

Depletion of Mutant p53 and Cytotoxicity of Histone Deacetylase Inhibitors

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Abstract

Mutant p53 is a cancer-specific target for pharmacologic intervention. We show that histone deacetylase inhibitors such as FR901228 and trichostatin A completely depleted mutant p53 in cancer cell lines. This depletion was preceded by induction of p53-regulated transcription. In cells with mutant p53 pretreated with histone deacetylase inhibitors, DNA damage further enhanced the p53 trans-function. Furthermore, histone deacetylase inhibitors were preferentially cytotoxic to cells with mutant p53 rather than to cells lacking wild-type p53. We suggest that, by either restoring or mimicking p53 trans-functions, histone deacetylase inhibitors initiate degradation of mutant p53. Because mutant p53 is highly expressed, a sudden restoration of p53-like functions is highly cytotoxic to cells with mutant p53. In a broader perspective, this shows how selectivity may be achieved by targeting a non-cancer-specific target, such as histone deacetylases, in the presence of a cancer-specific alteration, such as mutant p53. (Cancer Res 2005; 65(16): 7386-92)

Introduction

The p53 tumor suppressor is mutated in 50% of human cancers (1). Although mutant p53 renders cancer cells resistant to certain anticancer drugs, it is also a potential cancer-specific target for pharmacologic intervention (2–4). Given that mutant p53 is highly overexpressed, its sudden reactivation may be very toxic for a cell. Reintroduction of wild-type (wt) p53 by adenoviruses (Ad-p53) is predominantly cytotoxic to cancer cells that lack wt p53 (mutant p53 and null; ref. 5). However, it is currently technically impossible to introduce wt p53 all tumor cells. Another strategy is to reactivate mutant p53 using small molecular therapeutic agents that change conformation of mutant p53 (2–4). Although several compounds were described, their selectivity, specificity, and mechanism of action are still unclear (2, 6). Another strategy is to deplete mutant p53. For example, Hsp90-active agents (e.g., geldanamycin) moderately decrease mutant p53 (7). Although depletion of mutant p53 per se cannot and does not restore p53 functions, it may abolish dominant-positive effects (8, 9).

Conversely, restoration of p53 function, in theory, will deplete mutant p53. In addition, functional reactivation of mutant p53, which is initially overexpressed, will be especially toxic to such a cell. Wt p53 transactivates Mdm-2, which targets p53 for degradation.

In addition, p21 may also be required for p53 degradation (10, 11). By inducing Mdm-2 and p21, wt p53 stimulates its own degradation via the proteasome. Because mutant p53 cannot trans-activate Mdm-2 or p21, mutant p53 is not degraded and accumulates at high levels. Therefore, restoration of p53 function should result in degradation of mutant p53. Support of this idea can be found in observations that introduction of wt p53 leads to degradation of mutant p53 (12), and in experiments in which normal p53 function is imitated (13). For example, compounds that block degradation of Mdm-2 and p21 cause depletion of mutant p53 (13).

The histone deacetylase inhibitor FR901228 (FK228, depsipeptide) is currently undergoing clinical trials (14). Histone deacetylases inhibit the trans-activating functions of wt p53 (15, 16). It was expected that FR901228 would be predominantly cytotoxic to cells with wt p53. However, as shown by correlative studies, including data from the National Cancer Institute drug screen, FR901228 was less active in cells with wt p53 (17, 18). Without mechanistic explanation, the significance of the correlation between wt p53 status and relative resistance to FR901228 remained elusive. The effects of drugs on p53 were usually studied in cells with wt p53; histone deacetylase inhibitors exert neither consistent nor significant effects on wt p53 levels (19–21). Here we report that trichostatin A and FR901228 induce p53-regulated transcription in cells with mutant p53 resulting in complete depletion of the p53 protein. Furthermore, mutant p53, rather than loss of wt p53, was associated with increased cytotoxicity of histone deacetylase inhibitors.

Materials and Methods

Cell lines. Cancer cell lines with mutant p53, MDA-MB-231, DU145, and SKBr3 (mutations at residues 280, 274/223, and 175H, respectively), were obtained from American Type Culture Collection (Manassas, VA). Clones of A2780-1A9 cells with different p53 statuses, A2780-1A9 (wt p53), PTX22 (mutant p53), and PTX5 (pseudo-null p53), were previously described (22, 23).

