PUMA Induction by FoxO3a Mediates the Anticancer Activities of the Broad-Range Kinase Inhibitor UCN-01

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Abstract

Most targeted anticancer drugs are inhibitors of kinases that are aberrantly activated in cancer cells. However, the mechanisms by which kinase inhibitors suppress tumor growth remain unclear. In this study, we found that UCN-01, a staurosporine analogue and broad-range kinase inhibitor used in clinical trials, inhibits colon cancer cell growth by inducing apoptosis via PUMA, a BH3-only Bcl-2 family member and a p53 target. PUMA expression was markedly elevated in a p53-independent fashion following UCN-01 treatment. The induction of PUMA by UCN-01 was mediated by direct binding of FoxO3a to the PUMA promoter following inhibition of AKT signaling. Deficiency in PUMA abrogated UCN-01–induced apoptosis, caspase activation, and mitochondrial dysfunction, and rendered UCN-01 resistance in a clonogenic assay, whereas elevated PUMA expression or a BH3 mimetic sensitized UCN-01 induced apoptosis. Chemosensitization by UCN-01 seemed to involve simultaneous PUMA induction through both p53-dependent and p53-independent mechanisms. Furthermore, deficiency in PUMA suppressed the antitumor effects of UCN-01 in a xenograft model, concurrent with reduced apoptosis and caspase activation in vivo. These results suggest that PUMA-mediated apoptosis is pivotal for the anticancer activities of UCN-01, and possibly other clinically used kinase inhibitor drugs, and that PUMA manipulation may be useful for improving their anticancer activities. Mol Cancer Ther; 9(11); 2893–902. ©2010 AACR.

Introduction

Inhibition of aberrant kinase signaling in cancer cells represents one of the most effective approaches for anticancer therapy. Most recently approved anticancer drugs are inhibitors of kinase signaling pathways required for tumor cell growth. However, there is little understanding of how kinase inhibition leads to therapeutic response. Virtually all kinase inhibitor drugs inhibit multiple kinases, and their anticancer activities are often attributed to off-target effects. One critical anticancer mechanism of kinase inhibitors is their ability to induce apoptosis (1). For example, clinical response to epidermal growth factor receptor (EGFR)–targeted anticancer therapies is correlated with induction of apoptosis in tumor cells (2).

It is well known that several broad-range kinase inhibitors, such as staurosporine (STS) and its more selective derivative UCN-01 (7-hydroxystaurosporine), have anticancer activities. UCN-01 is being evaluated in several clinical trials as a single agent or a chemosensitizer (http://clinicaltrials.gov). It can potentiate cell cycle arrest and apoptosis induced by a variety of chemotherapeutic agents, such as cisplatin, topotecan, and 5-fluorouracil (3). UCN-01 inhibits a variety of kinases involved in regulating cell cycle progression and apoptosis, such as cyclin-dependent kinases (CDK), checkpoint kinase 1 (CHK1), protein kinase C (PKC), phosphoinositide-dependent protein kinase 1 (PDK1), and AKT (4). Although the effects of UCN-01 on cell cycle checkpoints have been well characterized, the mechanism by which UCN-01 promotes apoptosis remains unclear. Recent studies suggest that UCN-01 can modulate Bcl-2 family members to potentiate apoptosis in cancer cells (5, 6).

PUMA, p53-upregulated modulator of apoptosis, is a BH3-only Bcl-2 family member and a potent inducer of apoptosis. Transcription of PUMA is activated by p53 in response to DNA-damaging agents such as γ-irradiation and common chemotherapeutic drugs (7). PUMA binds to all five antiapoptotic Bcl-2 family members, such as Bcl-2 and Bcl-XL, which relieves their inhibition of Bax and Bak, leading to mitochondrial membrane permeabilization, and subsequently caspase cascade activation (7). PUMA knockout (KO) renders resistance to
p53-dependent apoptosis induced by genotoxic agents in human cancer cells and mice (8–10). Nevertheless, p53-dependent regulation of PUMA is dysfunctional in most cancer cells due to p53 abnormalities, causing survival of tumor cells and therapeutic resistance. PUMA also mediates p53-independent apoptosis induced by a variety of nongenotoxic stimuli, such as tumor necrosis factor-α (11), serum starvation (12), cytokine withdrawal (13), STS (10, 14), glucocorticoids (9, 10), and ischemia/reperfusion (15). Several transcription factors, including p65, p73, and forkhead box O3a (FoxO3a), have been implicated in p53-independent PUMA induction. For example, PUMA is induced in response to cytokine deprivation by FoxO3a (13, 16), whose activity is negatively regulated by phosphorylation via AKT (17).

