Tumor-Specific p73 Up-regulation Mediates p63 Dependence in Squamous Cell Carcinoma

Maurice Phillip DeYoung,1,2 Cory M. Johannessen,1,2 Chee-Onn Leong,1,2 William Faquin,2,3 James W. Rocco,1,2,4 and Leif W. Ellisen1,2

Abstract

p63 is essential for normal epithelial development and is overexpressed in the vast majority of squamous cell carcinomas (SCC). Recent work has shown that ΔNp63α is essential for survival of SCC cells, raising the possibility that the p63 pathway may be an attractive therapeutic target in these tumors. Nevertheless, it is unknown whether a therapeutic window exists for inhibiting p63 in tumor cells versus normal epithelia. Here, we show that SCC cells are uniquely dependent on ΔNp63α for survival, unlike normal p63-expressing epithelial cells, and that dependence is mediated through tumor-specific up-regulation of the related protein p73. In normal primary human keratinocytes, we find that inhibition of endogenous p63 by RNA interference (RNAi) induces p21CIP1 expression, inhibits cell cycle progression, and ultimately promotes cellular senescence. In contrast, p63 inhibition in SCC cells induces proapoptotic bcl-2 family members and rapidly triggers apoptosis. Expression of p73 is low in uncultured basal keratinocytes but is markedly up-regulated in both SCC cell lines and primary tumors in vivo. Whereas p21CIP1 induction following loss of p63 in normal cells is independent of p53 and p73, both proapoptotic gene induction and cell death following p63 RNAi in tumor cells are p73 dependent. Finally, ectopic p73 expression in primary keratinocytes does not affect baseline cell proliferation but is sufficient to trigger cell death following loss of p63. Together, these findings define a specific molecular mechanism of p63 dependence through p73 up-regulation, and they provide a rationale for targeting the p63 pathway as a therapeutic strategy in SCCs. (Cancer Res 2006; 66(19): 9362-8)

Introduction

The p53 family member p63 plays an essential role in epithelial development as evidenced by p63-null mice, which exhibit near complete absence of the epidermis, mammary, and prostate tissue and a profound failure in limb formation (1, 2). Although some controversy exists about the specific mechanisms responsible for this phenotype, recent reports strongly implicate growth arrest and senescence as major contributors to developmental failure following loss of p63 (3). In addition to its role in normal development, a potential role for p63 in tumorigenesis is supported by the finding that p63 is a target of genomic amplification and/or overexpression in >80% of primary head and neck squamous cell carcinomas (HNSCC) as well as other squamous epithelial malignancies (4–6). We recently showed that p63 is essential for survival of HNSCC cells at least in part through its ability to repress apoptosis mediated by proapoptotic isofoms of the p53 family member p73 (7). In contrast, cell death has not been observed following loss of p63 in a developmental context (1, 2), which may imply potentially divergent roles for p63 in development and tumorigenesis. Here, we show that the distinct phenotypes elicited following loss of p63 in normal cells versus tumor cells can be explained by up-regulation of transactivating p73 isofoms during tumorigenesis in vivo. We find that up-regulation of p73 is both necessary and sufficient to confer dependence on p63 for tumor cell survival. These findings suggest a potential therapeutic window for p63 pathway inhibition in squamous carcinomas.

Materials and Methods

Cell culture, immunoprecipitation, and immunoblot analysis. Primary human keratinocytes were prepared from foreskins as described (8) and were used at passages 2 to 4; human keratinocytes and OKF6 (OKF6-TERT1; ref. 9) were cultured as described (8). Primary murine keratinocytes were prepared as above from newborn pups and cultured in keratinocyte serum-free medium/epidermal growth factor/bovine pituitary extract (Invitrogen, Carlsbad, CA) with the addition of 20 ng/mL cholera toxin (Sigma-Aldrich, St. Louis, MO) and 0.05 mM/L calcium chloride. HNSCC-derived cell lines were the generous gift of David Sidransky (Johns Hopkins University, Baltimore, MD) and were cultured as described (7). Protein lysates from cells or tissues were extracted, and immunoprecipitation experiments were done using conditions and antibodies described previously (7).