Reagents. Paclitaxel (Taxol) was a Bristol-Myers product (Bristol-Myers, Princeton, NJ). Doxorubicin was obtained from Sigma (St. Louis, MO). Trichostatin A was obtained from Wako Pure Chemical Industries, Ltd. (Japan) and prepared as a 1 mg/mL stock in DMSO. FR901228 (FK228, depsipeptide) was obtained from the Chemistry and Synthesis Branch of National Cancer Institute (Bethesda, MD) and prepared as a 1 mg/mL stock solution in water. The active enantiomer of nutlin-3 (nutlin-3a) or the inactive enantiomer nutlin-3b was provided by Hoffmann-La Roche, Inc. (Nutley, NJ). These drugs were dissolved in DMSO and kept as 10 mmol/L stock solutions at –20°C.

Immunoblot analysis. Proteins were resolved on SDS-PAGE or NuPAGE 4% to 12% Bis-Tris gel with MOPS running buffer (NOVEX, San Diego, CA) according to the instructions of the manufacturer. Immunoblotting was done using rabbit polyclonal anti-human poly(ADP-ribose) polymerase (Upstate Biotechnology, Lake Placid, NY), mouse monoclonal anti-human p21 [Calbiochem (Cambridge, MA), or Transduction Laboratories (Lexington, KY)], rabbit polyclonal anti-acetylated histone H3 (Upstate

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Biotechnology), mouse monoclonal anti-human tubulin and anti-Lys40-acetotubulin (Sigma), anti-human p53 (Ab-6 and Ab-2; Calbiochem), anti-Mdm-2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-actin (AC-15; Sigma Aldrich, St. Louis, MO), anti-p53 antibody against mutant (pAB 240) and wt p53 (pAB 1620) Oncogene Science antibodies N3 and N5, anti-acetylated p53 (Lys320) antibody (Upstate Biotechnology), anti-acetylated p53 (Lys373) antibody (Upstate Biotechnology), and anti-acetylated p53 (Lys382) antibody (Oncogene Research Products). Secondary antibodies were horseradish peroxidase-linked anti-mouse or anti-rabbit immunoglobulin (Amersham, Piscataway, NJ). The membrane was developed using ECL Western blotting detection reagents (Amersham).

PCR amplification of p53 and β -actin. Total RNA was extracted using RNA STAT-60 (TEL-TEST, Inc., Friendswood, TX). Single-stranded oligo (dT)-primed cDNA was generated using MMLV reverse transcriptase (Life Technologies, Gaithersburg, MD). Oligonucleotide primers used for reverse transcription-PCR (RT-PCR) analysis of human p53 were 5' (sense) ³³⁷TTCTTGCATCTCTGGACAGCC³⁵⁷ and 3' (antisense) ¹⁰⁴²GGCCTCATT-CAGCTCTCGGAAC¹⁰²¹.

The amplification reaction was carried out for 30 cycles, and each cycle consisted of 94°C for 20 seconds, 57°C for 30 seconds, and 72°C for 1 minute, followed by a final 10-minute elongation at 72°C. Comparability of RNA quantities was ensured using β -actin as an internal standard (18). Oligonucleotide primers for human β -actin RNA amplification were (GenBank accession no. XM-004814) 5' (sense) ²⁰⁷TGGGCATGGGTCA-GAAGGAT²²⁶ and 3' (antisense) ⁴⁸⁸GAGCGGTACAGGGATAGCAC⁵⁰⁷.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells were plated on 96-well flat-bottomed plates (2,000 cells/well) and then exposed to the various agents. After 3 days, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution in PBS was added to each well for 4 hours. After removal of the medium, DMSO was added to each well to dissolve the formazan crystals. The absorbance at 540 nm was determined (15).

Number of dead and live cells. Cells were plated on 24-well plates in 1 mL of medium or on 96-well plates in 0.2 mL of medium, and were treated with drugs. After the indicated time, cells were counted in triplicate on a Coulter Z1 cell counter (Hialeah, FL). In addition, cells were incubated with trypan blue and the numbers of blue (dead) cells and transparent (live) cells were counted with a hemocytometer.

Immunocytochemistry. Cells were plated onto glass coverslips in 24-well plates. The following day, cells were treated with paclitaxel or Adriamycin for 16 hours, then washed with PBS and fixed with 3% paraformaldehyde for 30 minutes at room temperature followed by 5-minute incubation at -20°C with ice-cold methanol. Cells were then immunostained with anti-p53 antibody against mutant (pAB 240) and wt p53 (pAB 1620) Oncogene Science antibodies N3 and N5 (as described in ref. 7), anti-acetylated p53 (Lys320) antibody (Upstate Biotechnology), anti-acetylated p53 (Lys373) antibody (Upstate Biotechnology), and anti-acetylated p53 (Lys382) antibody (Oncogene Research Products), followed by secondary FITC-conjugated anti-mouse antibody (Vector Lab., Lincolnshire, IL) or Rhodamine Red-X anti-mouse antibody (Jackson Immunochemicals, West Grove, PA). Coverslips were mounted on glass slides and analyzed with a Zeiss LSM 510 microscope as previously described (24).

