PUMA Sensitizes Lung Cancer Cells to Chemotherapeutic Agents and Irradiation

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Abstract

Purpose: Lung cancer, the leading cause of cancer mortality worldwide, is often diagnosed at late stages and responds poorly to conventional therapies, including chemotherapy and irradiation. A great majority of lung tumors are defective in the p53 pathway, which plays an important role in regulating apoptotic response to anticancer agents. PUMA was recently identified as an essential mediator of DNA damage–induced and p53-dependent apoptosis. In this study, we investigated whether the regulation of PUMA by anticancer agents is abrogated in lung cancer cells and whether PUMA expression suppresses growth of lung cancer cells and/or sensitizes lung cancer cells to chemotherapeutic agents and irradiation through induction of apoptosis.

Experimental Designs: The expression of PUMA was examined in lung cancer cells with different p53 status treated with chemotherapeutic agents. An adenovirus expressing PUMA (Ad-PUMA), alone or in combination with chemotherapeutic agents or γ-irradiation, was used to treat lung cancer cells. The growth inhibitory and apoptotic effects of PUMA in vitro and in vivo were examined. The mechanisms of PUMA–mediated growth suppression and apoptosis were investigated through analysis of caspase activation and release of mitochondrial apoptogenic proteins. The cytotoxicities of PUMA on cancer and normal/nontransformed cells were compared. The efficacy of PUMA and p53 in suppressing the growth of lung cancer cells was also compared.

Results: We showed that the induction of PUMA by chemotherapeutic agents is abolished in p53-deficient lung cancer cells. PUMA expression resulted in potent growth suppression of lung cancer cells and suppressed xenograft tumor growth in vivo through induction of apoptosis. Low dose of Ad-PUMA significantly sensitized lung cancer cells to chemotherapeutic agents and γ-irradiation through induction of apoptosis. The effects of PUMA are mediated by enhanced caspase activation and release of cytochrome c and apoptosis-inducing factor into the cytosol. Furthermore, PUMA seems to be selectively toxic to cancer cells and more efficient than p53 in suppressing lung cancer cell growth.

Conclusions: Our findings indicate that PUMA is an important modulator of therapeutic responses of lung cancer cells and is potentially useful as a sensitizer in lung cancer therapy.

Lung cancer is the leading cause of cancer mortality worldwide (1). The current treatment options for patients with advanced disease are limited to chemotherapy and radiation therapy, which unfortunately produce a low rate of response and virtually no cure. The 5-year survival rate, currently at 14%, has only been marginally improved in the last two decades despite the development of new therapeutic agents and improved patient care. Novel therapeutic interventions are critical for improvement of the survival and prognosis of lung cancer patients.

Cancer development is a multistage process which involves a number of genetic and epigenetic changes in the genes controlling cell survival, cell death, cell-cell communication, cell-microenvironment interactions, and angiogenesis (2, 3). It has become increasingly clear that abnormalities in cell death pathways play an important role in tumorigenesis and the development of resistance to chemotherapy and radiation therapy (4, 5). Most of the agents used in cancer therapy directly or indirectly damage DNA and induce apoptosis. Defects in the apoptotic machinery can lead to multidrug resistance (6). Therapeutic manipulation of the apoptotic pathways has become an attractive avenue to improve the clinical response of lung cancer patients (7–9).

Defective p53 pathway is one of the most common signatures of human cancer (2). p53 mutations occur in ~50% of non–small-cell lung cancers and >70% of small-cell lung cancers (10). A major physiologic function of p53 is to kill damaged or stressed cells through induction of apoptosis (11). p53 induces apoptosis by transactivation of its downstream
apoptotic regulators. p53 mutations in cancer cells almost invariably abolish this activity, indicating that the apoptotic function of p53 is critical for its tumor suppressor activity (11). Restoration of the p53 pathway by activating p53 itself or p53 downstream targets has been explored to improve efficacy of anticancer therapies (12).