Lentiviral and retroviral production and infection. Short hairpin RNA (shRNA) lentiviral constructs were created by transferring the U6 promoter-shRNA cassette into a lentiviral backbone as described previously (7). High-titer amphotropic retroviral and lentiviral stocks were generated by cotransfection with packaging vectors into 293T cells, and viral supernatants were collected 48 hours later (10, 11). The targeted sequences for p63 were 5′-GGTTGAGGCGTATGATTGCT-3′ and 5′-GAGTTGGATGACTTCACCTT-3′. The targeted sequence for TAp73 was 5′-GGATCGCAGTTACCTT-3′.

Real-time quantitative reverse transcription-PCR analysis. Quantitative reverse transcription-PCR (RT-PCR) was done using iQ SYBR Green Supermix reagent (Bio-Rad, Hercules, CA) and the Opticon Real-time PCR Detection System (MJ Research, Waltham, MA). To determine relative copy numbers, full-length cDNA constructs for each isoform were serially diluted 10-fold (0.1-1.0 × 10−8 ng per reaction) and amplified to generate an eight-point standard curve. Each sample was assigned an expression value based on its threshold cycle (Ct) number and normalization to its own glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript levels. The quantitative RT-PCR primers used were as follows: TAp73, 5′-GCAC-CAGCTTGGACACCTCCT-3′ (forward) and 5′-GCAGATGAACTGGG-CCATGA-3′ (reverse), 167 bp. Other primer sequences as well as quantitative

Note: Current address for C.M. Johannessen: Genetics Division, Department of Medicine, Brigham and Women’s Hospital, Boston, MA 02115.

Requests for reprints: Leif W. Ellisen, Massachusetts General Hospital Cancer Center, 450 Vassar Street, Boston, MA 02114. Phone: 617-726-4315; E-mail: lellisen@partners.org

doi:10.1158/0008-5472.CAN-06-1619

Cancer Res 2006; 66(19): 9362-8

© 2006 American Association for Cancer Research.
Distinct Roles for p63 in Normal versus Tumor Cells

Figure 1. Distinct responses to p63 inhibition by RNAi in primary keratinocytes and HNSCC cells. A, inhibition of endogenous p63 causes induction of p21\(^{CIP1}\) in primary human keratinocytes (HK) and induces Puma and PARP cleavage in HNSCC cells (JHU-029 and JHU-011). Immunoblot of lysates from cells harvested 48 hours following infection with a lentiviral p63-directed shRNA construct (p63si) or the control vector (V). \(\beta\)-Tubulin (\(\beta\)-tub) serves as a loading control. B, knockdown of p63 inhibits cell cycle progression in human keratinocytes and induces apoptosis in HNSCC cells. Top, cell cycle profiles generated by flow cytometry analysis of BrdUrd/propidium iodide–stained primary human keratinocytes 48 hours following lentiviral infection. Mean of three independent experiments; bars, SD. C, expression of shRNA-resistant m\(\Delta\)Np63\(\alpha\) blocks induction of p21\(^{CIP1}\) in human keratinocytes and abolishes Puma induction and PARP cleavage in HNSCC cells (JHU-029). Immunoblot of lysates harvested 48 hours following p63 shRNA or control lentiviral vector infection. Note that epitope-tagged m\(\Delta\)Np63\(\alpha\) does not comigrate with endogenous \(\Delta\)Np63\(\alpha\). D, prolonged inhibition of p63 induces cellular senescence in primary human keratinocytes. Representative photomicrographs of human keratinocytes assayed for endogenous \(p63\)causes induction of \(p21\)CIP1 in human keratinocytes and HNSCC cells. In contrast, TAp63 isoforms are rare in these tissues compared to normal versus tumor cells.