Transient transfections. WWP-Luc, a p21 promoter-luciferase construct, and PG13-Luc, containing a generic p53 response element, were provided by Bert Vogelstein (Sidney Kimmel Cancer Center, Johns Hopkins Medical Institutions, Baltimore, MD) and Wafik S. El-Deiry (Department of Medicine, University of Pennsylvania, Philadelphia, PA). Bax-Luc was provided by Karen Vousden (Beatson Institute, Glasgow, United Kingdom). The control luciferase plasmid, pGL2-Control, driven by the SV40 promoter and enhancer sequences, was purchased from Promega (Madison, WI). Cells of 50,000 were plated on 24-well plates (Costar, Acton, MA). The next day, cells were transfected with plasmids as previously described (25).

Results

FR901228 depletes mutant p53. Using three cell lines harboring mutant p53 (MDA-MB231, DU145, and SKBr3, with

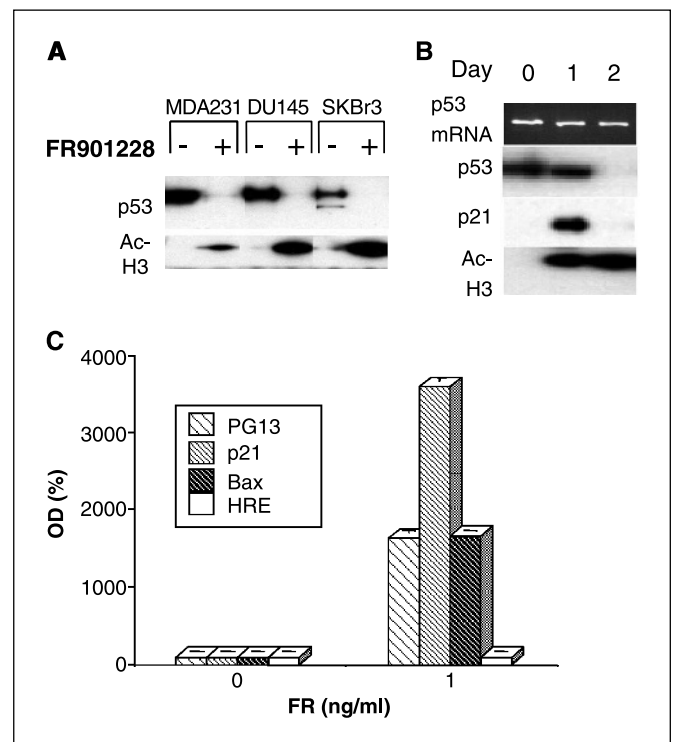


Figure 1. Effect of FR901228 on mutant p53 and p53-stimulated transcription. **A**, three cell lines harboring mutant p53 (MDA-MB231, DU145, and SKBr3) were treated with 1 ng/mL FR901228 for 3 days. Immunoblot assay for p53 and acetylated histone H3 was done. Acetylation of histone H3 was measured as a marker of inhibition of histone deacetylases. **B**, SKBr3 cells were treated with 1 ng/mL FR901228 for 1 and 2 days. Immunoblot assay for p53, p21, and acetylated histone H3 was done. In parallel, mRNA was isolated and RT-PCR for p53 mRNA was done. **C**, effect of FR901228 on p53-stimulated transcription. SKBr3 cells were transiently transfected with PG13-Luc, p21-Luc, Bax-Luc, or HRE-Luc as indicated. After 16 hours, cells were treated with 1 ng/mL FR901228. The luciferase activity was measured after an additional 48 hours.

mutations at residues 280, 274/223, and 175, respectively), we found that low concentrations of FR901228 caused a complete depletion of mutant p53 (Fig. 1). Histones were acetylated, consistent with the mechanism of action of FR901228. To determine whether depletion of p53 occurs at transcriptional or posttranscriptional levels, we next compared the effect of FR901228 on the p53 protein and mRNA. As shown in Fig. 1B, 2 days after the start of FR901228 treatment, mutant p53 protein had disappeared without a change in p53 mRNA levels. Therefore, p53 protein was depleted whereas its mRNA remained unchanged. This is consistent with the notion that p53 expression is regulated at protein levels.

