Arsenic Suppresses Cell Survival via Pirh2-mediated Proteasomal Degradation of ΔNp63 Protein

Received for publication, October 22, 2012, and in revised form, December 6, 2012. Published, JBC Papers in Press, December 27, 2012, DOI 10.1074/jbc.M112.428607

Wensheng Yan, Xiufang Chen, Yanhong Zhang, Jin Zhang, Yong-Sam Jung, and Xinbin Chen

From the Comparative Oncology Laboratory, University of California at Davis, Davis, California 95616 and the Department of Hematology, Hospital of Nantong University, Nantong, Jiangsu 226001, China

The transcription factor p63, a member of the p53 family, shares a high degree of sequence similarity with p53. Because of transcription from two distinct promoters, the p63 gene encodes two isoforms, TAp63 and ΔNp63. Although TAp63 acts as a tumor suppressor, ΔNp63 functions as an oncogene and is often overexpressed in squamous cell carcinomas. Thus, therapeutic agents targeting ΔNp63 might be used to manage tumors that overexpress ΔNp63. Here we found that arsenic trioxide, a frontline agent for acute promyelocytic leukemia, inhibits ΔNp63 but not TAp63 expression in time- and dose-dependent manners. In addition, we found that arsenic trioxide decreases the stability of ΔNp63 protein via a proteasome-dependent pathway but has little effect on the level of ΔNp63 transcript. Furthermore, we found that arsenic trioxide activates the Pirh2 promoter and consequently induces Pirh2 expression. Consistent with this, we found that knockdown of Pirh2 inhibits, whereas ectopic expression of Pirh2 enhances, arsenic-induced degradation of ΔNp63 protein. Importantly, we found that knockdown of ΔNp63 sensitizes, whereas ectopic expression of ΔNp63 inhibits, growth suppression induced by arsenic. Together, these data suggest that arsenic degrades ΔNp63 protein at least in part via Pirh2-dependent proteolysis and that inhibition of ΔNp63 expression facilitates tumor cells to arsenic-induced death.

Background: ΔNp63 exhibits an oncogenic potential and is often overexpressed in squamous cell carcinomas.

Results: Arsenic degrades ΔNp63 protein at least in part via Pirh2-mediated proteolysis, and inhibition of ΔNp63 expression facilitates tumor cells to arsenic-induced death.

Conclusion: Arsenic trioxide induces Pirh2 to target ΔNp63 for degradation.

Significance: Targeting ΔNp63 may be explored to manage tumors overexpressing ΔNp63.

Apoptosis (5, 6). In contrast, ΔNp63 loses such an activation domain but gains 14 unique residues at the N terminus. These 14 residues, together with the adjacent proline-rich region, constitute an activation domain for ΔNp63 (7, 8). Thus, ΔNp63 also possesses a transcriptional activity.

Although p53 functions as a classical tumor suppressor, the role for p63 in tumorigenesis is still uncertain. A study showed that p63+/− mice are predisposed to develop spontaneous tumors (9), suggesting that the p63 gene acts as a tumor suppressor. Consistently, TAp63 is found to induce senescence and suppress tumorigenesis in TAp63 conditional knockout mice (10). On the other hand, many studies have highlighted the oncogenic properties of ΔNp63. ΔNp63 is frequently found to be amplified and overexpressed in squamous cell carcinomas (11, 12). ΔNp63α overexpression promotes cell proliferation in vitro and tumor growth in vivo (13, 14). In addition, ΔNp63α represses apoptosis-related genes and, thereby, contributes to chemoresistance of hepatocellular carcinoma (15). In line with this, knockdown of ΔNp63α induces apoptosis and sensitizes cells to DNA damage (16). Clinically, high levels of ΔNp63 expression in tumors are associated with an aggressive phenotype and chemoresistance (17, 18).

The role of ΔNp63 in tumorigenesis might be partially due to its transcriptional activity. We found previously that GPX2 and BMP7, two direct targets of ΔNp63, inhibit oxidative-stress-induced apoptosis in a p53-dependent manner and are required for survival of tumor cells (19, 20). Other studies also found that ΔNp63 regulates genes involved in cell cycle progression and cell survival (2, 21). Interestingly, ΔNp63 was found to regulate the splicing pattern of CD44, which may affect the adhesion and metastasis of cancer cells (14). The oncogenic potential of ΔNp63 might be also due to its dominant-negative activities to suppress p53- or TAp63-mediated transactivation (2, 7, 15, 23). In addition, ΔNp63α is found to exhibit a survival function in squamous epithelial malignancy by repressing TAp73-dependent pro-apoptotic activity (24). However, the unique transcriptional and dominant-negative abilities in ΔNp63 may be explored for a new therapeutic approach modulating ΔNp63 expression to manage tumors that overexpress ΔNp63 but harbor TAp63, TAp73, and/or wild-type p53.
**ΔNp63, a Novel Target of Arsenic**