In this study, we investigated how PUMA is induced by the kinase inhibitors UCN-01 and STS, and its role in UCN-01–induced apoptosis and chemosensitization. We found that FoxO3a-mediated PUMA induction is pivotal for the anticancer effects of UCN-01. The results provide novel mechanistic insight into therapeutic response to kinase inhibitors and may have broad implications for their future applications.

Materials and Methods

Cell culture and treatment

The human colorectal cancer cell lines, including HCT116, RKO, Lim2405, LoVo [all wild-type (WT) p53], and HT29 and DLD1 (both mutant p53), were obtained from the American Type Culture Collection before 2002. The isogenic cell lines included the previously described p53-KO, p21-KO, PUMA-KO, p21-KO/PUMA-KO (from Dr. Bert Vogelstein, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins in 2002; ref. 8), p21-KO/p53 binding site–KO (BS-KO) HCT116 cells, and PUMA-KO DLD1 cells created in our laboratory in 2007 (14). Cell lines were last tested and authenticated for absence of Mycoplasma, genotypes, drug response, and morphology in our laboratory in April 2010.

All cell lines were cultured in McCoy’s 5A modified medium (Invitrogen) supplemented with 10% defined fetal bovine serum (HyClone), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were maintained in a 37°C incubator at 5% CO2. For drug treatment, cells were plated in 12-well plates at 20% to 30% density 24 hours before treatment. The DMSO (Sigma) stocks of the agents used, including UCN-01, STS, wortmannin, LY294002 (Sigma), triciribine (Enzo Life Sciences AG), and MK-2206 (MediMol, Centereach), were diluted into appropriate concentrations with the 0.9% NaCl.

Western blotting and treatments

Western blotting was done as previously described (11). The following antibodies were used: PUMA (8), FoxO3a (Millipore), phospho-FoxO3a (Cell Signaling), AKT (Cell Signaling), phospho-AKT (S473; Cell Signaling), active caspase-3 (Cell Signaling), cytochrome oxidase subunit IV (Invitrogen), cytochrome c (Santa Cruz Biotechnology), β-actin (Sigma), and α-tubulin (Oncogene Science).

Real-time reverse transcription-PCR

Total RNA was isolated from UCN-01– or STS-treated cells using the Mini RNA Isolation II kit (Zymo Research) according to the manufacturer’s protocol. Total RNA (1 μg) was used to generate cDNA using SuperScript II reverse transcriptase (Invitrogen). Real-time PCR was carried out as before for PUMA and GAPDH (11).

Transfection and small interfering RNA knockdown

Cells were transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. pCMV, FoxO3a triple mutant (FoxO3aTM; Addgene, deposited by Dr. Michael Greenberg at Harvard Medical School), and WT and constitutively active AKT vectors were used in the transfection. Small interfering RNA (siRNA) knockdown was done 24 hours before UCN-01 or STS treatment using 400 pmol of FoxO3a(ON-TARGETplus J-003007-10) or the control scrambled siRNA (Dharmacon).

Luciferase assays

PUMA luciferase reporter construct was generated by cloning a genomic fragment (WT fragment: −500 to +739 bp) containing two FoxO3a sites located within the first intron of PUMA into the pBV-Luc plasmid as previously described (12). Mutations were introduced into the FoxO3a binding sites using QuikChange XL Site-Directed Mutagenesis kit (Stratagene). For reporter assays, cells were transfected with the WT or mutant PUMA reporter along with the transfection control β-galactosidase reporter pCMVβ (Promega). Cell lysates were collected and luciferase activities were measured as previously described (12). All reporter experiments were done in triplicate and repeated three times.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was done using the Chromatin Immunoprecipitation Assay kit (Upstate Biotechnology) as previously described (14), with FoxO3a antibody for chromatin precipitation. The precipitates were analyzed by PCR using primers 5′-GGCGACAGGTGCTCGGC-3′ and 5′-TGGGTGTG-GCCGCCCTC-3′.

