. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999; 398:714-8.
18. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, Weinberg RA. Tumor spectrum analysis in p53-mutant mice. Curr Biol 1994; 4:1-7.
19. Keyes WM, Vogel H, Koster MI, Guo X, Qi Y, Petherbridge KM, Roop DR, Bradley A, Mills AA. p63 heterozygous mutant mice are not prone to spontaneous or chemically induced tumors. Proc Natl Acad Sci USA 2006; 103:8435-40.
20. Flores ER, Sengupta S, Miller JB, Newman JJ, Bronson R, Crowley D, Yang A, McKeon F, Jacks T. Tumor predisposition in mice mutant for p63 and p73: Evidence for broader tumor suppressor functions for the p53 family. Cancer Cell 2005; 7:363-73.
21. Mills AA, Qi Y, Bradley A. Conditional inactivation of p63 by Cre-mediated excision. Genesis 2002; 32:138-41.
22. 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, Donehower LA. p53 mutant mice that display early ageing-associated phenotypes. Nature 2002; 415:45-53.
23. Maier B, Gluba W, Bernier B, Turner T, Mohammad K, Guise T, Sutherland A, Thorner M, Scrable H. Modulation of mammalian life span by the short isoform of p53. Genes Dev 2004; 18:306-19.
24. Sommer M, Poliak N, Upadhyay S, Ratovitski E, Nelkin BD, Donehower LA, Sidransky D. ΔNp63α overexpression induces downregulation of sirt1 and an accelerated aging phenotype in the mouse. Cell Cycle 2006; 5.
25. Langley E, Pearson M, Faretta M, Bauer UM, Frye RA, Minucci S, Pelicci PG, Kouzarides T. Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. Embo J 2002; 21:2383-96.
26. Campisi J. Senescent cells, tumor suppression and organismal aging: Good citizens, bad neighbors. Cell 2005; 120:513-22.
27. Dimri GP. What has senescence got to do with cancer? Cancer Cell 2005; 7:505-12.
28. Ratovitski EA, Patturajan M, Hibi K, Trink B, Yamaguchi K, Sidransky D. p53 associates with and targets Delta Np63 into a protein degradation pathway. Proc Natl Acad Sci U S A 2001; 98:1817-22.
29. Liefer KM, Koster MI, Wang XJ, Yang A, McKeon F, Roop DR. Down-regulation of p63 is required for epidermal UV-B-induced apoptosis. Cancer Res 2000; 60:4016-20.
30. Keyes WM, Mills AA. p63: a new link between senescence and aging. Cell Cycle 2006; 5:260-5.
31. Tatar M, Bartke A, 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, Kuro-o M. Suppression of aging in mice by the hormone Klotho. Science 2005; 309:1829-33.
33. Nahor I, Abramovitch S, Engeland K, 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; 15:388-96.
34. Barbieri CE, Perez CA, Johnson KN, Ely KA, Billheimer D, Pietenpol JA. IGFBP-3 is a direct target of transcriptional regulation by ΔNp63α in squamous epithelium. Cancer Res 2005; 65:2314-20.
35. Osada M, Ohba M, Kawahara C, Ishioka C, Kanamaru R, Katoh I, Ikawa Y, Nimura Y, Nakagawara A, Obinata M, Ikawa S. Cloning and functional analysis of human p51, which structurally and functionally resembles p53. Nat Med 1998; 4:839-43.
36. Shimada A, Kato S, Enjo K, Osada M, Ikawa Y, Kohno K, Obinata M, Kanamaru R, Ikawa S, Ishioka C. The transcriptional activities of p53 and its homologue p51/p63: similarities and differences. Cancer Res 1999; 59:2781-6.
37. Dietz S, Rother K, Bamberger C, Schmale H, Mossner J, Engeland K. Differential regulation of transcription and induction of programmed cell death by human p53-family members p63 and p73. FEBS Lett 2002; 525:93-9.
38. Lo Iacono M, Di Costanzo A, Calogero RA, Mansueto G, Saviozzi S, Crispi S, Pollice A, La Mantia G, Calabro V. The Hay Wells syndrome-derived TAp63αQ540L mutant has impaired transcriptional and cell growth regulatory activity. Cell Cycle 2006; 5:78-87.
39. Westfall MD, Mays DJ, Sniezek JC, Pietenpol JA. The ΔNp63α phosphoprotein binds the p21 and 14-3-3σ promoters in vivo and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutations. Mol Cell Biol 2003; 23:2264-76.