PUMA was recently identified as a BH3-only Bcl-2 family protein and an essential mediator of p53-dependent and p53-independent apoptosis (13). PUMA is induced by DNA-damaging agents in a p53-dependent fashion and by non-genotoxic agents independent of p53 (14–16). Expression of PUMA rapidly kills a variety of human cancer cells (14, 15). PUMA is localized in the mitochondria and induces apoptosis by activating caspases through mitochondrial dysfunction (14, 15, 17). PUMA functions through other Bcl-2 family members, such as Bax, Bcl-2, and Bcl-XL. Deletion of PUMA in human cancer cells attenuated apoptotic response to p53, DNA-damaging agents, and hypoxia (17). PUMA-knockout mice recapitulated several major apoptotic deficiencies observed in p53-knockout mice (18, 19). However, the role of PUMA in therapeutic responses in lung cancer cells remains unclear.

In this study, we examined the regulation of PUMA by commonly used anticancer agents in lung cancer cells. We found that p53 mutations abolish the induction of PUMA by these agents. We also showed that PUMA is a potent and selective inducer of apoptosis in lung cancer cells and that expression of PUMA enhances the therapeutic responses of lung cancer cells to chemotherapeutic agents and γ-irradiation.

Materials and Methods

Cell culture and drug treatment. The cell lines used in the study were from American Type Culture Collection (Manassas, VA), except for the lung cancer cell lines 273T and 201T, which were from the University of Pittsburgh Cancer Institute lung cancer program, and the human primary fibroblast cell line WI-38, which was obtained from the Coriell Institute (Camden, NJ). All cell lines were maintained at 37°C and 5% CO₂. Cell culture media included RPMI 1640 (Mediatech, Herndon, VA) for all lung cancer cell lines, McCoy’s 5A (Invitrogen, Carlsbad, CA) for colorectal cancer cell line CHT116 and DLD1, DMEM (Mediatech) for 293 and 911 cells, and EMEM (Invitrogen) for WI-38. The cell culture media were supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen), except for EMEM, which was supplemented with 20% fetal bovine serum. Transfection was done with Lipofectamine 2000 (Invitrogen) following the instructions of the manufacturer. Reporter assays were carried out as previously described (14).

The anticancer drugs used in the study, including Adriamycin, oxaliplatin, cisplatin, 5-fluorouracil (5-FU), etoposide, and Taxol, were purchased from Sigma (St. Louis, MO). Caspase inhibitors, including the pan-caspase inhibitor Z-VAD-fmk, caspase-3 inhibitor Z-DEVD-fmk, caspase-8 inhibitor Z-IETD-fmk, and caspase-9 inhibitor Z-LEHD-fmk, were purchased from R&D Systems (Minneapolis, MN). All drugs and inhibitors were dissolved in DMSO and diluted to 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 16 to 18 hours before drug treatment or γ-irradiation.

Constructs and adenoviruses. The expression constructs used in the study included those expressing wild-type and different forms of mutant p53, including V143A, R175H, R249S, and R273H (20), and constructs expressing NH₂-terminal green fluorescent protein (GFP)–tagged proteins, including wild-type PUMA, PUMA lacking the BH3 domain (ΔBH3), or the mitochondrial targeting sequence of PUMA (C43; ref. 17). For reporter assays, the previously described PUMA luciferase reporter constructs Frag 1 and Frag 2 were cotransfected with the β-galactosidase reporter pCMVβ (Promega; ref. 14).

The recombinant adenoviruses Ad-PUMA, Ad-ΔBH3, and Ad-p53 were constructed using the Ad-Easy system as previously described (17, 21). High-titer viruses were produced in 293 cells and purified by CsCl gradient ultracentrifugation (22).