Arsenic is a metalloid with a substantial efficacy in treating patients with acute promyelocytic leukemia, myeloma, and myelodysplastic syndromes (25). Evidences showed that arsenic functions as an anticancer agent at least in part via targeting proteins for degradation (26–31). Recently, we found that arsenic targets mutant p53 for degradation, which mediates arsenic-induced growth suppression in solid tumor cells (32). The structural and functional similarity between ΔNp63 and mutant p53 prompts us to examine whether arsenic has an effect on ΔNp63 expression. Indeed, we found that arsenic induces ΔNp63 degradation via the proteasome-dependent pathway. Our finding suggests that targeting ΔNp63 with arsenic may be explored further to manage tumors that are carrying a high level of ΔNp63.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human keratinocyte cell line HaCaT, human cervical carcinoma cell line ME-180, and human pancreatic cancer cell line MIA PaCa-2 were cultured in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Hyclone). The human mammary epithelial cell line MCF10A was cultured in DMEM/F12 supplemented with 5% donor horse serum, 20 ng/ml of EGF, 10 μg/ml of insulin, 0.5 μg/ml of hydrocortisone, and 100 ng/ml of cholera toxin.

**Antibodies**—Mouse anti-p63(4A4) was purchased from Santa Cruz Biotechnology, Inc. Rabbit anti-Pirh2 was purchased from Bethyl Laboratories, Inc. Rabbit anti-actin and mouse anti-FLAG were purchased from Sigma.

**Plasmids**—Myc-tagged ΔNp63α and 2 × FLAG-tagged Pirh2 cDNAs in pcDNA3 expression vector were described previously (8, 33). To generate the luciferase reporter under the control of the Pirh2 promoter, a 2043-bp DNA fragment containing the Pirh2 promoter (from nucleotides −2000 to +43) was amplified using human genomic DNA with forward primer 5′-CTCGAGGCTATCTGTGAATGATATGCCAG-3′ and reverse primer 5′-AAGCTTCCATCTGCAATATGGCCTGAGT-3′. The PCR product, Pirh2–2000, was cloned into the pGEM-T-Easy vector and confirmed by DNA sequencing. Pirh2 cDNAs in pcDNA3 expression vector were described previously (8, 33). To generate the luciferase reporter under the control of the Pirh2 promoter, a 2043-bp DNA fragment containing the Pirh2 promoter (from nucleotides −2000 to +43) was amplified using human genomic DNA with forward primer 5′-CTCGAGGCTATCTGTGAATGATATGCCAG-3′ and reverse primer 5′-AAGCTTCCATCTGCAATATGGCCTGAGT-3′. The PCR product, Pirh2–2000, was cloned into the pGEM-T-Easy vector and confirmed by DNA sequencing.

**siRNA**—A siRNA against Pirh2 (5′-GTCTGTGTTATATAGGGACTGG-3′) and a scrambled siRNA (5′-CTCGAGGCTATCTGTGAATGATATGCCAG-3′) were purchased from Dharmacon RNA Technologies (Chicago, IL).

**Reverse Transcription PCR Assay**—Total RNA was isolated from cells using TRIzol reagent (Invitrogen). cDNA was synthesized using an Iscript™ cDNA synthesis kit (Bio-Rad). To measure ΔNp63 mRNA, RT-PCR was done with forward primer 5′-TGGCAAAATCTTGAGCCAGC-3′ and reverse primer 5′-GTCTGTGTTATATAGGGACTGG-3′. To measure Pirh2 mRNA, RT-PCR was done with forward primer 5′-CTGGAGCATTATGAGCAG-3′ and reverse primer 5′-TCTGCTAGGATTTAATGTC-3′. Actin was amplified with forward primer 5′-TCTATCGTGAGTGGGAG-3′ and reverse primer 5′-TGATCCACATCTGTGGAAG-3′.

**Protein Half-life Assay**—ME-180 cells were left untreated or pretreated with 10 μM arsenic trioxide for 1 h and then incubated with 50 μg/ml of cycloheximide to inhibit de novo protein synthesis for various times. The relative level of ΔNp63α protein was quantified by densitometry and normalized by the level of actin protein, which was then plotted versus time (h) to calculate the half-life of ΔNp63α.

**Proteasome Inhibition Assay**—Cells were seeded for 24 h, left untreated or pretreated with proteasome inhibitor MG132 (5 μM) for 4 h, and then treated with arsenic trioxide for various times.