Stable knockdown of FoxO3a

The sequence of FoxO3a siRNA used for transient knockdown was cloned into the pSUPER vector (OligoEngine). The short hairpin RNA (shRNA) construct was transfected into HCT116 cells, and cells were plated in 96-well plates in the presence of puromycin (2 μg/mL; Invitrogen). After puromycin-resistant clones were isolated, Western blotting was used to identify clones with reduced FoxO3a levels.
Analysis of apoptosis

Analysis of apoptosis by nuclear staining with Hoechst 33258 (Invitrogen) was done as previously described (18). Annexin V-propidium iodide (PI) staining was done using Annexin Alexa 488 (Invitrogen) and PI as described (14). For analysis of cytochrome c release, mitochondrial and cytosolic fractions were isolated by the differential centrifugation method previously described (19), and probed by Western blotting for cytochrome c. For colony formation assays, the treated cells were plated in 12-well plates at appropriate dilutions and allowed to grow for 10 to 14 days before staining with crystal violet (Sigma). For detection of mitochondrial membrane potential change, the treated cells were stained by MitoTracker Red CMXRos (Invitrogen) for 15 minutes at room temperature and then analyzed by flow cytometry.

Xenograft studies

All animal experiments were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Five- to 6-week-old female nu/nu mice (Charles River) were housed in a sterile environment with microisolator cages and allowed access to water and chow ad libitum. Mice were injected s.c. in both flanks with 4 × 10^6 p21-KO or p21-KO/PUMA-KO HCT116 cells. Following tumor growth for 7 days, mice were injected i.p. for 5 consecutive days with 9 mg/kg UCN-01 (20) diluted in 20 mmol/L sodium citrate buffer (pH 6). UCN-01 used for the animal
study was obtained from Kwoya Hakko Kirin Co. Ltd. through the Developmental Therapeutics Program at the National Cancer Institute. Mice were euthanized when tumors reached $\sim 1\text{ cm}^3$ in size. Tumors were dissected and fixed in 10% formalin before paraffin embedding.

**Immunostaining**

Immunostaining was carried out on 5-μm paraffin-embedded tumor sections. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and active caspase-3 immunofluorescence were done as previously described (21), with an Alexa Fluor594-conjugated secondary antibody (Invitrogen) for detection. Phospho-AKT and phospho-FoxO3a immunohistochemistry was done using the same antibodies used for Western blotting, and the avidin-biotin complex/3,3′-diaminobenzidine method for detection.

**Statistical analysis**

Statistical analyses were carried out using GraphPad Prism IV software. P values were calculated by the Student's t test and considered significant if $P < 0.05$. The means ± 1 SD were displayed in the figures.

**Results**

**PUMA is induced by UCN-01 in a p53-independent manner**

The requirement for PUMA in STS-induced apoptosis (10, 14) prompted us to investigate whether it contributes to apoptosis induced by UCN-01, a STS derivative and a more selective kinase inhibitor that has been tested in clinical trials (Fig. 1A). PUMA was found to be induced by 0.1 to 1.0 μmol/L UCN-01 in a dose-dependent manner in both WT and p53-KO HCT116 colon cancer cells (Fig. 1B). Both PUMA mRNA and protein were induced by UCN-01 within several hours, with the peak level of PUMA mRNA induction at 8 hours (Fig. 1C, left), and that of protein at 24 hours (Fig. 1C, right). This induction time course was similar to that of STS (Supplementary Fig. S1A and B), which also induced PUMA independently of p53 (Supplementary Fig. S1C). The induction of PUMA by UCN-01 or STS could be blocked by the transcription inhibitor actinomycin D (data not shown). Furthermore, UCN-01 treatment resulted in PUMA induction in five additional colon cancer cell lines regardless of their p53 status (Fig. 1D). Taken together,
these data indicate that PUMA can be induced by the broad-spectrum kinase inhibitor UCN-01 through p53-independent transcription.

**FOXO3a directly activates PUMA transcription following UCN-01 treatment**

PUMA can be induced by the transcription factor FoxO3a in T and mast cells following cytokine deprivation (13, 16). Because UCN-01 was shown to inhibit AKT (22), which phosphorylates FoxO3a and prevents its nuclear localization (17), we determined whether FoxO3a and AKT are involved in PUMA induction by UCN-01. Expression of the constitutively active FoxO3aTM induced PUMA in p53-KO cells (Supplementary Fig. S2A). siRNA depletion of FoxO3a in WT and p53-KO HT116 cells abrogated PUMA induction by UCN-01 or STS (Fig. 2A; data not shown), indicating the requirement of FoxO3a for the induction of PUMA following kinase inhibition.