Activation of p53-regulated transcription. As shown in Fig. 1B, the depletion of mutant p53 protein by day 2 was preceded by a dramatic transient induction of p21 at day 1. This induction of p21 may indicate reactivation of normal p53 function. However, previous studies have shown that histone deacetylase inhibitors, including FR901228, can induce p21 in a p53-independent manner (26–28). Therefore, we investigated the effects of FR901228 on three p53-responsive constructs (p21-Luc, Bax-Luc, and PG13-Luc). The Bax promoter, like p21, is a physiologic target of wt p53. Because the affinity of p53 for the Bax promoter is lower than its affinity for the p21 promoter, a much higher activity of wt p53 is required to induce Bax-Luc than to induce p21-Luc and PG13-Luc (25). Unlike the p21 and Bax promoters, PG13-Luc is an artificial construct. PG13-Luc,

which contains 13 repeats of the p53-binding site, is unresponsive to other transcription factors and is p53 specific. In addition, we investigated a construct that contains three HIF-1 binding sites [hypoxia-responsive element-luciferase (HRE-Luc)] and is unresponsive to p53 (25). All three p53-dependent constructs were induced by 1 ng/mL (2 nmol/L) FR901228. HRE-Luc, a reporter activated by hypoxia-inducible factor, was not induced. This indicates the induction of Bax-Luc, p21-Luc, and PG13-Luc was selective. This induction of p53-dependent transcription was transient and declined between 48 and 72 hours when 1 ng/mL FR901228 was used and by 36 to 48 hours when 10 to 100 ng/mL (20-200 nmol/L) FR901228 was added (data not shown).

FR901228 and doxorubicin are synergistic. DNA-damaging drugs activate wt p53 but not mutant p53. We reasoned that if FR901228 restores wt p53 function to a mutant p53, then it might potentiate doxorubicin, a DNA-damaging drug. As shown in Fig. 2A, both high (10 ng/mL) and low (1 ng/mL) concentrations of FR901228 depleted mutant p53 by 36 hours. However, low doses of FR901228 did not cause cleavage of poly(ADP-ribose) polymerase at that time (Fig. 2A, FR901228 1 ng/mL, Doxorubicin "No"). Thus, depletion of p53 preceded apoptosis. Doxorubicin alone did not induce poly(ADP-ribose) polymerase cleavage in SKBr3 cells. Although neither doxorubicin nor low doses of FR901228 induced poly(ADP-ribose) polymerase cleavage, their combination resulted in poly(ADP-ribose) polymerase cleavage (Fig. 2A). This was confirmed by the MTT assay (Fig. 2B). SKBr3 cells were treated either with FR901228 alone or with a combination of FR901228 and doxorubicin. Whereas 1 ng/mL FR901228 only minimally affected cell survival, the addition of either 40 or 400 ng/mL doxorubicin resulted in greater cell death (Fig. 2C, arrow). Also, doxorubicin potentiated FR901228-induced p53-dependent transcription (Fig. 2C). To exclude nonspecific cytotoxic effects, we used the ratio PG13-Luc to HRE-Luc. We conclude that doxorubicin enhanced the p53-stimulated transcription in FR901228-pretreated cancer cells with mutant p53 (Fig. 2C).

Effects of FR901228 on mutant conformation and acetylation. We next investigated whether p53 changed its conformation after treatment with FR901228. Mutant p53 is recognized by p240 antibody, whereas wt p53 is recognized by p1620 antibody. Because the immunoblot method denatures proteins, both mutant and wt conformations are not detectable by immunoblot but can be detected by immunochemistry. As shown in Fig. 3A, there was a strong nuclear staining with antibodies against mutant p53 conformation in DU145 cells, which disappeared after treatment with FR901228. There was no staining with p1620 antibodies neither before nor after treatment with FR901228 (data not shown). The same result was obtained with SKBr3 cells. Although we could not detect wt conformation, it is still possible that once such conformation is acquired, p53 degrades.

Using three different anti-acetylated p53 antibodies (Lys320, Lys373, and Lys382), we observed a strong nuclear staining after treatment with FR901228 (Fig. 3A). Unfortunately, this staining was not necessarily p53 specific because it was also observed in PC3 (p53 null) cell line. By analyzing these antibodies on immunoblot, we found that they recognize a protein with MW 14 kDa (possibly, acetylated histones), which was strongly induced by FR901228 (Fig. 3C). At that point, due to nonspecificity of all available antibodies, we conclude that we have no evidence for acetylation of p53.