**Luciferase Assay**—The dual luciferase assay was done in triplicate according to the instructions of the manufacturer (Promega). Briefly, 0.5 μg of a luciferase reporter and 3 ng of pRL-SV40-Renilla luciferase reporter (Promega) were cotransfected into HaCaT cells by using the Expressfect transfection reagent (Denville). The fold increase in relative luciferase activity is a product of the luciferase activity induced by arsenic treatment divided by that induced by mock treatment.

**Cell Survival Assay**—To determine whether ectopically expressed ΔNp63α makes tumor cells resistant to arsenic trioxide treatment, ME-180 cells were transiently transfected with pcDNA3 or pcDNA3-myc-ΔNp63α for 1 day and then left untreated or treated with 7.5 μM arsenic trioxide for 2 days. The survival cells were collected and counted.

To determine whether knockdown of ΔNp63 sensitizes tumor cells to arsenic trioxide treatment, ME-180 cells were transfected with scrambled siRNA or siRNA against p63 for 1 day and then left untreated or treated with 7.5 μM arsenic trioxide for 2 days. The survival cells were collected and counted.

**Statistics**—All experiments were performed in triplicates. Two-group comparisons were analyzed by two-sided Student’s t test. p values were calculated, and p < 0.05 was considered significant.

**RESULTS**

**Arsenic Trioxide Inhibits ΔNp63 Expression in Time- and Dose-dependent Manners**—Arsenic trioxide is a therapeutic agent for acute promyelocytic leukemia, which potentially targets proteins for degradation (26–31). We found previously that arsenic-induced degradation of mutant p53 leads to growth suppression (32). ΔNp63, a member of p53 family, shares the high degree of sequence similarity to mutant p53 (1) and confers a proliferative and chemoresistant advantage to tumor cells (16, 18). Thus, we rationalized that targeting ΔNp63 with arsenic may be explored as a therapeutic strategy to tumors that carry a high level of ΔNp63.

Studies showed that although TAp63 is expressed at a very low level, ΔNp63, especially ΔNp63α, is highly expressed in MCF10A mammary epithelial cells, HaCaT keratinocyte cells, and ME-180 cervical carcinoma cells (34–36). Thus, the effect of arsenic on ΔNp63 expression was examined in these cells. We found that upon treatment with 10 μM arsenic trioxide, the
level of ΔNp63α protein was promptly decreased in ME-180 cells, reaching maximum reduction within 12–24 h (Fig. 1A, top panel). The level of actin protein was used as a loading control (Fig. 1A, bottom panel). Similar results were found in HaCaT cells (Fig. 1B) and MCF10A cells (C).

To determine the dose-response relationship between arsenic and ΔNp63α expression, ME-180 cells were treated with various doses of arsenic trioxide for 12 h. We found that the level of ΔNp63α protein in ME-180 cells was decreased markedly by arsenic trioxide at a concentration of as low as 2.5 μM (Fig. 1D). This concentration is close to that for degradation of mutant p53 in cultured cells (32) and the plasma peak values of 1.5–3.4 μM in acute promyelocytic leukemia patients treated with arsenic trioxide (37). As arsenic concentration was increased to 5 μM, the level of ΔNp63α was decreased further in ME-180 cells (Fig. 1D). However, the arsenic-induced decrease of ΔNp63α protein was not further enhanced at concentrations of more than 5 μM (Fig. 1D). A similar result was seen in HaCaT cells (Fig. 1E) and MCF10A cells (F).

Because TAp63 isoforms function as tumor suppressors by inducing senescence (10) and preventing invasiveness and metastasis (38), it is vitally important to determine how arsenic alters TAp63 expression in tumor cells. For this purpose, MIA PaCa-2 cells, which carry a high level of TAp63α, were left untreated or treated with arsenic trioxide as indicated (Fig. 1, G and H). Interestingly, we found that arsenic trioxide had little, if any, effect on the level of TAp63α protein in MIA PaCa-2 cells (Fig. 1, G and H).

Arsenic Trioxide Decreases the Stability of ΔNp63 Protein but Has Little Effect on the Level of ΔNp63 Transcript—To examine whether arsenic-induced reduction of ΔNp63 is through a post-translational mechanism, ME-180 cells were treated with 50 μg/ml of cycloheximide for various times in the absence or presence of arsenic trioxide. The relative level of ΔNp63α protein was quantified by densitometry and normalized by level of actin protein, which was then plotted versus time (h) to calculate the half-life of ΔNp63α. We found that the half-life for ΔNp63α protein was decreased from about 2 h in the control cells to about 1 h in cells treated with arsenic trioxide (Fig. 2, A and B).

Next, to test whether ΔNp63 transcription is suppressed by arsenic trioxide, RT-PCR analysis was performed to measure the level of ΔNp63 transcript in ME-180, HaCaT, and MCF10A cells, which were mock-treated or treated with 10 μM arsenic trioxide as indicated. The actin transcript was measured as a control. We found that the level of ΔNp63 mRNA was not obviously altered by arsenic trioxide in ME-180 (Fig. 2C), HaCaT (D), and MCF10A (E) cells. These results suggest that arsenic trioxide does not inhibit ΔNp63 transcription but rather decreases the stability of ΔNp63 protein.