A conserved FoxO binding element (AAACA) was previously identified in the human and mouse PUMA promoters (13). We noted that in addition to this site, there is another FoxO3a binding element two bases apart within the first intron of PUMA, but in the reverse orientation (Fig. 2B). To determine the role of these putative FoxO3a binding sites in PUMA induction, luciferase reporters containing a 1.1-kb fragment including these two sites were constructed and analyzed. Both FoxO3a binding sites were also mutated to dissect out their specific role in PUMA induction (Fig. 2B). Transfection of FoxO3aTM or treating cells with UCN-01 or STS markedly activated the WT PUMA reporter. In contrast, the binding site mutations abolished the responsiveness of the PUMA reporter to FoxO3aTM and also abrogated the effects of STS and UCN-01 (Fig. 2C). To determine whether FoxO3a directly activates the PUMA promoter, ChIP was done on lysates from STS- or UCN-01–treated cells using the FoxO3a antibody. The binding of FoxO3a to the PUMA promoter was significantly enhanced after treatment with either compound (Fig. 2C). Taken together, these data suggest that FoxO3a can directly bind to the PUMA promoter to activate its transcription following STS or UCN-01 treatment.

**AKT inhibition drives PUMA induction by UCN-01**

We then determined whether AKT, an antiapoptotic kinase often activated aberrantly in cancer cells, is involved in the effect of UCN-01 on PUMA expression. UCN-01 treatment markedly inhibited AKT phosphorylation at S473, concurrent with reduced FoxO3a phosphorylation (Fig. 3A), which is known to prevent its nuclear translocation and ensuing transactivation (17). On the other hand, transfection with WT or constitutively active AKT induced FoxO3a phosphorylation, suppressed basal PUMA expression (Fig. 3B), and overcame the induction of PUMA by UCN-01 (Fig. 3C). Furthermore, treating cells with the AKT inhibitor triciribine or MK-2206, an AKT inhibitor in clinical trials, was sufficient to induce PUMA after diminished AKT and FoxO3a phosphorylation (Fig. 3D). In addition, PUMA could also be induced similarly by the phosphatidylinositol 3-kinase (PI3K) inhibitors wortmannin and LY294002 in p53-KO cells (Supplementary Fig. S2B). Therefore, the induction of PUMA by
FoxO3a following UCN-01 treatment seemed to be mediated through AKT inhibition.

**PUMA plays an essential role in UCN-01–induced apoptosis**

To investigate the role of PUMA in UCN-01–induced apoptosis, we analyzed p21-KO cells with different PUMA status because they often have a more pronounced apoptotic phenotype due to defects in cell cycle checkpoints (23). UCN-01 treatment induced dose-dependent apoptosis in p21-KO cells, with >50% apoptotic cells detected following treatment with 3 μmol/L UCN-01 (Fig. 4A). Remarkably, UCN-01–induced apoptosis was almost completely abolished in PUMA-deficient p21-KO cells (Fig. 4A). The requirement of PUMA in UCN-01–induced apoptosis is not cell line specific, as the apoptosis was also abolished in the previously described PUMA-KO DLD1 colon cancer cells (Supplementary Fig. S3A; ref. 14). Analysis of apoptosis by Annexin V/PI staining confirmed reduced apoptosis in PUMA-deficient cells (Supplementary Fig. S3B). PUMA deficiency abrogated UCN-01–induced caspase-9 and caspase-3 activation (Fig. 4B), cytosolic release of cytochrome c (Fig. 4C), as well as mitochondrial membrane potential change (Supplementary Fig. S3C). PUMA-deficient cells were also found to be significantly more resistant to UCN-01 than WT cells in a long-term clonogenic assay (Fig. 4D). As a control, we also analyzed p21-KO cells with a deletion of the p53 binding sites in the PUMA promoter (p21-KO/BS-KO), which abolishes apoptosis induced by p53 and DNA-damaging agents (14). The apoptotic effect of UCN-01 remained intact in these cells, confirming the dispensability of p53. Furthermore, transient knockdown of FoxO3a by a siRNA or stable depletion of FoxO3a with a shRNA suppressed PUMA transcription and UCN-01–induced apoptosis (Supplementary Fig. S3D; data not shown). Collectively, these results show that PUMA is essential for UCN-01–induced apoptosis in colon cancer cells.