Results and Discussion

Endogenous p63 inhibition elicits distinct phenotypes in normal versus tumor cells. The \(p63\) gene is expressed as multiple protein isoforms. Two distinct promoters produce isoforms that either contain or lack the NH\(_2\)-terminal transactivation domain (TAp63 and \(\Delta\)Np63, respectively), whereas multiple splicing events cause three COOH-terminal variants (12). \(\Delta\)Np63\(\alpha\) is the predominant p63 isoform expressed in basal epithelial cells in vivo, in primary human keratinocytes, and in HNSCC-derived cell lines (Fig. 1A; refs. 7, 12). In contrast, TAp63 isoforms are rare in these cells and are usually undetectable by immunoblot analysis.
consistent with our finding that ΔNp63 mRNA is >100-fold more abundant than TAp63 mRNA in these cells (data not shown; ref. 7).

To compare the role of p63 in normal primary human keratinocytes and HNSCC-derived cells, we used a lentiviral RNA interference (RNAi) approach to efficiently inhibit endogenous ΔNp63a expression in these cells (7). Using two independent shRNA species, we observe comparable knockdown of p63 in human keratinocytes and HNSCC cells as assessed by real-time quantitative RT-PCR and immunoblot analysis (Fig. 1A; data not shown). Induction of the cell cycle inhibitor p21CIP1 was observed following knockdown of p63 expression by RNAi in primary human keratinocytes (Fig. 1A) and was associated with an increase in G1-phase cells and a marked decrease in S phase (Fig. 1B). A second p63-directed shRNA targeting all p63 isoforms produced essentially identical induction of p21CIP1 and decrease in cell cycle progression (data not shown). These findings support the function of ΔNp63a as a mediator of proliferation in basal epithelial cells, and they agree with a previous report suggesting that ΔNp63a functions as a

![Figure 2](image_url)

A, p21CIP1 induction and cell cycle inhibition following p63 RNAi are p53 independent. Left, immunoblot of lysates from primary murine keratinocytes harvested 48 hours following infection with a murine p63-directed shRNA lentivirus or the control vector. Note the decreased basal expression of p21CIP1 in p53−/− keratinocytes. Right, quantitation of G1-phase cells by BrdUrd/propidium iodide staining and flow cytometry analysis 48 hours following p63 shRNA lentivirus or control vector infection. B, p21CIP1 induction and cell cycle inhibition following p63 RNAi are not blocked by knockdown of TAp73. Left, human keratinocytes stably expressing a TAp73-directed shRNA or control lentiviral vector were subsequently infected with a p63 shRNA-expressing lentivirus or control vector. Lysates were harvested at 48 hours for immunoblot analysis. Right, quantitation of G1-phase cells following infection with TAp73 and p63-directed shRNA lentiviral vectors as at left. P values in (A) and (B) are derived from two-tailed Student’s t test.

C, Puma induction, PARP cleavage, and cell death following p63 knockdown in HNSCC cells are TAp73 dependent. JHU-011 cells were treated as in (B) and then harvested at 48 hours for immunoblot analysis (left) or at 96 hours for Annexin V/propidium iodide staining (right). Note that these cells express both TAp73a and TAp73β. Columns, mean of three independent experiments; bars, SD.
Distinct Roles for p63 in Normal versus Tumor Cells

Little or no evidence of cell death was observed in primary human keratinocytes following lentiviral p63 knockdown (Fig. 1B). Instead, morphologic features of cellular senescence were observed in a subset of cells, and these changes correlated with staining for SA-β-gal (Fig. 1D). This senescence phenotype is highly reminiscent of that observed following conditional ablation of p63 in the mouse in vivo (3). In contrast to these effects in primary human keratinocytes, ablation of p63 by RNAi in HNSCC cells had no effect on p21<sup>CIP1</sup> expression but instead induced the proapoptotic bcl-2 family member Puma and triggered apoptosis as evidenced by cleavage of poly(ADP-ribose) polymerase (PARP)-1, an apoptotic hallmark (Fig. 1A). Apoptosis following p63 knockdown specifically in HNSCC cells was confirmed in two independent HNSCC cell lines as previously shown (Fig. 1A and B; ref. 7).