Arsenic Trioxide Degrades ΔNp63 Protein via the Proteasome-dependent Pathway—Because the stability of ΔNp63 protein was decreased by arsenic, we further explored whether ΔNp63 is degraded via the proteasome-dependent pathway. To test this, ME-180 cells were left untreated or treated with 4 μM MG132, an inhibitor of 26 S proteasome, in the absence or presence of arsenic trioxide. We found that arsenic-induced ΔNp63 degradation was almost abolished by MG132 (Fig. 3A). Similarly, we found that arsenic-induced degradation of ΔNp63 protein was inhibited by MG132 in HaCaT cells (Fig. 3B). Taken together, these results suggest that arsenic-induced ΔNp63 degradation is at least in part via the proteasome-dependent pathway.

Arsenic Trioxide Induces Expression of Pirh2 E3 Ligase—Next, we sought to identify the proteins that may mediate arsenic-induced ΔNp63 degradation. Multiple E3 ligases were reported to degrade p63 protein. For example, MDM2 and Fbw7 cooperate to induce p63 protein degradation following DNA damage and cell differentiation (39). WW domain-containing E3 ubiquitin protein ligase 1 targets p63 protein for degradation through a ubiquitin-proteasome-dependent pathway. Because Pirh2 is a WW-containing E3 ligase that binds p63, and because arsenic induces expression of Pirh2 in cultured cells (38), we hypothesized that arsenic induces expression of Pirh2 to mediate arsenic-induced ΔNp63 degradation.
Arsenic-induced degradation of Np63 protein is at least in part mediated by the Pirh2 E3 ligase. Here, multiple E3 ligases, known to degrade p63 or other p53 family members, were screened in arsenic-treated cells with a Western blot assay. We found that upon treatment with arsenic trioxide, the level of Pirh2 protein was promptly increased in HaCaT cells (Fig. 4A) and ME-180 cells (B). To further examine whether arsenic increased the level of Pirh2 mRNA, RT-PCR analysis was performed with HaCaT cells, which were left untreated or pretreated with 4 μM MG132 for 4 h and then left untreated or treated with arsenic trioxide for 7 h. We also found that when combined with arsenic treatment, the level of Np63α was further decreased in ME-180 cells with p63 knockdown (Fig. 6A, compare lane 4 with lanes 1–3). In addition, we found that short-term knockdown of Np63 alone had little effect on cell survival in ME-180 cells (Fig. 6B). However, we found that upon arsenic treatment, Np63 knockdown significantly reduced the number of survival cells by 42.2% as compared with that in control cells (Fig. 6B).

Next, we tested whether ectopic expression of Np63 is capable of making tumor cells resistant to arsenic treatment. For this purpose, ME-180 cells were transiently transfected with an empty vector or a vector expressing Np63α for 24 h and then treated with or without arsenic trioxide for 48 h. We found that the level of Np63α was increased significantly in ME-180 cells transfected with a vector expressing Np63α.
regardless of arsenic treatment (Fig. 7A, compare lanes 1 and 3 with lanes 2 and 4, respectively). As expected, we found that arsenic also decreased the ectopically expressed ΔNp63α protein (Fig. 7A, compare lane 2 with lane 4). Furthermore, we found that upon arsenic treatment, ectopic expression of ΔNp63 significantly increased the number of survival cells by 1.6-fold of that in cells transfected with an empty vector. We would like to mention that short-term overexpression of ΔNp63 alone had little effect on cell survival in ME-180 cells (Fig. 7B). Together, these results suggest that ΔNp63 plays a role in arsenic-induced inhibition on cell survival and that knockdown of ΔNp63 sensitizes tumor cells to arsenic trioxide.

### DISCUSSION

ΔNp63 is often highly expressed in squamous cell carcinomas (11, 12, 44). In addition to transactivation of pro-survival genes, ΔNp63 protein is dominant-negative over TAp63, TAp73, and wild-type p53 (2, 7, 15, 23, 24). Thus, the imbalance of ΔNp63 with TA isoforms of the p53 family members and wild-type p53 may lead to tumorigenesis via altered expression.