**PUMA mediates chemosensitization by UCN-01 and enhances its anticancer activity**

UCN-01 has mostly been used as a chemosensitizer and can potentiate the effects of common chemotherapeutic drugs such as cisplatin (3). Because both UCN-01
and cisplatin induce PUMA-dependent apoptosis (Fig. 4; Supplementary Fig. S4A and B), we asked whether chemosensitization by UCN-01 involves PUMA induction. A combination of UCN-01 and cisplatin induced PUMA at a much higher level in HCT116 cells compared with UCN-01 or cisplatin alone (Fig. 5A), consistent with two distinct, p53-dependent and p53-independent, mechanisms of PUMA induction by these agents. Apoptosis and caspase-3 activation was also markedly enhanced by the combination treatment in HCT116 cells (Fig. 5A and B). However, the enhanced apoptosis and caspase activation by the combination treatment was greatly reduced in the PUMA-KO cells (Fig. 5B; Supplementary Fig. S4C), suggesting that p53-independent induction of PUMA by UCN-01 mediates its chemosensitization effect. We also found that an adenovirus expressing PUMA (Ad-PUMA), but not a BH3 domain−deleted mutant (Ad-ΔBH3), enhanced UCN-01−induced apoptosis in HCT116 cells (Fig. 5C). Furthermore, GX15-070, a PUMA-like BH3 mimetic compound capable of binding to all five antiapoptotic Bcl-2 family members, significantly enhanced UCN-01−induced apoptosis (Fig. 5D). These observations suggest that manipulating apoptosis regulators such as PUMA can potentiate the anticancer effects of kinase inhibitors.

**PUMA contributes to the antitumor activity of UCN-01 in a xenograft model**

To determine whether the PUMA-dependent apoptotic effect of UCN-01 contributes to its antitumor activity in vivo, p21-KO and p21-KO/PUMA-KO HCT116 cells were injected into nude mice to establish xenograft tumors. Tumor-bearing mice were treated with 9 mg/kg of UCN-01 for 5 days as previously described (20), and tumor volumes were measured every 2 to 3 days for 3 weeks. The p21-KO tumors responded to the UCN-01 treatment with slower growth and were roughly half the size of the untreated tumors on day 12 following treatment (Fig. 6A). In contrast, the p21-KO/PUMA-KO tumors were indistinguishable from the untreated mice and did not respond to UCN-01 treatment (Fig. 6A). UCN-01 treatment suppressed AKT and FoxO3a phosphorylation (Fig. 6B) and induced PUMA expression in the p21-KO tumors (Fig. 6C). TUNEL and active caspase-3 staining revealed significant apoptosis induction in the p21-KO tumors, which was almost completely abolished in the p21-KO/PUMA-KO tumors (Fig. 6D; Supplementary Fig. S5). These data clearly show the necessity of PUMA for the in vivo antitumor and apoptotic effects of UCN-01.

**Discussion**

Our results provide novel mechanistic insight into the anticancer mechanism of the broad-range kinase inhibitor UCN-01. Most of the previous studies have focused on the effect of UCN-01 on cell cycle checkpoints, which is widely believed to be its major mode of action in...
anticancer therapy. Several reports suggest that UCN-01 can potentiate apoptosis via the mitochondrial pathway (24–26). However, the exact mechanism underlying this activity is unclear, and the role of apoptosis in the therapeutic response to UCN-01 remains conjecture. Our results show for the first time that UCN-01 treatment leads to PUMA induction by FoxO3a following AKT inhibition. Induction of PUMA accounts for most, if not all, of in vitro and in vivo activities of UCN-01 against colon cancer cells, and such a requirement for PUMA was not affected by the genetic background and p53 status. In addition to PUMA, other BH3-only proteins, such as Bim, may also contribute to the effect of UCN-01 on other tumor types (6). It is notable that UCN-01 induces apoptosis in solid tumor cells typically at micromolar concentrations, which are significantly higher than those required for checkpoint inhibition (27, 28). The UCN-01 dose used in our study (1.0 μmol/L) is similar to those used in the previous apoptosis studies (5, 29, 30). Pharmacokinetic analyses showed that UCN-01 plasma concentrations can be as high as 30 μmol/L (31, 32), suggesting that apoptosis-inducing concentrations are achievable.