To confirm that the cellular effects observed following p63 RNAi were specifically due to the loss of ΔNp63α protein, we sought to “rescue” the effects of the p63 RNAi by constitutively expressing murine ΔNp63α (mΔNp63α), which is insensitive to the human-specific p63 shRNA by virtue of a nucleotide sequence difference in the targeted region. Constitutive retroviral expression of mΔNp63α in human keratinocytes blocked both p21<sup>CIP1</sup> induction and cell cycle arrest following p63 shRNA treatment, whereas in JHU-029 retroviral expression of mΔNp63α prevented induction of Puma and significantly blocked PARP cleavage and cell death following endogenous p63 knockdown (Fig. 1C; data not shown). Taken together, these data show the specificity of the RNAi-mediated inhibition of ΔNp63α, and they argue that ΔNp63α plays a critical role in regulating cellular proliferation in primary basal epithelial cells while promoting cell survival in HNSCC cells.

p63-dependent regulation of p21<sup>CIP1</sup> and cell cycle progression are independent of p53 and TAp73. To determine whether ΔNp63α functions to suppress p53-dependent activation of p21<sup>CIP1</sup> in epithelial cells, we compared the effects of endogenous p63 knockdown in keratinocytes derived from p53-null mice or heterozygous littermates. As predicted, p53 inactivation significantly lowered the basal level of p21<sup>CIP1</sup> expression (Fig. 2A) and it abolished the strong induction of p21<sup>CIP1</sup> observed in p53 heterozygous cells following doxorubicin treatment (data not shown; ref. 14). In contrast, p53 nullizygosity did not abolish p21<sup>CIP1</sup> induction or cell cycle inhibition following p63 RNAi (Fig. 2A). Similar results were obtained in primary human keratinocytes in which p53 was inactivated by expression of a COOH-terminal truncated p53 fragment (p53DD) that functions as a potent inhibitor of p53 function (data not shown; ref. 15). These data show that p53 transcriptional activity is not required for induction of p21<sup>CIP1</sup> following elimination of repressive ΔNp63α in both human and murine primary keratinocytes (13). Similarly, we previously showed that induction of Puma and cell death in HNSCC cells following p63 inhibition was p53 independent, as both occurred in JHU-011 cells that lack wild-type p53 (Fig. 1A; ref. 7). Taken together, these findings suggest that the distinct transcriptional responses to inhibition of endogenous p63 in normal primary keratinocytes and HNSCC cells are not mediated in either case through activation of p53.

We next wished to determine whether endogenous TAp73 is required for p21<sup>CIP1</sup> up-regulation and cell cycle inhibition following loss of p63. p73 is expressed at low levels in primary human keratinocytes, and based on its migration, the major p73 isoform detectable by immunoblot analysis is TAp73<sup>B</sup> (Fig. 2B; data not shown). Primary human keratinocytes were infected with a lentiviral shRNA that specifically targets the NH<sub>2</sub>-terminal transactivation domain of p73 followed by brief drug selection. This pooled population was then infected with the p63-directed shRNA. Loss of TAp73 had no effect on the induction of p21<sup>CIP1</sup> or on cell cycle inhibition following p63 RNAi (Fig. 2B), showing that cell cycle regulation mediated by p63 does not require trans-activating isoforms of p73. This conclusion is supported by data presented in Fig. 4A, showing that ectopic expression of TAp73 in human keratinocytes does not enhance p21<sup>CIP1</sup> induction following p63 RNAi. In contrast to these results in normal human keratinocytes, we recently showed that the apoptotic program triggered following loss of p63 in HNSCC cells was highly dependent on TAp73 (7). To extend this previous work, we infected JHU-011 cells with a TAp73-directed shRNA lentivirus followed by p63 knockdown. Ablation of TAp73 expression in these tumor cells virtually completely eliminated Puma induction, PARP cleavage, and cell death following p63 knockdown (Fig. 2C). Taken together, these findings show that, unlike cell death following p63 inhibition in HNSCC tumor cells, p63-mediated cell cycle effects in primary human keratinocytes are both p53 and p73 independent (7).