---

**FIGURE 4. Arsenic trioxide induces expression of Pirh2 E3 ligase.** A and B, Western blot analyses were prepared with extracts from HaCaT (A) and ME-180 (B) cells left untreated or treated with 5 μM arsenic trioxide for 3–9 h and then probed with antibodies against Pirh2 and actin, respectively. C, the level of Pirh2 transcript is increased by arsenic trioxide. RT-PCR analysis was performed with total RNAs isolated from HaCaT cells left untreated or treated with 5 μM arsenic trioxide for 6 or 12 h. Actin mRNA was amplified as a loading control. D, schematic presentation of the Pirh2 promoter luciferase reporters. E, arsenic treatment transactivates the Pirh2 promoter. The dual luciferase assay was performed with HaCaT cells that were cotransfected with 0.5 μg of a luciferase reporter (LUC) and 3 ng of pRL-SV40-Renilla vector for 24 h and then left untreated or treated with 5 μM arsenic trioxide for 8 h. *, p < 0.05.

---

**FIGURE 5. Knockdown of Pirh2 inhibits whereas ectopic expression of Pirh2 promotes arsenic-induced degradation of ΔNp63 protein.** A and B, Western blot analyses were prepared with extracts from ME-180 (A) and HaCaT (B) cells that were transfected with scrambled siRNA or siRNA against Pirh2 for 3 days and then treated with 5 μM arsenic trioxide for 9 h. The blots were then probed with antibodies against Pirh2, ΔNp63α, and actin, respectively. C and D, Western blot analyses were prepared with extracts from ME-180 (C) and HaCaT (D) cells transfected with pcDNA3 or pcDNA3–2×FLAG-Pirh2 for 2 days and then treated with 5 μM arsenic trioxide for 9 h. The blots were then probed with antibodies against the FLAG tag, ΔNp63α, and actin, respectively.

---

**FIGURE 6. Knockdown of ΔNp63 sensitizes tumor cells to arsenic treatment.** A, Western blot analysis was performed with extracts from ME-180 cells that were transfected with scrambled siRNA or siRNA against p63 for 1 day and then untreated or treated with 7.5 μM arsenic trioxide for 2 days. The blots were then probed with antibodies against p63 and actin, respectively. B, ME-180 cells were treated as in A. The survival cells were collected and counted. *, p < 0.05.

---

**FIGURE 7. Ectopic expression of ΔNp63 makes cells resistant to arsenic treatment.** A, Western blot analysis was performed with extracts from ME-180 cells that were transfected with pcDNA3 or pcDNA3–Myc-ΔNp63 for 1 day and then left untreated or treated with 7.5 μM arsenic trioxide for 2 days. The blots were then probed with antibodies against p63 and actin, respectively. B, ME-180 cells were treated as in A. The survival cells were collected and counted. *, p < 0.05.
ΔNp63, a Novel Target of Arsenic

of genes related to cell cycle arrest, apoptosis, and inhibition of metastasis. Here we showed that arsenic trioxide, a frontline therapeutic agent for acute promyelocytic leukemia, inhibits ΔNp63 but not TAp63 expression in time- and dose-dependent manners. In addition, we found that arsenic trioxide destabilizes ΔNp63 protein via the proteasome-dependent pathway. Importantly, we found that knockdown of ΔNp63 sensitizes whereas ectopic expression of ΔNp63 inhibits growth suppression induced by arsenic trioxide. Thus, identification of arsenic trioxide as an effective agent for ΔNp63 but not TAp63 degradation provides a promising approach to promptly decrease the level of ΔNp63 in tumor cells. This finding is of vital importance and potentially developed as a therapeutic strategy to tumors highly expressing ΔNp63, given that high ΔNp63 expression confers proliferative and chemoresistant advantage to tumor cells (16–18).

In an effort to probe into the mechanisms by which arsenic promotes ΔNp63 degradation, we found that arsenic potently up-regulates the level of Pirh2 E3 ligase. Knockdown of Pirh2 inhibits whereas ectopic expression of Pirh2 promotes arsenic-induced degradation of ΔNp63 protein. Pirh2, a p53-induced RING finger E3 ubiquitin ligase, is involved in the negative regulation of both TAp63 and ΔNp63 expression through physical interaction and ubiquitin-mediated and proteasome-dependent proteolysis (43). Interestingly, we found that the protein level of ΔNp63 but not TAp63 is decreased by arsenic. This result is similar to the selective decrease of mutant p53 protein, but not wild-type p53 protein, in arsenic-treated cells (32). This may be due to arsenic-activated signal pathways related to reactive oxygen species (45, 46). Consistent with the postulation, reactive oxygen species generated from hypoxia and reoxygenation increase the expression of p63 protein in human lymphocytes (47), a group of cells in which TAp63 is dominantly expressed (48).