Apoptosis and growth assays. After treatment, attached and floating cells were harvested and analyzed for apoptosis by nuclear staining with propidium iodide (23). A minimum of 300 cells were analyzed for each treatment. The propidium iodide–stained cells were analyzed by flow cytometry to determine the fraction of sub-G₀ cells. Apoptosis was also analyzed by staining cells with Annexin V-Alexa 594 (Molecular Probes, Carlsbad, CA) counterstaining with 4′,6-diamidino-2-phenylindole, followed by flow cytometry. Cell growth was measured by

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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 the CellTiter 96 AQueous One Solution (Promega, Madison, WI) following the instructions of the manufacturer. A_{570} nm was measured using a Victor III (Perkin-Elmer/Wallace) plate reader. Each experiment was done in triplicate and repeated at least twice.

**Western blotting and antibodies.** Western blotting was done as previously described (24). The antibodies used for Western blotting included rabbit polyclonal antibodies against PUMA (17), caspase 9 (Cell Signaling Technology, Danvers, MA), caspase 3 (Stressgen, San Diego, CA), and HA (Santa Cruz Biotechnology, Santa Cruz, CA); monoclonal antibodies against α-tubulin (Oncogene Sciences, San Diego, CA), p21 (Oncogene Sciences), cytochrome c (BD Biosciences, San Jose, CA), cytochrome oxidase subunit IV (Molecular Probes), caspase 8 (Cell Signaling Technology), and p53 (DO1, Santa Cruz Biotechnology); and a goat antibody against apoptosis-inducing factor (Santa Cruz Biotechnology).

**Cellular fractionation.** Floating and attached cells were harvested from two 75-cm² (T75) flasks by centrifugation, resuspended in PBS, centrifuged at 10,000 g for 15 minutes to obtain cytosolic (supernatant) and mitochondrial (pellet) fractions.

**Reverse transcription-PCR.** Total RNA was isolated using the RNAGents Total RNA Isolation System (Promega) according to the instructions of the manufacturer. First-strand cDNA was synthesized using Superscript II reverse transcriptase (Invitrogen). Reverse transcription-PCR was done to amplify PUMA using the cycle conditions previously described (14). The primers used to amplify PUMA included 5′-ttcagcactgctgctcg-3′ and 5′-ccagttctgactcagc-3′. The primers for amplifying the control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5′-ctcagccacatggggaaggtga-3′ and 5′-atgattcggtctgctgata-3′.

**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 were established by s.c. injection of 4 × 10⁶ A549 or H1299 cells into both flanks of 5- to 6-week-old female athymic nude mice (Harlan, Indianapolis, IN). Tumor treatment was initiated by injecting each tumor-bearing mouse 5- to 6-week-old female athymic nude mice (Harlan, Indianapolis, IN). Western blotting and antibodies. The cells infected by Ad-PUMA, but not those infected by Ad-ΔBH3, had virtually no effect on cell growth compared with the untreated controls (Fig. 2A).

**Statistical analysis.** Statistical analysis was done using GraphPad Prism IV software. P values were calculated by Student’s t test or two-way ANOVA. The mean ± SD were displayed in the figures.

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**Results**

**PUMA Induction by chemotherapeutic agents is abolished in p53-deficient lung cancer cells.** PUMA is normally expressed at low levels in human tissues but can be induced by p53 or DNA-damaging agents (14, 15). To investigate the regulation of PUMA by anticancer agents in lung cancer cells, eight lung cancer cell lines with different p53 status were treated with Adriamycin (0.2 μg/mL, a DNA-damaging agent producing double-stranded DNA breaks and a chemotherapeutic drug commonly used for treating lung cancer (25). The expression of PUMA and p21, which mediate p53-dependent apoptosis and cell-cycle arrest, respectively, were analyzed (11). Both PUMA and p21 were found to be induced by Adriamycin in the wild-type p53 cell lines A549 and 128.88T, but not in the p53-mutant cell lines DMS53, 201T, 273T, and H1752 and the p53-null cell lines H1299 and Calu 1 (Fig. 1A). PUMA was also found to be induced only in the wild-type p53 lung cancer cells by another chemotherapeutic agent, 5-FU (50 μg/mL), and γ-irradiation (8 Gy; data not shown).