40. Calabro V, Mansueto G, Santoro R, Gentilella A, Pollice A, Ghioni P, Guerrini L, La Mantia G. Inhibition of p63 transcriptional activity by $p14^{Arf}$: Functional and physical link between human ARF tumor suppressor and a member of the p53 family. Mol Cell Biol 2004; 24:8529-40.
41. Lowe SW, Sherr CJ. Tumor suppression by Ink4a-Arf: Progress and puzzles. Curr Opin Genet Dev 2003; 13:77-83.
42. Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP, Pelicci PG. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature 2000; 406:207-10.
43. Ferbeyre G, de Stanchina E, Querido E, Baptiste N, Prives C, Lowe SW. PML is induced by oncogenic ras and promotes premature senescence. Genes Dev 2000; 14:2015-27.
44. Salomoni P, Pandolfi PP. The role of PML in tumor suppression. Cell 2002; 108:165-70.
45. de Stanchina E, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G, Lowe SW. PML is a direct p53 target that modulates p53 effector functions. Mol Cell 2004; 13:523-35.
46. Bernardi R, Scaglioni PP, Bergmann S, Horn HF, Vousden KH, Pandolfi PP. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. Nat Cell Biol 2004; 6:665-72.
47. Alcalay M, Tomassoni L, Colombo E, Stoldt S, Grignani F, Fagioli M, Szekely L, Helin K, Pelicci PG. The promyelocytic leukemia gene product (PML) forms stable complexes with the retinoblastoma protein. Mol Cell Biol 1998; 18:1084-93.
48. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 2005; 8:19-30.
49. Hibi K, Trink B, Patturajan M, Westra WH, Caballero OL, Hill DE, Ratovitski EA, Jen J, Sidransky D. AIS is an oncogene amplified in squamous cell carcinoma. Proc Natl Acad Sci USA 2000; 97:5462-7.
50. 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, Trink B. ΔNp63α and TAp63α regulate transcription of genes with distinct biological functions in cancer and development. Cancer Res 2003; 63:2351-7.
51. 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, Trink B. ΔNp63α up-regulates the Hsp70 gene in human cancer. Cancer Res 2005; 65:758-66.
52. Barbieri CE, Barton CE, Pietenpol JA. ΔNp63α expression is regulated by the phosphoinositide 3-kinase pathway. J Biol Chem 2003; 278:51408-14.
53. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2002; 2:489-501.
54. Rocco JW, Leong CO, Kuperwasser N, DeYoung MP, Ellisen LW. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. Cancer Cell 2006; 9:45-56.
55. Patturajan M, Nomoto S, Sommer M, Fomenkov A, Hibi K, Zangen R, Poliak N, Califano J, Trink B, Ratovitski E, Sidransky D. ΔNp63 induces β-catenin nuclear accumulation and signaling. Cancer Cell 2002; 1:369-79.
56. Zmijewski JW, Jope RS. Nuclear accumulation of glycogen synthase kinase-3 during replicative senescence of human fibroblasts. Aging Cell 2004; 3:309-17.
57. Turenne GA, Price BD. Glycogen synthase kinase3 β phosphorylates serine 33 of p53 and activates p53's transcriptional activity. BMC Cell Biol 2001; 2:12.
58. Cui R, He J, Mei R, de Fromentel CC, Martel-Planche G, Taniere P, Hainaut P. Expression of p53, p63, and p73 isoforms in squamous cell carcinoma and adenocarcinoma of esophagus. Biochem Biophys Res Commun 2005.
59. Koster MI, Kim S, Mills AA, DeMayo FJ, Roop DR. p63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev 2004; 18:126-31.
60. Koster MI, Lu SL, White LD, Wang XJ, Roop DR. Reactivation of developmentally expressed p63 isoforms predisposes to tumor development and progression. Cancer Res 2006; 66:3981-6.
61. Sbisa E, Mastropasqua G, Lefkimmiatis K, Caratozzolo MF, D'Erchia AM, Tullo A. Connecting p63 to cellular proliferation: The example of the adenosine deaminase target gene. Cell Cycle 2006; 5:205-12.
62. Di Como CJ, Urist MJ, Babayan I, Drobnjak M, Hedvat CV, Teruya-Feldstein J, Pohar K, Hoos A, Cordon-Cardo C. p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002; 8:494-501.
63. Nylander K, Vojtesek B, Nenutil R, Lindgren B, Roos G, Zhanxiang W, Sjostrom B, Dahlqvist A, Coates PJ. Differential expression of p63 isoforms in normal tissues and neoplastic cells. J Pathol 2002; 198:417-27.