Previously, Pirh2 was regarded as an oncogene because of overexpression in tumor tissues (49, 50) and targeting several expressed (48). Interestingly, we found that the protein level of ΔNp63 but not TAp63 is decreased by arsenic. This level of ΔNp63 and potentially developed as a therapeutic strategy to tumors (16–18).

and has transcriptional repressor activity that is reduced by Hay-Wells syndrome-derived mutations. Mol. Cell. Biol. 23, 2264–2276

3. King, K. E., and Weinberg, W. C. (2007) p63: defining roles in morphogenesis, homeostasis, and neoplasia of the epidermis. Mol. Carcinog. 46, 716–724

4. Vanbokhoven, H., Melino, G., Candi, E., and Declercq, W. (2011) p63, a story of mice and men. J. Invest. Dermatol. 131, 1196–1207

5. Osada, M., Ohba, M., Kawahara, C., Ishioka, C., Kanamaru, R., Katoh, I., Ikawa, Y., Nimura, Y., Nakagawara, A., Obinata, M., and Ikawa, S. (1998) A new human p53 homologue. Nat. Med. 4, 839–843

6. Trink, B., Okami, K., Wu, L., Sriruanpong, V., Jen, J., and Sidransky, D. (1998) A new human p53 homologue. Nat. Med. 4, 747–748

7. Yang, A., Kaghad, M., Wang, Y., Gillette, E., Fleming, M. D., Dötsch, V., Andrews, N. C., Caput, D., and McKeon, F. (1998) p53 homolog at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. Mol. Cell 2, 305–316

8. Dohn, M., Zhang, S., and Chen, X. (2001) p63α and ΔNp63α can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. Oncogene 20, 3193–3205

9. Flores, E. R., Sengupta, S., Miller, J. B., Newman, J. J., Bronson, R., Crowley, D., Yang, A., McKeon, F., and Jacks, T. (2005) Tumor predisposition in mice mutant for p63 and p73. Evidence for broader tumor suppressor functions for the p53 family. Cancer Cell 7, 363–373

10. Guo, X., Keyes, W. M., Papazoglou, C., Zuber, J., Li, W., Lowe, S. W., Vogel, H., and Mills, A. A. (2009) TAp63 induces senescence and suppresses tumorigenesis in vivo. Nat. Cell Biol. 11, 1451–1457

11. Massion, P. P., Tafslan, P. M., Jamshedur Rahman, S. M., Yildiz, P., Shyr, Y., Edgerton, M. E., Westfall, M. D., Roberts, J. R., Pietenpol, J. A., Carbone, D. P., and Gonzalez, A. L. (2005) Significance of p63 amplification and overexpression in lung cancer development and prognosis. Cancer Res. 65, 7113–7121

12. Nonaka, D. (2012) A study of ΔNp63 expression in lung non-small cell carcinomas. Am. J. Surg. Pathol. 36, 895–899

13. Hibi, K., Trink, B., Patturajan, M., Westra, W. H., Caballero, O. L., Hill, D. E., Ratovitski, E. A., Jen, J., and Sidransky, D. (2000) AL5 is an oncogene amplified in squamous cell carcinoma. Proc. Natl. Acad. Sci. U.S.A. 97, 5462–5467

14. Boldrup, L., Coates, P. I., Gu, X., and Nylander, K. (2007) ΔNp63 isoforms regulate CD44 and keratins 6, 4, 16 and 19 in squamous cell carcinoma of head and neck. J. Pathol. 213, 384–391

15. Mundt, H. M., Stremmel, W., Melino, G., Kramer, P. H., Schilling, T., and Müller, M. (2010) Dominant negative (ΔN) p63α induces drug resistance in hepatocellular carcinoma by interference with apoptosis signaling pathways. Biochem. Biophys. Res. Commun. 396, 335–341

16. Li, X., Chen, J., Yi, Y., Li, C., and Zhang, Y. (2012) DNA damage down-regulates ΔNp63α and induces apoptosis independent of wild type p53. Biochem. Biophys. Res. Commun. 423, 338–343

17. Mitani, Y., Li, J., Weber, R. S., Lippman, S. L., Flores, E. R., Caulin, C., and El-Naggar, A. K. (2011) Expression and regulation of the ΔN and TAp63 isoforms in salivary gland tumorigenesis clinical and experimental findings. Am. J. Pathol. 179, 391–399

18. Zangen, R., Ratovitski, E., and Sidransky, D. (2005) ΔNp63α levels correlate with clinical tumor response to cisplatin. Cell Cycle 4, 1313–1315

19. Yan, W., and Chen, X. (2006) GIPX2, a direct target of p63, inhibits oxidative stress-induced apoptosis in a p53-dependent manner. J. Biol. Chem. 281, 7856–7862

20. Yan, W., and Chen, X. (2007) Targeted repression of bone morphogenetic protein 7, a novel target of the p53 family, triggers proliferative defect in ΔNp63-deficient breast cancer cells. Cancer Res. 67, 9117–9124

21. Barbieri, C. E., Perez, C. A., Johnson, K. N., Ely, K. A., Billheimer, D., and Pietenpol, J. A. (2005) IGFBP-3 is a direct target of transcriptional regulation by ΔNp63α in squamous epithelium. Cancer Res. 65, 2314–2320

