Chemosensitization of head and neck cancer cells by PUMA

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Abstract

Head and neck squamous cell carcinoma (HNSCC) ranks the eighth most common cancer worldwide. The patients often present with advanced disease, which responds poorly to chemoradiation therapy. PUMA is a BH3-only Bcl-2 family protein and a p53 target that is required for apoptosis induced by p53 and various chemotherapeutic agents. In this study, we found that PUMA induction by chemotherapeutic agents is abrogated in most HNSCC cell lines. Adenoviral gene delivery of PUMA induced apoptosis and chemosensitization more potently than did adenoviral delivery of p53 in HNSCC cells. Finally, we showed that PUMA suppressed the growth of HNSCC xenograft tumors and sensitized them to cisplatin through induction of apoptosis. Our data suggest that absence of PUMA activation in HNSCC cells contributes to chemoresistance and that gene therapy with PUMA might be an efficient substitute for p53 to enhance the responses of HNSCC cells to chemotherapy. [Mol Cancer Ther 2007;6(12):3180–8]

Introduction

Head and neck cancer ranks the eighth most common cancer worldwide (1). About 95% of head and neck tumors are squamous cell carcinoma (HNSCC). Conventional treatment options for HNSCC include surgery, radiation therapy, and chemotherapy. Unfortunately, 70% or more of the patients present with stage III or IV disease with a 5-year survival rate of 30% in spite of treatment (1). The mortality is mostly due to a high rate of tumor recurrence and the development of second primary tumors (2). Therefore, a better understanding of the molecular mechanisms of HNSCC pathogenesis is expected to facilitate the development of novel therapies for this disease.

Emerging evidence suggests that deregulated programmed cell death or apoptosis is a major contributor to tumor initiation, progression, and development of acquired resistance to anticancer therapies (3–6). Apoptosis induced by a wide range of anticancer agents is largely mediated through the intrinsic pathway by the Bcl-2 family proteins (7–9). An increased understanding of deregulated apoptosis in cancer has led to efforts to restore apoptosis in cancer cells for therapeutic purposes (10). For example, a defective p53 pathway is one of the most common signatures of human cancer and is associated with poor response to therapy in some cancers including HNSCC (11). Several recent reports using sophisticated animal models showed that restoration of p53 function induced tumor regression, owing in part to p53-induced apoptosis (12–14). The combination of p53 restoration with DNA-damaging agents further increased the survival of tumor-bearing mice in a lymphoma model (12). Moreover, p53 replacement gene therapy has been tested extensively in preclinical and clinical settings and shown to improve the therapeutic responses of a number of solid tumors including HNSCC (15, 16). Most of these studies also showed that the enhanced therapeutic effect is associated with increased apoptosis (15, 16). Several recent studies also showed that small-molecule compounds that mimic the functions of the BH3 domain or the mitochondrial apoptopgenic protein Smac sensitized cancer cells or xenograft tumors to conventional chemotherapies by enhancing mitochondria-mediated apoptosis (17, 18).

We and others discovered PUMA as a transcriptional target of p53 and a potent apoptosis inducer in cancer cells (19–21). PUMA is a member of the BH3-only Bcl-2 protein family, members of which are suggested to initiate apoptosis in a tissue and stimulus-specific manner (6, 22). Biochemically, PUMA functions through Bax/Bak by antagonizing the antiapoptotic activities of the Bcl-2 like proteins, including, but not limited to, Bcl-2 and Bcl-xL, to trigger mitochondrial dysfunction and caspase activation (23, 24). Mounting evidence suggests that PUMA is an essential mediator of apoptosis induced by a wide range of anticancer agents. For example, PUMA is induced by DNA-damaging agents in a p53-dependent manner (19, 23, 25). Targeted deletion of PUMA resulted in resistance to apoptosis induced by p53, DNA-damaging agents, γ-irradiation, and hypoxia in human colorectal cancer cells (23). Moreover, studies with PUMA-deficient mice also showed that PUMA is required for apoptosis induced by oncogenes and DNA-damaging agents in several cell types.
(26, 27). Although PUMA has been shown to regulate anti-cancer drug-induced apoptosis in a variety of cell types, its role in the therapeutic response of HNSCC cells has not been studied.