To determine whether the deficiency in PUMA induction is at the mRNA level, semiquantitative reverse transcription-PCR was used to examine PUMA in cells with and without Adriamycin treatment. PUMA transcripts were found to be increased in the wild-type p53 cells but not in the p53-mutant or p53-null cells (Fig. 1B). To test whether p53 activates the PUMA promoter in lung cancer cells, PUMA reporter constructs, along with wild-type p53 and several tumor-derived p53 mutants (10), were cotransfected into H1299 cells. The reporter containing the p53 responsive elements was activated by wild-type p53 but not by p53 mutants (Fig. 1C).

These results indicate that the induction of PUMA by chemotherapeutic agents in lung cancer cells is mediated by p53 and that this induction is abolished in p53-deficient lung cancer cells.

**PUMA profoundly suppresses growth of lung cancer cells through induction of apoptosis.** The proapoptotic function of PUMA and lack of PUMA induction in p53-deficient lung cancer cells prompted us to investigate whether PUMA suppresses lung cancer cell growth. Six lung cancer cell lines (A549, Caul 1, 128.88T, H1299, H1752, and DMS53) were infected with an adenovirus expressing PUMA (Ad-PUMA) or a control adenovirus (Ad-ΔBH3) expressing PUMA lacking the BH3 domain, which is essential for its proapoptotic function (14). For all six cell lines, at least 80% of the cells were infected by Ad-PUMA and Ad-ΔBH3 as indicated by the GFP signal (data not shown). After infection for 48 hours, cells were analyzed by 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Ad-PUMA was found to cause profound growth suppression in all the cell lines whereas Ad-ΔBH3 had virtually no effect on cell growth compared with the untreated cells (Fig. 2A).

The cells infected by Ad-PUMA, but not those infected by Ad-ΔBH3, underwent massive apoptosis revealed by nuclear staining, cell cycle analysis, and Annexin V staining (Fig. 2B and C). Activation of caspases 3, 8, and 9 was detected in all cell lines after Ad-PUMA infection and correlated with growth suppression (Fig. 2A and D). Interestingly, DMS53 cells were most sensitive to Ad-PUMA infection, showing signs of late apoptosis 48 hours after infection, including PUMA degradation and fully activated caspases (Fig. 2A and D; ref. 14). To examine whether PUMA-induced apoptosis in lung cancer cells is mediated through mitochondrial pathway (26, 27), cytosolic fractions were isolated from H1299 cells infected with Ad-PUMA and Ad-ΔBH3 for 48 hours, and then analyzed by...
Western blotting. Cytochrome c and apoptosis-inducing factor were found to be released into the cytosol in the cells infected with Ad-PUMA but not in those infected with Ad-ΔBH3 (Fig. 2E).

These data show that PUMA is a potent inducer of growth suppression and apoptosis in lung cancer cells and that PUMA promotes the release of mitochondrial apoptogenic proteins and caspase activation to induce apoptosis in lung cancer cells.

**PUMA sensitizes lung cancer cells to chemotherapeutic agents and γ-irradiation.** Abnormalities of apoptosis regulation have been shown to contribute to the development of resistance to chemotherapy and radiation therapy in cancer cells (4–6). The important role of PUMA in DNA damage-induced and p53-dependent apoptosis suggests that elevated PUMA expression may restore sensitivity of cancer cells to anticancer agents. To test this hypothesis, A549 lung cancer cells were treated with low dose of Ad-PUMA [10 multiplicity of infection (MOI)], alone or in combination with γ-irradiation or chemotherapeutic agents, including Taxol, 5-FU, oxaliplatin, cisplatin, etoposide, and Adriamycin. PUMA was found to significantly enhance the growth inhibitory effects of these chemotherapeutic drugs and γ-irradiation, with the synergy most pronounced when combined with DNA-damaging agents, including Adriamycin, cisplatin, etoposide, and γ-irradiation (Fig. 3A). For example, as much as 8-fold increase in growth suppression was achieved when Ad-PUMA was combined with Adriamycin. We also determined the IC_{50} of several chemotherapeutic agents in A549 cells with or without Ad-PUMA and found that Ad-PUMA significantly lowered the IC_{50} of these agents by 3-fold (Adriamycin) to over 10-fold (Taxol and 5-FU; Fig. 3B; Table 1).