22. Hakem, A., Bohgaki, M., Lemmers, B., Tai, E., Salmena, L., Matysiak-Zablocki, E., Jung, Y. S., Karaskova, J., Kaustov, L., Duan, S., Madore, J., Boutros, P., Sheng, Y., Chesi, M., Bergsagel, P. L., Perez-Ordonez, B., Mes-Masson, A. M., Penn, L., Squire, J., Chen, X., Jurisica, I., Arrowsmith, C., Sanchez, O., Benchimol, S., and Hakem, R. (2011) Role of Pirh2 in medi-
23. Crook, T., Nicholls, J. M., Brooks, L., O’Nions, J., and Alliday, M. J. (2000) High level expression of ΔN-p63, a mechanism for the inactivation of p53 in undifferentiated nasopharyngeal carcinoma (NPC)? *Oncogene* **19**, 3439–3444

24. Rocco, J. W., Leong, C. O., Kuperwasser, N., DeYoung, M. P., and Ellisen, L. W. (2006) p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. *Cancer Cell* **9**, 45–56

25. Emadi, A., and Gore, S. D. (2010) Arsenic trioxide, an old drug rediscovered.

26. Zhang, X. W., Yan, X. J., Zhou, Z. R., Yang, F. F., Wu, Z. Y., Sun, H. B., Liang, W. X., Song, A. X., Lallemant-Breitenbach, V., Jeanne, M., Zhang, Q. Y., Yang, H. Y., Huang, Q. H., Zhou, G. B., Tong, J. H., Zhang, Y., Wu, J. H., Hu, H. Y., de Thé, H., Chen, S. J., and Chen, Z. (2010) Arsenic trioxide controls the fate of the PML-RARα oncoprotein by directly binding PML. *Science* **328**, 240–243

27. Jeanne, M., Lallemant-Breitenbach, V., Ferhi, O., Koken, M., Le Bras, M., Duffort, S., Peres, L., Berthier, C., Soilihi, H., Raught, B., and de Thé, H. (2010) PML/RARA oxidation and arsenic binding initiate the antileukemia response of A2O3. *Cancer Cell* **18**, 88–98

28. Shackelford, D., Kenific, C., Blustajn, A., Waxman, S., and Ren, R. (2006) Targeted degradation of the AML1/MDS1/EVI1 oncoprotein by arsenic trioxide. *Cancer Res.* **66**, 11360–11369

29. Kim, J., Lee, J. J., Kim, J., Gardner, D., and Beachy, P. A. (2010) Arsenic antagonizes the Hedgehog pathway by preventing ciliary accumulation and reducing stability of the Gli2 transcriptional effector. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 13432–13437

30. Mann, K. K., Colombo, M., and Miller, W. H., Jr. (2008) Arsenic trioxide decreases AKT protein in a caspase-dependent manner. *Mol. Cancer Ther.* **7**, 1680–1687

31. Zhang, Q. Y., Mao, J. H., Liu, P., Huang, Q. H., Lu, J., Xie, Y. Y., Weng, L., Zhang, Y., Chen, Q., Chen, S. J., and Chen, Z. (2009) A systems biology understanding of the synergistic effects of arsenic sulfide and Imatinib in BCR/ABL-associated leukemia. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 3378–3383

32. Yan, W., Zhang, Y., Zhang, J., Liu, S., Cho, S. I., and Chen, X. (2011) Mutant p53 protein is targeted by arsenic for degradation and plays a role in arsenic-mediated growth suppression. *J. Biol. Chem.* **286**, 17478–17486

33. Jung, Y. S., Liu, G., and Chen, X. (2010) Pirh2 E3 ubiquitin ligase targets DNA polymerase η for 20 S proteasomal degradation. *Mol. Cell. Biol.* **30**, 1041–1048

34. Debnath, J., Muthuswamy, S. K., and Brugge, J. S. (2003) Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods* **30**, 256–269

35. Carroll, D. K., Carroll, J. S., Leong, C. O., Cheng, F., Brown, M., Mills, A. A., Brugge, J. S., andEllisen, L. W. (2006) p63 regulates an adhesion programme and cell survival in epithelial cells. *Nat. Cell Biol.* **8**, 551–561

36. Zhang, J., Jun Cho, S., and Chen, X. (2010) RNPC1, an RNA-binding protein and a target of the p53 family, regulates p63 expression through mRNA stability. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 9614–9619

37. Shen, Y., Shen, Z. X., Yan, H., Chen, J., Zeng, X. Y., Li, J. M., Li, X. S., Wu, W., Xiong, S. M., Zhao, W. L., Tang, W., Wu, F., Liu, Y. F., Niu, C., Wang, Z. Y., Chen, S. J., and Chen, Z. (2001) Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia. A comparison with conventional dosage. *Leukemia* **15**, 735–741