FR901228 and trichostatin-A have similar effects on p53. Histone deacetylase inhibitors possess overlapping and distinct activities. For example, trichostatin A causes acetylation of tubulin, whereas FR901228 does not (17). Initially, we found that 1 ng/mL (3 nmol/L) trichostatin A did not deplete mutant p53. However, trichostatin A and FR901228 differ in their potency as cytotoxic drugs. FR901228 and trichostatin A inhibited proliferation of SKBr3 cells with IC₅₀ concentrations of 0.7 ng/mL (1.4 nmol/L) and 12 ng/mL (36 nmol/L), respectively (Fig. 4A). Taking this into account, we showed that, at equitoxic concentrations, both inhibitors depleted mutant p53 (Fig. 4B). Furthermore, at comparably toxic concentrations, both trichostatin A and FR901228 induced PG13-Luc. This induction occurred early (12 hours) and further increased by 24 hours (Fig. 4C and D).

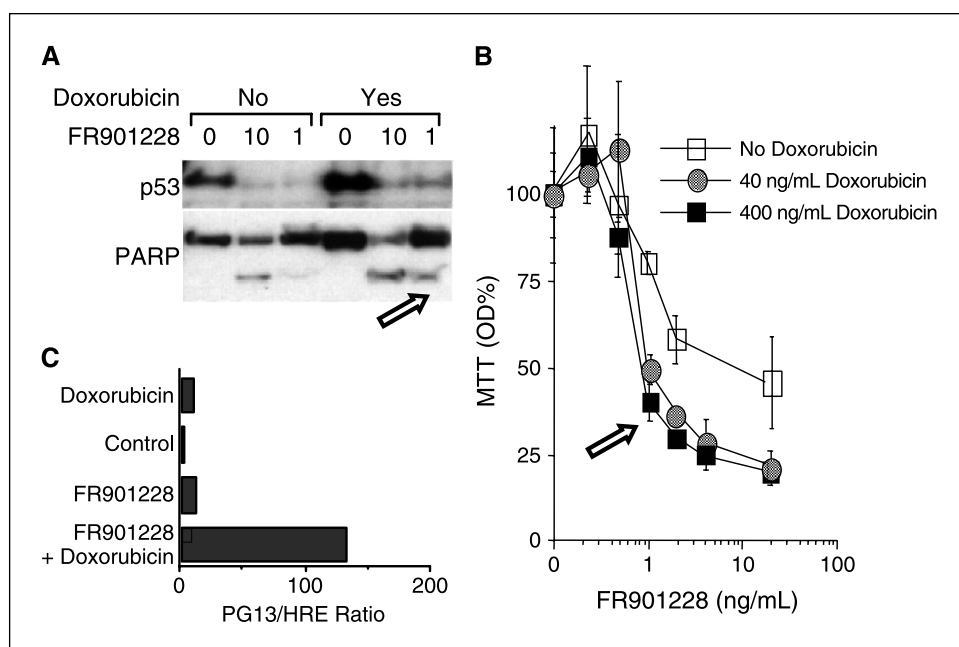


Figure 2. Synergism between FR901228 and doxorubicin. **A**, SKBr3 cells were treated with the indicated concentrations of FR901228 (1 or 10 ng/mL) with or without doxorubicin for 36 hours. Immunoblot assay for p53 and poly(ADP-ribose) polymerase was done as described in Materials and Methods. **B**, SKBr3 cells were treated with increasing concentrations of FR901228 (X axis) without or with doxorubicin (40 or 400 ng/mL) for 3 days. MTT assay was done as described in Materials and Methods. The data are plotted as percent of control. **C**, effects of FR901228 on p53-stimulated transcription. SKBr3 cells were transiently transfected with either PG13-Luc or HRE-Luc. After 16 hours, cells were treated with 40 ng/mL doxorubicin, 1 ng/mL FR901228, or doxorubicin and FR901228. Twenty-four hours later, cells were lysed and luciferase activity was measured. Columns, ratio of PG13 to HRE.