We then determined whether PUMA expression sensitizes lung cancer cells to the anticancer agents through induction of apoptosis. A549 cells are resistant to apoptosis induced by Adriamycin (up to 2 μg/mL) and γ-irradiation (up to 8 Gy; data not shown).

**Table 1.** IC_{50} of the chemotherapeutics in A549 cells with or without Ad-PUMA

<table>
<thead>
<tr>
<th>Drug</th>
<th>-Ad-PUMA</th>
<th>+Ad-PUMA</th>
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<tbody>
<tr>
<td>Taxol</td>
<td>74.0 nmol/L</td>
<td>5.0 nmol/L</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>110.7 μmol/L</td>
<td>18.5 μmol/L</td>
</tr>
<tr>
<td>5-FU</td>
<td>77.8 μg/mL</td>
<td>7.5 μg/mL</td>
</tr>
<tr>
<td>Etoposide</td>
<td>48.0 μg/mL</td>
<td>6.0 μg/mL</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>0.76 μg/mL</td>
<td>0.22 μg/mL</td>
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not shown). Ad-PUMA (10 MOI) and Adriamycin (0.2 μg/mL) alone did not induce significant apoptosis in A549 cells (Fig. 4A). However, almost 90% of cells underwent apoptosis after the combination treatment for 72 hours (Fig. 4A). Similarly, a combination of γ-irradiation (8 Gy) with Ad-PUMA also led to a markedly enhanced apoptotic response in A549 cells [Fig. 4B]. In contrast, the control Ad-ΔBH3 had no effect when combined with Adriamycin or γ-irradiation (Fig. 4A and B). Analysis of apoptosis using other approaches, including nuclear staining and cell cycle analysis, confirmed these results (data not shown). Furthermore, the apoptosis was accompanied by enhanced activation of caspases 3, 8, and 9 (Fig. 4C), as well as release of cytochrome c and apoptosis-inducing factor into the cytosol (Fig. 4E). Pretreating the cells with caspase inhibitors significantly decreased the apoptosis and growth inhibitory effects of the combination treatments (Fig. 4D and data not shown). Similarly, PUMA was found to enhance apoptosis induced by Adriamycin and γ-irradiation in 128.8T cells (data not shown).

These data indicate that PUMA can synergize with different chemotherapeutic agents and irradiation to trigger the release of cytochrome c and apoptosis-inducing factor and caspase activation to initiate apoptosis in lung cancer cells.

**PUMA is selectively toxic to cancer cells and more potent than p53 in growth suppression.** Selectivity, or therapeutic index, is an important issue to consider for any therapeutic agent.
Thus, the toxicities of PUMA in several cancer and normal/nontransformed cell lines were evaluated. Cells were transfected with a vector expressing NH2-terminal GFP-fused wild-type PUMA, a control vector expressing PUMA lacking the BH3 domain (∆BH3), or a vector expressing the mitochondrial localization sequence of PUMA (C43; ref. 17). Because cells killed by PUMA do not express significant amount of GFP (examples shown in Fig. 5A), the toxicities of PUMA to different cell lines can be compared through analysis of GFP-positive cells after transfection. This assay is similar to the widely used β-galactosidase cotransfection method in assessing cytotoxicity (28). The fractions of GFP-positive cells were indistinguishable in all cell lines after transfection with the control vectors expressing ∆BH3 or C43 (Fig. 5A and B). However, transfection with wild-type PUMA diminished GFP-positive cells in the cancer cell lines, including the lung cancer cell lines A549, H1299, Calu 1, 128.88T, and 201T and the colon cancer cell lines HCT116 and DLD1. In contrast, PUMA has little effect on the normal/nontransformed cell lines, including the primary human fibroblast cell line WI-38, the immortalized but nontransformed kidney epithelial cell line 293, and the retinal epithelial cell line 911 (P < 0.0001; Fig. 5B). These results suggest that PUMA is selectively toxic to cancer cells although the underlying mechanism of this selectivity remains unclear.