In this study, we examined PUMA expression in HNSCC cells following treatment with anticancer agents and its role in the induction of apoptosis and chemosensitization in vitro and in vivo. Our results showed that PUMA is not induced by commonly used chemotherapies in most HNSCC cell lines. High levels of adenoviral delivered PUMA expression alone induce extensive apoptosis in HNSCC cells, whereas lower levels enhance the therapeutic response of HNSCC cells to chemotherapy.

Materials and Methods

Cell Culture and Drug Treatment

The cell lines UPCI:SCC003, UPCI:SCC104, UPCI:SCC105, UPCI:SCC116 (28, 29), 1483 (30), UPCI-15B (15B; ref. 31), UM-SCC-22A, UM-SCC-22B, JHU-012 (32), and two early-passage normal human keratinocyte primary cultures, HN1771 and HN1871 (28), used in the study were from the University of Pittsburgh Cancer Institute (UPCI) Head and Neck cancer program. All cell lines were maintained at 37°C in 5% CO2. Cell culture media included DMEM (Mediatech) for 1483 and 15B cells, RPMI 1640 (Cellgro) for JHU-012 cells, and EMEM (Invitrogen) for the UPCI:SCC cell lines and the primary cultures. The cell culture media were supplemented with 10% fetal bovine serum (HyClone), 100 units/mL penicillin, and 100 µg/mL streptomycin (Invitrogen). The anticancer drugs used in the study, including Adriamycin, cisplatin, 5-fluorouracil (5-FU), etoposide, mitomycin C, and Taxol, were purchased from Sigma. Caspase inhibitors including the pan-caspase inhibitor Z-VAD-fmk, caspase-3 inhibitor Z-DEVD-fmk, and caspase-9 inhibitor Z-LEHD-fmk were purchased from R&D Systems. All drugs were dissolved in DMSO and diluted to the appropriate concentrations with cell culture media. Some cells were exposed to γ-irradiation at 8 Gy. For combination treatments, cells were infected with adenoviruses for 24 h before drug treatment or γ-irradiation in virus-free media.

Adenoviruses

The recombinant adenoviruses, Ad-PUMA, Ad-ΔBH3, and Ad-p53, constructed using the Ad-Easy system, were previously described (23, 33). High-titer viruses were produced in HEK-293 cells and purified by CsCl gradient ultracentrifugation.

Apoptosis and Growth Assays

After treatment, adherent and floating cells were harvested and analyzed for apoptosis by nuclear staining with Hoechst 33342. A minimum of 300 cells were analyzed for each treatment. The Hoechst-stained cells were analyzed by flow cytometry to determine the fraction of sub-G1 cells. Cell growth was measured by the 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay in 96-well plates (2,500 cells per well) using CellTiter 96 AQueous One Solution (Promega) following the manufacturer’s instructions. A490 nm was measured with a Victor III (Perkin-Elmer/Wallace) plate reader. Each experiment was done in triplicate and repeated at least twice. For colony formation assays, equal numbers of cells were subjected to various treatments and plated into 6- or 12-well plates at different dilutions. Colonies were visualized by crystal violet staining 11 to 14 days after plating as previously described (33).

Western Blotting and Antibodies

Western blotting was done as previously described (19). The antibodies used for Western blotting included those against caspase-3 (Stressgen), caspase-9 (Cell Signaling Technology), α-tubulin, p21 (EMD Chemicals, Inc.), hemagglutinin (for Ad-PUMA and Ad-ΔBH3), p53, p27, c-Myc, cyclin D1 (Santa Cruz Biotechnology), and PUMA (23).

Xenograft Tumors and Tissue Staining

All animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Xenograft tumors (~50–100 mm3) were established by s.c. injection of 5 × 106 1483 cells into both flanks of 5- to 6-week-old female athymic nude mice (Harlan). A total of three intratumoral injections of Ad-PUMA or Ad-ΔBH3 in 100 µL of PBS were administered (on days 1, 3, and 5). In the combination experiment, cisplatin (3 mg/kg/d) was injected i.p. into the mice (on days 6–10). Tumor growth was monitored every other day with calipers to calculate tumor volumes according to the formula (length × width2) / 2. To avoid potential systemic effects of different viruses, Ad-PUMA and Ad-ΔBH3 were injected into separate tumors in the same animals. Six tumors were analyzed for each group.