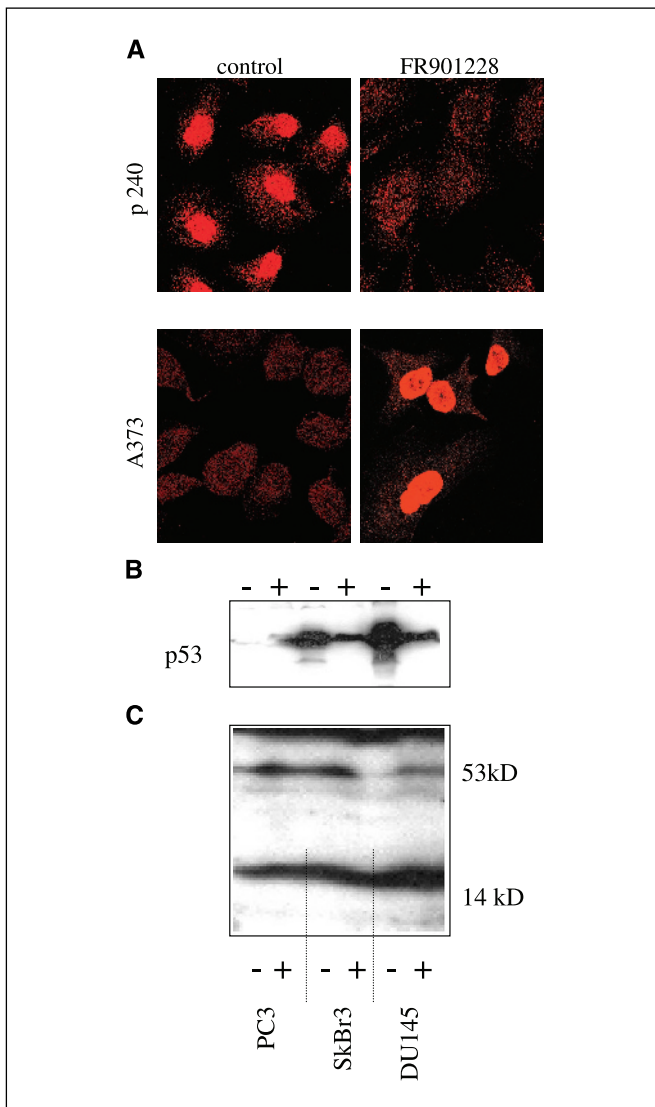


Figure 3. The effects of FR901228 on conformation and acetylation of mutant p53. **A**, DU145 cells were incubated with or without 10 ng/mL FR901228 for 2 days and immunofluorescence with anti-mutant p53 antibodies that recognize p240 mutant conformation epitope (*top*) and A373 antibodies that recognize acetylated p53 (*bottom*) was done as described in Materials and Methods. **B-C**, PC3M, DU145, and SKBr3 cells, if indicated (+), were treated with 10 ng/mL FR901228 and after 2 days immunoblots with anti-p53 (Ab2; **A**) and anti-acetylated p53 (A373; **B**) were done as described in Materials and Methods. (A373 recognizes a 14 kDa protein that is induced after FR901228, presumably acetylated histones).

Mutant p53 sensitizes cells to FR901228 and trichostatin A.

If histone deacetylase inhibitors restore wt p53 function to mutant p53, then histone deacetylase inhibitors should be preferentially cytotoxic in cells with mutant p53 but not in p53-null cells. To address this question, we took advantage of three well-characterized cell lines of identical genetic background with wt p53 (A2780 (1A9) cells), mutant p53 (PTX22 cells), and pseudo-null p53 (PTX15 cells; refs. 22, 23). Both PTX22 and PTX15 are paclitaxel resistant. PTX22 harbors a mutation in p53, leading to its functional inactivation and overexpression. In contrast, as we have previously shown (18), PTX15 cells do not express detectable p53mRNA or protein, although previous DNA sequencing revealed a wt p53 (pseudo-null). PTX22 cells harboring a mutant p53 were

more sensitive to both FR901228 and trichostatin A than PTX15 cells (Fig. 5). In PTX22 cells, FR901228 induced p21 and Mdm-2 in a dose-dependent manner. At 24 hours, this induction was accompanied by down-regulation of mutant p53 (Fig. 6A) with complete disappearance of p53 by 48 hours (data not shown). In contrast, in PTX15 cells, which possess a pseudo-null p53 status, FR901228 did not significantly induce either p21 or Mdm-2 (Fig. 6A). To exclude the possibility that FR901228 had no effect on PTX15 cells, we showed comparable histone acetylation in both PTX22 and PTX15 cells (Fig. 6A).