64. Hedvat CV, Teruya-Feldstein J, Puig P, Capodieci P, Dudas M, Pica N, Qin J, Cordon-Cardo C, Di Como CJ. Expression of p63 in diffuse large B-cell lymphoma. Appl Immunohistochem Mol Morphol 2005; 13:237-42.
65. Pruneri G, Fabris S, Dell'Orto P, Biasi MO, Valentini S, Del Curto B, Laszlo D, Cattaneo L, Fasani R, Rossini L, Manzotti M, Bertolini F, Martinelli G, Neri A, Viale G. The transactivating isoforms of p63 are overexpressed in high-grade follicular lymphomas independent of the occurrence of p63 gene amplification. J Pathol 2005; 206:337-45.
66. Fukushima N, Satoh T, Sueoka N, Sato A, Ide M, Hisatomi T, Kuwahara N, Tomimasu R, Tsuneyoshi N, Funai N, Sano M, Tokunaga O, Sueoka E. Clinico-pathological characteristics of p63 expression in B-cell lymphoma. Cancer Sci 2006.
67. Carroll DK, Carroll JS, Leong CO, Cheng F, Brown M, Mills AA, Brugge JS, Ellisen LW. p63 regulates an adhesion programme and cell survival in epithelial cells. Nat Cell Biol 2006; 8:551-61.
68. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253:49-53.
69. Moll UM, Slade N. p63 and p73: Roles in development and tumor formation. Mol Cancer Res 2004; 2:371-86.

70. Tonon G, Wong KK, Maulik G, Brennan C, Feng B, Zhang Y, Khatry DB, Protopopov A, You MJ, Aguirre AJ, Martin ES, Yang Z, Ji H, Chin L, Depinho RA. High-resolution genomic profiles of human lung cancer. Proc Natl Acad Sci USA 2005; 102:9625-30.
71. Mills AA. p63: Oncogene or tumor suppressor? Curr Opin Genet Dev 2006; 16:38-44.
72. Perez-Losada J, Wu D, DelRosario R, Balmain A, Mao JH. p63 and p73 do not contribute to p53-mediated lymphoma suppressor activity in vivo. Oncogene 2005; 24:5521-4.
73. Lazzerini Denchi E, Attwooll C, Pasini D, Helin K. Deregulated E2F activity induces hyperplasia and senescence-like features in the mouse pituitary gland. Mol Cell Biol 2005; 25:2660-72.
74. 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.
75. 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.
76. 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.
77. 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.
78. Gaiddon C, Lokshin M, Ahn J, Zhang T, Prives C. A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. Mol Cell Biol 2001; 21:1874-87.
79. Maisse C, Guerrieri P, Melino G. p73 and p63 protein stability: The way to regulate function? Biochem Pharmacol 2003; 66:1555-61.
80. Petitjean A, Cavard C, Shi H, Tribollet V, Hainaut P, Caron de Fromentel C. The expression of TA and ΔNp63 are regulated by different mechanisms in liver cells. Oncogene 2005; 24:512-9.
81. Senoo M, Manis JP, Alt FW, McKeon F. p63 and p73 are not required for the development and p53-dependent apoptosis of T cells. Cancer Cell 2004; 6:85-9.
82. Dohn M, Zhang S, Chen X. p63α and ΔNp63α can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. Oncogene 2001; 20:3193-205.
83. Harmes DC, Bresnick E, Lubin EA, Watson JK, Heim KE, Curtin JC, Suskind AM, Lamb J, DiRenzo J. Positive and negative regulation of deltaN-p63 promoter activity by p53 and ΔNp63α contributes to differential regulation of p53 target genes. Oncogene 2003; 22:7607-16.
84. Waltermann A, Kartasheva NN, Dobbelstein M. Differential regulation of p63 and p73 expression. Oncogene 2003; 22:5686-93.
85. Li N, Li H, Cherukuri P, Farzan S, Harmes DC, DiRenzo J. TAp63γ regulates expression of ΔN0p63 in a manner that is sensitive to p53. Oncogene 2006; 25:2349-59.
86. Antonini D, Rossi B, Han R, Minichiello A, Di Palma T, Corrado M, Banfi S, Zannini M, Brissette JL, Missero C. An autoregulatory loop directs the tissue-specific expression of p63 through a long-range evolutionarily conserved enhancer. Mol Cell Biol 2006; 26:3308-18.