Adenovirus-mediated transfer of p53 has been extensively explored in cancer gene therapy (12). To test the potential use of PUMA in cancer gene therapy, Ad-PUMA and an adenovirus expressing p53 (Ad-p53) constructed in the same system were compared for their potency in suppressing the growth of lung cancer cells. Strikingly, Ad-PUMA was found to be at least 5- to 10-fold more potent than Ad-p53 in suppressing the growth of H1299 and A549 cells (Fig. 5C). We observed much higher expression of PUMA but lower expression of p21 in the cells infected with Ad-PUMA compared with those infected with Ad-p53 at the same titer (Fig. 5D). We also compared the chemosensitization effects of Ad-p53 to those of Ad-PUMA and found that Ad-p53 (10 MOI) resulted in little enhancement of these effects in A549 cells (data not shown). These results show that PUMA is
more potent than p53 in growth suppression and chemosensitization in lung cancer cells.

**PUMA suppresses tumor growth in vivo.** To determine whether PUMA confers antitumor activity in vivo, A549 xenograft tumors (\(\sim 50-100\) mm\(^3\)) were treated with three injections of Ad-PUMA and the control Ad-\(\Delta\)BH3 at \(5 \times 10^8\) plaque-forming units (Fig. 6A). To avoid potential systemic effects of different viruses, Ad-PUMA and Ad-\(\Delta\)BH3 were injected into separate tumors in the same animals. Ad-\(\Delta\)BH3 did not have any effect on tumor growth compared with PBS alone (data not shown), with tumors reaching eight times the initial volumes in 24 days (Fig. 6A and B). In contrast, tumors subjected to Ad-PUMA treatment grew much slower and reached less than twice the initial volume, with at least 80% growth suppression compared with those treated by Ad-\(\Delta\)BH3 (\(P < 0.01\); Fig. 6A and B). Ad-PUMA was also used to treat H1299 xenograft tumors and found to suppress tumor growth by >80% (Fig. 6C). GFP fluorescence patterns indicated that transgenes were highly expressed in the tumor cells 48 hours following the second injection (Fig. 6D). Analyzing tumor histology by H&E staining and apoptosis in situ by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling staining revealed significant cell loss and large fractions of apoptotic cells in the tumors treated with Ad-PUMA but not in those treated with Ad-\(\Delta\)BH3 or PBS alone (Fig. 6D). These data show that PUMA can effectively inhibit growth of established tumors in vivo through induction of apoptosis.

### Discussion

Defects in the p53 pathway, which often abolish p53-mediated apoptotic response to DNA damage, are thought to play an important role in the development of drug resistance in cancer cells (29, 30). Although a number of proapoptotic proteins have been identified as p53 targets, only a few have been shown to play an important role in p53-dependent apoptosis in gene-targeting studies (13, 31). PUMA was identified as a p53 downstream gene that plays a critical role in p53-dependent apoptosis. Targeted deletion of PUMA in human colorectal cancer cells resulted in resistance to apoptosis induced by p53, the DNA-damaging agent Adriamycin, and hypoxia (17). Two recent studies using PUMA-knockout mice indicated that PUMA is an essential mediator of p53-dependent and p53-independent apoptosis in vivo (18, 19). It has also been shown that the apoptotic mediators of p53, including PUMA, the BH3-only proteins Bid and Noxa, and the death receptor DR5, can be induced by chemotherapeutics and irradiation in tissue-specific patterns, which correlated with radiosensitivity in these tissues (32). These observations, the prevalence of p53 mutations in lung cancer, as well as the ineffectiveness of current lung cancer treatments, prompted us to investigate the role of PUMA in the therapeutic responses of lung cancer cells.