Tumor-specific up-regulation of TAp73 in primary HNSCC in vivo. Our findings that TAp73 was dispensable for cell cycle regulation following inhibition of p63 in primary human keratinocytes but was an essential mediator of apoptosis following p63 inhibition in HNSCC prompted us to ask whether TAp73 might be differentially expressed in normal epithelia versus HNSCC tumors. We first examined p73 mRNA expression using isoform-specific quantitative RT-PCR in multiple human tumor-derived HNSCC cell lines compared with either primary human keratinocytes or primary immortalized human oral keratinocytes (OKF6; ref. 9). We designed isoform-specific primers that amplify either TAp73 or, alternatively, isoforms lacking the transactivation domain (ΔNp73 and ΔNp73, hereafter referred to as ΔNp73). The levels of TAp73 were dramatically elevated (18- to 30-fold) in all three HNSCC cell lines compared with human keratinocytes and OKF6, whereas ΔNp73 levels were not significantly increased in HNSCC cells versus primary human keratinocytes or OKF6 (Fig. 3A). Consequently, TAp73 was markedly more abundant than ΔNp73 in HNSCC cells, whereas in human keratinocytes and OKF6, ΔNp73 is the more abundant isoform (Fig. 3A). As predicted, we found that high levels of proapoptotic TAp73 isoforms were associated with up-regulation of inhibitory ΔNp63 isoforms (3.5- to 8-fold) in proliferating tumor cells (data not shown; ref. 7). Thus, both TAp73 and ΔNp63 are overexpressed in HNSCC tumor cells versus nontransformed keratinocytes, with a much larger relative increase in TAp73 levels.

Reports have varied about the expression of p73 in primary HNSCC, and few studies have focused on the expression of specific p73 isoforms (16–18). We wished to determine whether TAp73 is overexpressed in primary HNSCC versus normal epithelia and whether this protein is physically associated with inhibitory ΔNp63α in vivo as we observed in HNSCC cells (7). We first examined p63 expression by immunohistochemistry in a series of 11 primary, untreated invasive HNSCC tumor biopsy specimens, which were shown by pathologic review to contain a high fraction (>75%) of tumor cells. As expected, p63 expression was robust in the malignant cells in all these specimens (Fig. 3B; data not shown). We next examined isoform-specific expression of p73 mRNA in these tumor specimens and in normal human epidermis by quantitative RT-PCR. Consistent with our results in HNSCC cell

www.aacrjournals.org
lines, 9 of 11 tumor specimens exhibited dramatically elevated levels of TAp73 mRNA relative to normal epidermis, with a mean expression ratio in tumor/normal of 19.5. As was observed in cultured cells, tumor-specific up-regulation of TAp73 was also manifested by a marked increase in the TAp73/ΔNp63 expression ratio in tumor samples compared with normal epidermis (Fig. 3C). As predicted, the absolute level of ΔNp63 mRNA was greater than TAp73 in all specimens (Fig. 3C). Of note, ΔNp73 expression was not significantly up-regulated in tumors (mean ΔNp73 expression ratio in tumor/normal, 1.08). These results suggest that both ΔNp63 and TAp73 isoforms are selectively overexpressed in primary HNSCC versus normal epithelia in vivo.