Two additional tumors in each group were harvested during the experiments and embedded in optimum cutting temperature compound (Tissue-Tek). In the single-agent experiments, tumors were harvested 48 h after the second Ad-PUMA treatment (day 5). In the combination experiments, tumors were harvested following the second treatment of cisplatin (day 8). Mice were injected i.p. with a single dose of bromodeoxyuridine (BrdUrd, dissolved in PBS to a final concentration of 30 mg/mL) at 150 mg/kg of body weight, 2 h before sacrifice to label cells in S phase. Histologic and immunofluorescence analysis were done using 5-µm frozen sections. The expression of transgene was determined by fluorescence microscopic analysis of green fluorescent protein. All images were acquired with a Nikon TS800 fluorescence microscope using SPOT camera imaging software.

Terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining on frozen sections was done with recombinant terminal transferase (Roche) and dUTP-Alexa 594 (Invitrogen) according to the manufacturers’ instructions and counterstained with 4',6-diamidino-2-phenylindole. Apoptotic cells were counted under a fluorescence microscope in randomly chosen fields, and the apoptosis index was calculated as a percentage of TUNEL-positive cells in at least 1,000 scored cells.

BrdUrd incorporation was visualized with anti–BrdUrd-Alexa 594 antibody (Molecular Probes) on frozen sections.
and nuclei were visualized with 4',6-diamidino-2-phenylindole. The proliferation index was calculated by counting cells under a fluorescence microscope in several randomly chosen fields. The BrdUrd index was calculated as a percentage of BrdUrd-positive cells in at least 1,000 scored cells.

**Statistical Analysis**

Statistical analysis was done with GraphPad Prism IV software. P values were calculated with Student’s t test or two-way ANOVA. P < 0.05 was considered significant. The mean values ± SD were displayed in the figures.

**Results**

**PUMA Induction by Chemotherapeutic Agents Is Abolished in Head and Neck Cancer Cells**

PUMA is normally expressed at low basal levels and can be induced by p53 or DNA-damaging agents (19, 20, 26, 27, 34). To investigate the regulation of PUMA by anticancer agents in head and neck cancer cells, eight HNSCC cell lines were treated with two commonly used chemotherapeutic agents, 5-FU (50 μg/mL) and Adriamycin (0.2 μg/mL), which are known to induce PUMA in colon and lung cancer cells with wild-type p53 (19, 34). The expression of PUMA was analyzed by Western blotting. PUMA was not induced by 5-FU in six of eight cell lines, and only slightly induced in two cell lines (UPCI:SCC104 and UM-SCC-22B; Fig. 1A). Similar results were obtained with Adriamycin (data not shown). We then treated three HNSCC cell lines with cisplatin, an alkylating agent commonly used in HNSCC treatment. PUMA was not induced in any of these lines by cisplatin even when p53 was induced (15B; Fig. 1B). Four of the nine HNSCC lines were reported to have wild-type p53 (Supplementary Table S1; refs. 30, 32, 35, 36). Unlike in the cancer cell lines, PUMA was found to be induced by 5-FU and Adriamycin in two normal oral keratinocyte primary cultures treated with 5-FU (50 μg/mL) or Adriamycin (Adr; 0.2 μg/mL). D, the relative levels of PUMA in the cell lines or primary cultures following 5-FU treatment were analyzed and quantitated by densitometry.

**PUMA Induces Extensive Apoptosis in HNSCC Cells**

The lack of PUMA induction in HNSCC cells prompted us to investigate the effects of PUMA expression on growth and apoptosis in these cells. Six head and neck cancer cell lines were infected with an adenovirus expressing PUMA (Ad-PUMA) or a control adenovirus (Ad-DH3) expressing PUMA lacking the BH3 domain, which is essential for its proapoptotic function (23). All of the cell lines were infected by 40 MOI (multiplicity of infection) of adenovirus, with efficiencies between 80% and 100% based on GFP-positive cells (Fig. 2B and data not shown). Ad-PUMA, but not Ad-DH3, caused massive apoptosis in these cells 48 h after infection, as determined...
PUMA Induces Apoptosis and Suppresses Growth More Potently than Does p53 in HNSCC Cells