Our study showed that lung cancer cells with abnormalities in p53 are deficient in the activation of PUMA by...
Chemotherapeutics and irradiation, which is likely due to deficiency in the activation of PUMA promoter by p53. These results suggest that lack of PUMA induction may contribute to the development of resistance to anticancer agents in lung cancer. Delivery of PUMA into lung cancer cells through adenoviral gene transfer resulted in apoptosis and enhanced sensitivity to chemotherapeutic agents and \(\gamma\)-irradiation, suggesting that adequate levels of PUMA are crucial for triggering apoptotic responses to these agents. Interestingly, PUMA seems to be the most effective in enhancing growth suppression and apoptosis when combined with DNA-damaging agents, such as Adriamycin, etoposide, cisplatin, and \(\gamma\)-irradiation (Fig. 3A). This observation is consistent with the notion that PUMA plays a critical role in DNA damage–induced apoptosis (17–19). PUMA also synergized with other classes of chemotherapeutic agents, including the microtubule poison Taxol and antimetabolite 5-FU, at lesser but still significant levels (Fig. 3A). These observations suggest that PUMA is rather a broad-spectrum chemosensitizer and radiosensitizer of lung cancer cells and warrants further evaluation.

Restoration of the p53 pathway by introducing p53 itself or p53 downstream targets into cancer cells has become an attractive approach in cancer gene therapy (12). Nonreplicating adenoviruses expressing p53 have been shown to suppress cell growth and induce apoptosis in several types of cancer \(in vitro\) and \(in vivo\) (33, 34). Radiation therapy and p53 adenoviral gene transfer were shown to have synergistic growth-suppressive effects in a variety of cancer cells, including those of lung, colorectal, ovarian, and head and neck cancer (35–38). Clinical trials testing combinations of p53 gene replacement with chemotherapy have yielded some encouraging results in lung cancer patients (33, 34). Depending on its expression level, p53 can induce cell cycle arrest or apoptosis in lung cancer cells (39). In our study, Ad-PUMA seems to be more potent than Ad-p53 at the same titer in growth suppression, apoptosis induction, and chemosensitization (Fig. 5C and data not shown). This can be explained by the much higher levels of PUMA but lower levels of p21 induced by Ad-PUMA compared with Ad-p53 (Fig. 5D), as high level of p21 is known to inhibit apoptosis (17). These observations suggest that PUMA might be a more effective radiosensitizer and chemosensitizer for lung tumors compared with p53. However, this possibility needs to be further evaluated in lung cancer cells and other \(in vivo\) tumor models.

A recent study showed that PUMA can regulate diverse apoptotic pathways by antagonizing all known antiapoptotic Bcl-2 family members, including Bcl-2, Bcl-X\(_L\), Bcl-w, Mcl-1, and A1, which are frequently overexpressed in cancer cells, suggesting that PUMA may be useful for targeting a variety of apoptotic defects in cancer cells (40). PUMA was also found to be required for apoptosis induced by oncogenes c-myc and E1A (18, 19). In addition, insulin-like growth factor 1 and...
epidermal growth factor signaling pathways are frequently deregulated in cancer and can suppress PUMA expression in serum-starved tumor cells (16, 18, 19). Because tumor cell growth often relies on overexpression of oncogenes or antiapoptotic proteins (3), these observations may explain why tumor cells are much more sensitive to PUMA than normal cells (Fig. 5B).

References


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