Given our observation that p63 dependence in HNSCC cells required p73 but did not require functional p53, we asked whether p53 inactivation was observed in primary HNSCC exhibiting ΔNp63 and TAp73 overexpression. We stained the 11 tumors for p53 protein by immunohistochemistry. Six of 11 tumors showed overexpression of p53 by immunohistochemistry consistent with mutational inactivation of p53. Notably, six of seven tumors with the highest level of TAp73/ΔNp63 expression exhibited p53 overexpression, whereas the four tumors with the lowest TAp73/ΔNp63 expression did not (Fig. 3C). These data further support our finding that p63 dependence in HNSCC does not require functional p53.

To confirm expression of p73 protein and its abundance in primary tumors versus normal cells, we used immunoprecipitation/immunoblot analysis of lysates derived from these primary tumors versus normal skin biopsies. We detected robust expression of p73 protein in tumor-derived lysates while only detecting minimal p73 expression in normal epidermises (Fig. 3D). The p73 protein detected comigrated with TAp73, consistent with our quantitative RT-PCR data showing that TAp73 isoforms were by far the most abundant isoforms expressed in these tumors (data not shown). Finally, we did immunoblot analysis for p63 in the immunoprecipitated product. ΔNp63α was readily detectable in the immunoprecipitate following

![Figure 3. TAp73 is up-regulated in primary HNSCC tumors in vivo. A, TAp73 is up-regulated in HNSCC-derived cell lines relative to primary human keratinocytes or immortalized primary oral keratinocytes (OKF6) and is the major p73 isoform expressed in tumor cells. Left, real-time quantitative RT-PCR was used to measure p73 isoform-specific mRNA levels normalized to GAPDH expression. TAp73 and ΔNp73 mRNA levels in human keratinocytes are arbitrarily designated as 1.0. Right, molar ratio of TAp73/ΔNp73 mRNA was calculated based on cDNA standard curves for both isoforms (see Materials and Methods). Columns, mean of three independent reactions; bars, SD. B, representative standard H&E staining (left) and immunohistochemical analysis (right) of benign oral mucosa (Normal) and primary HNSCC (Tumor) specimens for p63 expression. In normal tissue, p63 expression is restricted to the basal and immediate suprabasal layers, whereas in HNSCC, p63 is highly and diffusely expressed in invasive tumor cells. C, increased TAp73/ΔNp63 ratio reflects increased TAp73 expression and correlates with p53 inactivation in primary HNSCC tumor specimens (T1-T11) compared with normal (N) or dysplastic (dys) epidermis. The mean ratio of TAp73/ΔNp63 mRNA was assayed by quantitative RT-PCR and calculated using standard cDNA templates. Asterisk, p53 overexpression as assessed by immunohistochemistry. Note that ΔNp63 is more abundant than TAp73 in each case. The mean ratio value for normal epidermis was derived from multiple specimens. Columns, mean of three independent reactions; bars, SD. D, TAp73 protein is overexpressed and physically associates with ΔNp63α in primary HNSCC tumors. Top, 1.0 mg of protein lyase from normal skin and three primary HNSCC tumor specimens was immunoprecipitated (IP) using a p73 antibody followed by immunoblot analysis for both p73 and p63. Note that the p73 antibody used for immunoprecipitation was previously shown to be non-cross-reactive with p63 (7). Bottom, immunoblot analysis of these lysates for p63 shows close correlation between the total amount of ΔNp63α protein and the p73-associated fraction. β-Tubulin serves as a loading control.](image_url)
Ectopic expression of TAp73β is sufficient to render primary human keratinocytes p63-dependent for survival. A, ectopic expression of TAp73β is well tolerated in human keratinocytes yet selectively promotes Puma expression and PARP cleavage following p63 RNAi. Human keratinocytes stably expressing retroviral TAp73β or the control vector were infected with a p63-directed shRNA lentivirus or the control vector. Lysates were harvested 48 hours after lentiviral infection for immunoblot analysis. Note that p21cip1 induction is not enhanced by TAp73β expression. B, apoptosis was assessed in cells treated as in (A) by Annexin V/propidium iodide staining of unfixed cells 96 hours following lentiviral infection. Columns, mean percentage of apoptotic cells from three independent experiments; bars, SD.