p53 depletion is partially Mdm-2 dependent. As shown in Fig. 6A, FR901228 induces Mdm-2 in PTX22 (mutant p53) but not in PTX15 cells, consistent with the notion that FR901228 activates p53-dependent transcription. Does Mdm-2 in turn participate in down-regulation of p53? In fact, it is known that Mdm-2 causes degradation of both mutant and wt p53 (12). However, because Mdm-2 is not expressed in cells with mutant p53, mutant p53 is not degraded in such cell lines. The introduction of wt p53 results in Mdm-2 induction and in degradation of both mutant and wt p53 (12). Here we observed induction of Mdm-2 in PTX22 cells treated with FR901228, which was accompanied by depletion of mutant p53 (Fig. 6A). To investigate whether the depletion of p53 is Mdm-2 dependent, we took advantage of Nutlin-3a, a recently developed selective inhibitor of Mdm-2 (29, 30) Nutlin-3a, but not its inactive analogue (nutlin-3b), partially prevented degradation of mutant p53 caused by FR901228 (Fig. 6C). Thus, we conclude that Mdm-2 is involved in FR901228-induced depletion of p53.

Discussion

Histone deacetylase inhibitors represent a novel class of antineoplastic agents (31–34). We found that FR901228 and trichostatin A completely depleted mutant p53 in all cell lines tested. Concentrations as low as 1 ng/mL FR901228 caused disappearance of mutant p53 by 36 to 72 hours. Inhibitors of Hsp90 such as geldanamycin can slightly decrease mutant p53 (7). It has been reported that high concentrations of trichostatin A (250 ng/mL) and FR901228 (25 ng/mL) affect Hsp90 and decrease levels of Hsp90-associated proteins (21). This Hsp90-dependent decline in p53 levels caused by high concentrations of histone deacetylase inhibitors and by geldanamycin is fast (occurring at 6 hours) and small (7, 21, 35, 36). Furthermore, Hsp90-active agents neither induce p21 and Mdm-2 nor have effects on other p53-dependent transcription processes (7, 35, 36). Therefore, inhibition of Hsp90 cannot explain the reappearance of p53 functions followed by disappearance of p53 caused by low concentrations of histone deacetylase inhibitors. We showed that marked activation of p53-dependent transcription measured using three luciferase vectors, p21-Luc, PG13 Luc, and Bax-Luc, preceded the depletion of mutant p53. Expression of endogenous p21 and Mdm-2 was also noted. Thus, an outburst of p53-like activity at 12 to 48 hours was followed by complete depletion of mutant p53. As predicted, by sensing wt p53 function, cells initiate degradation of mutant p53. In other words, the appearance of wt p53 function (trans-activation of Mdm-2 and p21) leads to degradation of mutant p53, without a change in mRNA levels. This sequence of events—induction of p53 function, depletion of mutant p53, and normalization of p53-stimulated transcription—is consistent with a pharmacologic rescue of mutant p53. Also, histone deacetylase inhibitors can imitate p53 functions by inducing a variety of p53-inducible genes in a p53-independent