UCN-01 is commonly used as a chemosensitizer in combination with other anticancer agents. It has additive or even synergistic effects on apoptosis induced by a variety of commonly used chemotherapeutic drugs, such as 5-fluorouracil (33), topotecan (34), tamoxifen (35), and mitomycin C (36). UCN-01 can also potentiate several nongenotoxic agents, such as the CDK inhibitor roscovitine (37), the mTOR (mammalian target of rapamycin) inhibitor rapamycin (38), the PKC activator PMA (phorbol 12-myristate 13-acetate; ref. 25), and the NF-κB inhibitor BAY 11-7082 (39). Research into its mechanism of action revealed activation of the extracellular signal-regulated kinase (ERK) pathway by UCN-01 (40), supporting a rationale of inhibiting the proliferative ERK signal to enhance the activity of UCN-01. It is exciting that several ERK inhibiting agents were found to potentiate UCN-01–induced apoptosis, such as the mitogen-activated
protein/ERK kinase (MEK) inhibitor PD184352 (41), the farnesyltransferase inhibitor L744832 (42), and the Hsp90 antagonist 17-AAG (17-allylamino-demethoxygeldanamycin; ref. 43). These studies highlight the multitudinous ways UCN-01 can be used in chemotherapy, as well as the importance of investigating UCN-01 modes of action to design rational combinations. Our results suggest that enhanced PUMA induction may be a novel mechanism of chemosensitization by UCN-01. The expression of PUMA is controlled by several proapoptotic transcription factors in a cell type– and stimuli-dependent manner (7). Combinations of different classes of PUMA-inducing agents may allow for simultaneous PUMA induction via multiple pathways, thereby lowering the threshold proapoptotic activity required for apoptosis induction.

Targeting cancer-specific molecular changes has brought new hope to therapeutic intervention of cancer. Most of the targeted drugs proven to be clinically useful are inhibitors of the kinase signaling pathways that are required for tumor cell growth. In addition to STS and UCN-01, PUMA can also be induced by other kinase inhibitors, such as the MEK inhibitor U0126 (44), PI3K inhibitors wortmannin and LY294002 (12, 45), the human EGFR/vascular endothelial growth factor receptor inhibitor BMS-690514 (46), and the farnesyltransferase inhibitor BMS-214662 (47). It has recently been shown that PUMA can be induced by the clinically used EGFR inhibitors, gefitinib and erlotinib, in head and neck cancer cells, and its expression correlated with therapeutic response to the EGFR inhibitors (48). Therefore, PUMA may play a broad functional role in determining response to targeted therapies, and the induction of PUMA may serve as a marker for predicting therapeutic response to these agents.

Maintenance of tumor phenotypes is dependent on the suppression of apoptosis by certain prosurvival proteins because neoplastic transformation would normally trigger an apoptotic response (49). The ability to reactivate the induction of apoptosis in cancer cells is an attractive approach for anticancer therapy. It is perhaps not surprising that apoptosis has emerged as a critical therapeutic endpoint of kinase inhibitors (1). The induction of PUMA by kinase inhibitors primarily occurs via p53-independent mechanisms. Such induction would bypass the p53 network that is required for the efficacy of common chemotherapeutics. Another way of circumventing the p53 pathway would be to use compounds that directly target the apoptotic machinery. For example, agents that mimic the BH3 domains of the proapoptotic Bcl-2 family members, such as ABT-263 and GX15-070, are currently being tested in clinical trials (50). The effectiveness of Ad-PUMA and GX15-070 in enhancing UCN-01–induced apoptosis supports a rationale for combining kinase inhibitors and apoptosis-targeting agents.

In conclusion, we showed that PUMA is a critical mediator of therapeutic response to UCN-01, and possibly other kinase inhibitors, in colon cancer cells. Our study may serve as a proof of principle for targeting PUMA to improve therapeutic effects of kinase inhibitors, for using PUMA expression as a biomarker for predicting therapeutic response, and for rational design of new therapeutic regimens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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