Numerous in vitro and in vivo studies have shown that p53 adenovirus (Ad-p53) induces apoptosis and growth suppression in cancer cells (16). To test the potential use of PUMA in cancer gene therapy, Ad-PUMA and Ad-p53, an adenovirus expressing p53 constructed in the same system, were compared for their potency in apoptosis induction and growth suppression in 1483 and 15B cells. These two cell lines were selected because of their robust growth and ability to form xenograft tumors in nude mice (1483). Using several titers of Ad-PUMA and Ad-p53, we found that Ad-p53 was inefficient in apoptosis induction in 1483 and 15B cells, even at 80 and 160 MOI, compared with Ad-PUMA at 40 and 80 MOI (Fig. 3A and data not shown). Ad-p53 was able to induce apoptosis at titers >200 MOI in some HNSCC lines (data not shown). This result was also confirmed by analyzing the sub-G1 fraction of cells using flow cytometry (Supplementary Fig. S1A).6 Because p53 induces growth suppression and/or apoptosis, depending on the cellular context, we compared the effects of Ad-PUMA and Ad-p53 on cell growth by the MTS assay. Ad-PUMA seemed to suppress cell proliferation better than Ad-p53 in 1483 and 15B cells at 24 and 48 h (Fig. 3B).

Consistent with the above observations, Ad-PUMA, but not Ad-p53, was found to induce activation of caspase-3 and caspase-9 by Western blotting (Fig. 3C). PUMA-induced apoptosis was efficiently blocked by the pan-caspase inhibitor Z-VAD in 1483 and 15B cells. The caspase-3 and caspase-9 inhibitors also blocked PUMA-induced apoptosis, but to a lesser extent (Supplementary Fig. S1B and C).6 In addition, Ad-p53 induced high levels of p21, but not PUMA, consistent with its growth-suppressive effects on HNSCC cells (Fig. 3C and B). We also examined expression of several other cell cycle–related proteins and found that p27 and cyclin D1 levels were suppressed by PUMA but not by p53 (Fig. 3C). c-Myc levels were not significantly affected by PUMA or p53 (Fig. 3C). These results show that PUMA is more potent than p53 in apoptosis induction and growth suppression in HNSCC cells.
The important role of PUMA in DNA damage–induced apoptosis predicts that elevated PUMA expression can sensitize cancer cells to some anticancer agents. To test this hypothesis, 1483 cells were treated with a lower dose of Ad-PUMA (10 MOI, which is not sufficient to induce apoptosis) alone or in combination with γ-irradiation or chemotherapeutic agents including mitomycin C, Adriamycin, cisplatin, etoposide, Taxol, and 5-FU. PUMA, but not PUMA-D\(\text{BH3}\), was found to significantly enhance the growth inhibitory effects of these agents (Fig. 4A). Interestingly, PUMA seems to synergize best with several agents that induce either DNA strand breaks or cross-links, such as mitomycin C, Adriamycin, cisplatin, and γ-irradiation (Fig. 4A). For example, growth suppression increased >5-fold when Ad-PUMA was combined with mitomycin C or Adriamycin compared with the drug alone or in combination with Ad-\(\text{BH3}\). Furthermore, we found that PUMA significantly enhanced the growth inhibitory effects of Adriamycin at concentrations between 0.05 and 0.8 μg/mL in both 1483 and 15B cells (Fig. 4B).

We then tested whether apoptosis induction is a major mechanism of PUMA-mediated chemosensitization. Ad-PUMA (10 MOI) and Adriamycin (0.2 μg/mL) alone did not induce significant apoptosis in 15B cells, but their combination dramatically increased apoptosis (Fig. 4C). In contrast, the combination of Ad-p53 (10 MOI) and Adriamycin did not result in an obvious increase in apoptosis. Analyzing apoptosis using other approaches, including cell cycle analysis and Annexin V staining, confirmed these results (data not shown). As we expected, the Adriamycin and Ad-PUMA combination resulted in significant activation of caspase-3 and caspase-9, whereas the single agent or the Adriamycin and Ad-p53 combination did not (Fig. 4D). These data indicate that PUMA synergizes with different chemotherapeutic agents and γ-irradiation to induce growth suppression and apoptosis in HNSCC cells.