38. Su, X., Chakravarti, D., Cho, M. S., Liu, L., Gi, Y. J., Lin, Y. L., Leung, M. L., El-Naggar, A., Creighton, C. J., Suraokar, M. B., Wistuba, I., and Flores, E. R. (2010) TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. *Nature* **467**, 986–990

39. Galli, F., Rossi, M., D’Alessandra, Y., De Simone, M., Lopardo, T., Haupt, Y., Alsheich-Bartok, O., Anzi, S., Shaulian, E., Calabrò, V., La Mantia, G., and Guerri, L. (2010) MDM2 and Fbw7 cooperate to induce p63 protein degradation following DNA damage and cell differentiation. *J. Cell Sci.* **123**, 2423–2433

40. Li, Y., Zhou, Z., and Chen, C. (2008) WW domain-containing E3 ubiquitin protein ligase 1 targets p63 transcription factor for ubiquitin-mediated proteasomal degradation and regulates apoptosis. *Cell Death Differ.* **15**, 1941–1951

41. Rossi, M., Aquelain, R. I., Neale, M., Candi, E., Salomoni, P., Knight, R. A., Croce, C. M., and Melino, G. (2006) The E3 ubiquitin ligase Itch controls the protein stability of p63. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12753–12758

42. Rossi, M., De Simone, M., Pollice, A., Santoro, R., La Mantia, G., Guerri, L., and Calabrò, V. (2006) Itch/AIP4 associates with and promotes p63 protein degradation. *Cell Cycle* **5**, 1816–1822

43. Jung, Y. S., Qian, Y., Yan, W., and Chen, X. (2012) Pirh2 E3 ubiquitin ligase modulates keratinocyte differentiation through p63. *J. Invest. Dermatol.*, in press

44. Murata, K., Ota, S., Niki, T., Goto, A., Li, C. P., Ruriko, U. M., Ishikawa, S., Aburutani, H., Kuriyama, T., and Fukayama, M. (2007) p63. Key molecule in the early phase of epithelial abnormality in idiopathic pulmonary fibrosis. *Exp. Mol. Pathol.* **83**, 367–376

45. Pellicano, H., Feng, L., Zhou, Y., Carew, J. S., Hileman, E. O., Plunkett, W., Keating, M. J., and Huang, P. (2003) Inhibition of mitochondrial respiration: a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism. *J. Biol. Chem.* **278**, 37832–37839

46. Jing, Y., Dai, J., Chalmers-Redman, R. M., Tatton, W. G., and Waxman, S. (1999) Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. *Blood* **94**, 2102–2111

47. Choi, J. Y., Kim, B. M., Kim, Y. J., Woo, H. D., and Chung, H. W. (2007) Hypoxia/reoxygenation-induced cytotoxicity in cultured human lymphocytes. *Biochem. Biophys. Res. Commun.* **352**, 366–371

48. Di Como, C. J., Urist, M. J., Babayan, I., Drobnjak, M., Hedvat, C. V., Teruya-Feldstein, J., Pohar, K., Hoo, A., and Cordon-Cardo, C. (2002) p63 expression profiles in human normal and tumor tissues. *Clin. Cancer Res.* **8**, 494–501

49. Wang, X. M., Yang, L. Y., Guo, L., Fan, C., and Wu, F. (2009) p53-induced RING-H2 protein, a novel marker for poor survival in hepatocellular carcinoma after hepatic resection. *Cancer* **115**, 4554–4563

50. Logan, I. R., Gaughan, L., McCracken, S. R., Sapountzi, V., Leung, H. Y., and Robson, C. N. (2006) Human PIRH2 enhances androgen receptor signaling through inhibition of histone deacetylase I and is overexpressed in prostate cancer. *Mol. Cell. Biol.* **26**, 6502–6510

51. Leng, R. P., Lin, Y., Ma, W., Wu, H., Lemmers, B., Chung, S., Parant, J. M., Lozano, G., Hakem, R., and Benchimol, S. (2003) Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. *Cell* **112**, 779–791

52. Jung, Y. S., Qian, Y., and Chen, C. (2011) The p73 tumour suppressor is targeted by Pirh2 RING finger E3 ubiquitin ligase for the proteasome-dependent degradation. *J. Biol. Chem.* **286**, 35388–35395

53. Hattori, T., Isobe, T., Abe, K., Kitukuchi, H., Kitagawa, K., Oda, T., Uchida, C., and Kitagawa, M. (2007) Pirh2 promotes ubiquitin-dependent degradation of the cyclin-dependent kinase inhibitor p27Kip1. *Cancer Res.* **67**, 10789–10795