Distinct Roles for p63 in Normal versus Tumor Cells

immunoprecipitation for p73 (Fig. 3D). The amount of ΔNp63α associated with p73 correlated strongly with its abundance as assessed by direct immunoblot analysis of lysates from these same tumors (Fig. 3D). Together, these data argue strongly that, in both HNSCC-derived cell lines and primary HNSCC tumors, TAp73 is highly up-regulated and is physically associated with ΔNp63α.

Ectopic TAp73β overexpression is sufficient to induce p63 dependence. Finally, we wished to test directly whether overexpression of TAp73 in normal primary cells would be sufficient to alter their response to p63 inhibition, rendering them p63 dependent for viability. Retroviral TAp73β expression alone in primary human keratinocytes had no effect on cell viability or cellular proliferation (data not shown). However, following p63 knockdown, cells expressing exogenous TAp73β showed induction of Puma, cleavage of PARP, and, at later time points, apoptosis (Fig. 4). Control vector–infected cells did not exhibit any of these features following inhibition of p63. Of note, p21cip1 was induced in both TAp73β-expressing and control vector–infected human keratinocytes on loss of p63, and its induction was somewhat attenuated in TAp73β-expressing cells. Together, these findings show that up-regulation of TAp73β is sufficient to render normal epithelial cells dependent on p63 for their survival.

Our results implicate tumor-specific up-regulation of TAp73 as a key molecular feature that mediates cellular dependence on p63 for survival. Conceivably, p73 up-regulation during tumorigenesis may serve to promote apoptosis and thereby eliminate nascent tumor cells exhibiting a variety of genetic alterations (19). Accordingly, inhibition of p73-dependent apoptosis may provide the selective pressure for the up-regulation of ΔNp63α that is observed in the majority of squamous tumors, thereby allowing such tumor cells to survive despite high levels of p73. Given that p63 up-regulation is among the most common molecular abnormalities in these tumors, potential therapeutic implications of these findings bear consideration. Targeting p63 itself in these cancers may be problematic, given the consequences of conditional p63 ablation in adult mice (3). However, it remains conceivable that transient or localized p63 inhibition might provoke a limited or reversible inhibition of proliferation in normal cells while inducing irreversible death in tumor cells. Similarly, although p63 inhibition may provoke limited cell death in vitro under different experimental conditions (20), we observe under all conditions that tumor cells with high levels of p73 exhibit a dramatic increase in death compared with untransformed cells. A more detailed understanding of the biochemistry involved in p63-dependent inhibition of p73 might well provide novel means of targeting this pathway to therapeutic advantage.

Acknowledgments

Received 5/2/2006; revised 7/7/2006; accepted 8/7/2006.

Grant support: NIH RO1 DE15945 (L.W. Ellisen), the Avon Foundation (L.W. Ellisen), the Norman Knight Head and Neck Cancer Research Fund (J.W. Rocco), and funds from the Danny Miller Chair in Head and Neck Molecular Biology (J.W. Rocco).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Nick Vidnovic and Avi Sofer for critical review of the manuscript.

References

7. Rocco JW, Leong CO, Kuperwasser N, et al. p63 mediates survival in squamous cell carcinoma by...
13. Westfall MD, Mays DJ, Sniezek JC, Pietenpol JA. The ΔNp63α phosphoprotein binds the p21 and 14-3-3α promoters in vivo and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutants. Mol Cell Biol 2003;23:2264–76.
Tumor-Specific p73 Up-regulation Mediates p63 Dependence in Squamous Cell Carcinoma

Maurice Phillip DeYoung, Cory M. Johannessen, Chee-Onn Leong, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/19/9362

Cited articles
This article cites 20 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/19/9362.full.html#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:
/content/66/19/9362.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.