**PUMA Sensitizes HNSCC Cells to Chemotherapeutic Agents and γ-Irradiation**

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**PUMA Suppresses HNSCC Tumor Growth In vivo**

To determine whether PUMA confers antitumor activity in vivo, established 1483 xenograft tumors (~100 mm\(^3\)) were treated with three intratumoral injections of Ad-PUMA (\(5 \times 10^8\) plaque-forming units), Ad-\(\text{BH3}\), or PBS.
Ad-ΔBH3 did not have an effect on tumor growth compared with PBS alone, with tumors reaching 12 times their initial volumes in 26 days (Fig. 6A and B). In contrast, Ad-PUMA treatment resulted in 67% growth suppression ($P < 0.001$; Fig. 5A). Analyzing tissue sections from tumors harvested 48 h after the second injection revealed that the transgenes were highly expressed in the tumors (Fig. 5B, GFP). Ad-PUMA, but not Ad-ΔBH3 treatment, significantly increased apoptosis and decreased proliferation in the tumors as assessed by TUNEL and BrdUrd staining, respectively (Fig. 5B). These data show that PUMA effectively inhibits the growth of established HNSCC tumors through apoptosis induction.

**PUMA Sensitizes HNSCC Xenografts to Cisplatin**

PUMA was found to enhance growth suppression induced by cisplatin in both short-term and long-term growth assays in vitro (Figs. 4A and 6A). We wanted to determine whether such effects can be obtained in the 1483 xenograft tumor model. To see the potential additive or synergistic effects, we used suboptimal doses of Ad-PUMA ($1.25 \times 10^8$ plaque-forming units/d for 3 days) and cisplatin (3 mg/kg/d for 5 days) in these experiments. Ad-PUMA or cisplatin treatment alone resulted in $\sim 35\%$ ($P < 0.01$) and 16% ($P < 0.01$) growth inhibition compared with Ad-ΔBH3 or PBS control, respectively (Fig. 6B). Analysis of tumor sections after the second cisplatin injection (day 8) revealed that the Ad-PUMA and cisplatin combination resulted in a significant increase in apoptosis and a decrease in cell proliferation compared with Ad-PUMA alone or the Ad-ΔBH3 and cisplatin combination (Fig. 6C and Supplementary Fig. S2). These results suggest that increased PUMA levels enhance the therapeutic responses of HNSCC tumors to cisplatin through apoptosis induction.

**Discussion**

**PUMA** is located in an area of chromosome 19q13.3 that frequently undergoes loss of heterozygosity in HNSCC, but the *PUMA* gene is not found to be mutated or silenced in HNSCC (32). Our study showed that PUMA induction by chemotherapeutic agents is abrogated in most HNSCC cells. Perhaps it is deregulated due to the defects in upstream signaling molecules including p53. Adenoviral

![Figure 4](mct.aacrjournals.org) PUMA sensitizes HNSCC to chemotherapeutic agents through apoptosis induction. In combination treatments, cells were infected by the indicated adenovirus at 10 MOI, then treated by chemotherapeutic agents or radiation in virus-free medium. A, 1483 cells were treated with chemotherapeutic agents and γ-irradiation alone, or in combination with Ad-PUMA or Ad-ΔBH3. Cell growth was measured at 48 h by the MTS assay. The ratios of growth inhibition conferred by the combinations over drug alone. MMC, mitomycin C; Oxa, oxaliplatin; Etp, etoposide; IR, γ-irradiation. B, the effects on the growth of cells treated with Adriamycin at different concentrations combined with Ad-PUMA or Ad-ΔBH3 were determined in 1483 and 15B cells. C, the cells were subjected to indicated treatments for 24 and 48 h. Apoptosis was quantitated by nuclear fragmentation assay. Adriamycin, 0.2 μg/mL. D, the expression of PUMA (ΔBH3), p53, p21, caspase-3, and caspase-9 was analyzed by Western blotting. Arrows, active forms of caspases.
gene delivery of PUMA resulted in apoptosis and enhanced sensitivity to chemotherapeutic agents and γ-irradiation in HNSCC cells, suggesting that adequate levels of PUMA are crucial for triggering apoptotic responses to these agents. Lack of PUMA induction may contribute to the chemoresistant phenotype of HNSCC cells. Our data also suggest that PUMA might be particularly useful when combined with DNA-damaging agents, such as mitomycin C, Adriamycin, cisplatin, or γ-irradiation, to induce cell killing, which is generally believed to be more effective in cancer treatment than cytostasis (4, 5). Inhibition of cell proliferation has been attributed to the antitumor effects of chemotherapy. Our data indicate that Ad-PUMA negatively affects several cell cycle regulators, suggesting a possible mechanism for enhanced growth suppression when combined with chemotherapy (Figs. 3C and 4A). These observations are consistent with the findings that PUMA plays a critical role in DNA damage–induced apoptosis and sensitizes lung and esophageal cancer cells to chemotherapeutic agents (23, 26, 27, 34, 37). It will be interesting to further investigate whether PUMA is an effective radiosensitizer and chemosensitizer in other tumor types.

The prevalence of p53 dysfunction in cancer and the importance of p53-mediated apoptosis in tumor initiation and progression have made p53 a very appealing target for cancer therapy (15). A recent study showed that restoration of p53 function in vivo induced tumor regression through apoptosis induction, and its combination with DNA-damaging agents enhanced therapeutic efficacy in a lymphoma model (12). These observations complemented the numerous earlier studies, in which nonreplicating adenoviruses expressing p53 were shown to suppress cancer cell growth and induce apoptosis (16). Combinations of p53 gene therapy with chemotherapy or radiation exhibit synergistic antitumor effects in clinical trials as well as in various in vitro and in vivo models (16). Particularly, adenoviral p53 gene therapy has been explored as a new modality in head and neck cancer, primarily due to its anatomic location and the morbidity and mortality largely related to local recurrence (2, 16). Just a few years ago, Gendicine, a p53 adenovirus, became the first approved cancer gene therapy product in the world to treat head and neck cancer in China and has shown encouraging results (38). Our data suggest that PUMA induces apoptosis and chemosensitization more potently than p53 itself in HNSCC cells, consistent with the findings reported in lung and esophageal cancer cells (34, 37). Based on these observations, PUMA might be a more effective radiosensitizer and chemosensitizer than p53 in solid tumors. However, intratumoral injection of Ad-PUMA has limitations in clinical settings, and therefore it will be interesting to explore other means of delivering PUMA gene to tumor cells.

One limitation of p53 gene therapy is for tumors that are positive for human papilloma virus. Studies indicate that 20% to 25% of head and neck cancers contain oncogenic human papilloma viruses, whereas >95% of cervical squamous cell carcinomas are linked to persistent human papilloma virus infection (39). The human papilloma virus–encoded E6 oncoprotein promotes p53 degradation and inactivation and renders tumors resistant to p53 gene therapy (39). PUMA has not been shown to be subjected to E6-mediated degradation. In addition, we and others have shown that some tumor cells are resistant to Ad-p53–induced apoptosis due to high levels of the
cyclin-dependent kinase inhibitor p21, a p53 target primarily involved in cell cycle arrest (refs. 23, 40; Fig. 4D). On the other hand, Ad-PUMA induces apoptosis in a variety of cancer cells independent of their p53 status (refs. 23, 34, 37; Fig. 2A). These observations suggest that selectively activating apoptosis rather than cell cycle arrest is therapeutically desirable. Based on its biological properties, PUMA gene therapy might be particularly useful in tumors that are resistant to p53 gene therapy.

Emerging evidence suggests that the BH3-only proteins are critical initiators of apoptosis and function in a tissue- and stimulus-specific manner (9, 22). Most BH3-only proteins selectively antagonize a subset of the antiapoptotic Bcl-2 family members, whereas PUMA, tBid, and Bim antagonize all known members including Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1, some of which are frequently overexpressed in cancer (22, 41). This might explain why PUMA and Bim are more potent in promoting apoptosis in a wide range of cancer cells compared with other BH3-only proteins (22). The studies with knockout animals also suggest that PUMA seems to be a major mediator of apoptosis induced by DNA damage, oncogenes, and growth factor deprivation in several cell types, and it integrates death signals in both a p53-dependent and a p53-independent manner (21, 26, 27). Tumor cells overcome a great deal of apoptotic pressure often by deregulating both antiapoptotic and proapoptotic proteins, and thus become highly dependent on these oncogenic changes (3). The so-called “oncogenic addition” of cancer cells might explain why proapoptotic genes like p53 or PUMA seem to be less toxic to normal cells and offer some degree of much needed therapeutic index in cancer treatment.

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