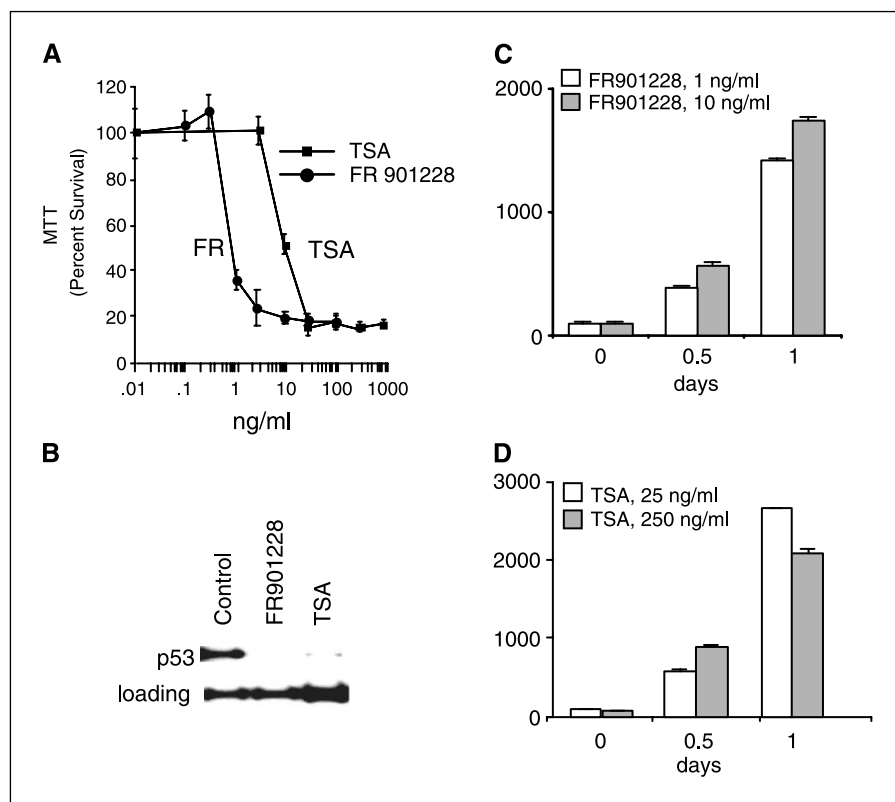


Figure 4. Comparison of FR901228 and trichostatin A. *A*, SKBr3 cells were treated with either FR901228 or trichostatin-A (TSA) for 3 days. MTT assay was done as described in Materials and Methods. *Points*, percent of control; *bars*, SD. *B*, SKBr3 cells were treated with 1 ng/mL FR901228 or 10 ng/mL trichostatin A for 3 days. Immunoblot assay for p53 was done as described in Materials and Methods. *C* and *D*, effects of FR901228 and trichostatin A on p53-stimulated transcription. SKBr3 cells were transiently transfected with PG13-Luc. After 16 hours, cells were treated with 1 and 10 ng/mL FR901228 (*C*) or 25 and 250 ng/mL trichostatin A (*D*). At the indicated times, cells were lysed and luciferase activity was measured.

manner. For example, induction of p21 by histone deacetylase inhibitors does not require p53 (26–28). Both reactivation of mutant p53 and p53-independent transactivation may occur simultaneously. Then, rescue of p53 is superimposed on imitation of p53 functions. Therefore, it is difficult to distinguish “reactivation with imitation” from “pure imitation.”

To address this question, we compared parental A2780 (1A9) cells with wt p53 and two drug-resistant clones lacking functional p53: PTX22, harboring a mutant p53, and PTX15, a cell line previously shown to possess a pseudo-null wt p53, with absence of wt p53 protein. In these three cell lines with the same genetic background, the order of cytotoxicity was mutant p53 > wt p53 > null. The sensitivity of PTX22 cells (mutant p53) to FR901228 and trichostatin A is notable because these cells are highly resistant to paclitaxel. If anything, these cells may be resistant to other

anticancer drugs. Yet, these were more sensitive to trichostatin A and FR901228 than parental cells (Fig. 4). These results suggest that the presence of mutant p53, rather than the loss of wt p53, renders cells sensitive to histone deacetylase inhibitors. High induction of p21 and Mdm-2 in PTX22 cells and almost undetectable induction in PTX15 cells following treatment with FR901228 are consistent with pharmacologic rescue (activation) of mutant p53. Because mutant p53 is highly expressed, reactivation of mutant p53 is very toxic for PTX22 cells. In this scenario, histone deacetylase inhibitors are more toxic to cells with mutant p53 than to cells lacking p53.

Because histone deacetylases are necessary for all cells, histone deacetylase inhibitors are cytotoxic to both normal and tumor cells with wt p53 or mutant p53. Yet, even intermediate selectivity towards cells with mutant p53 can be clinically important. In a

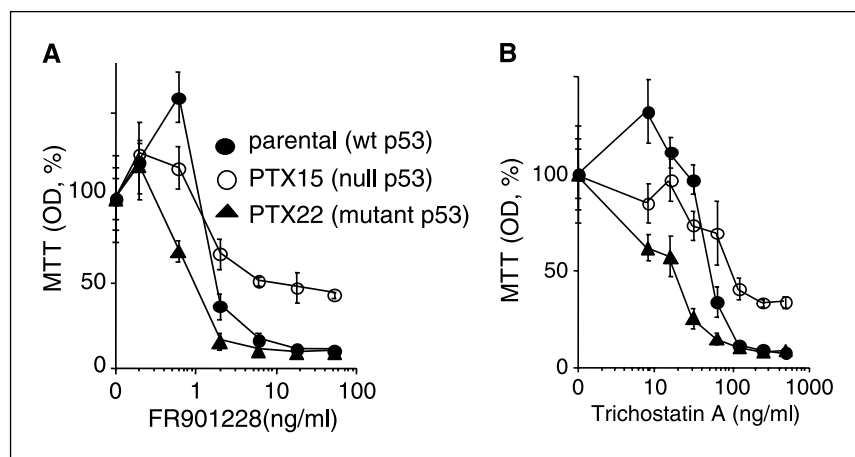
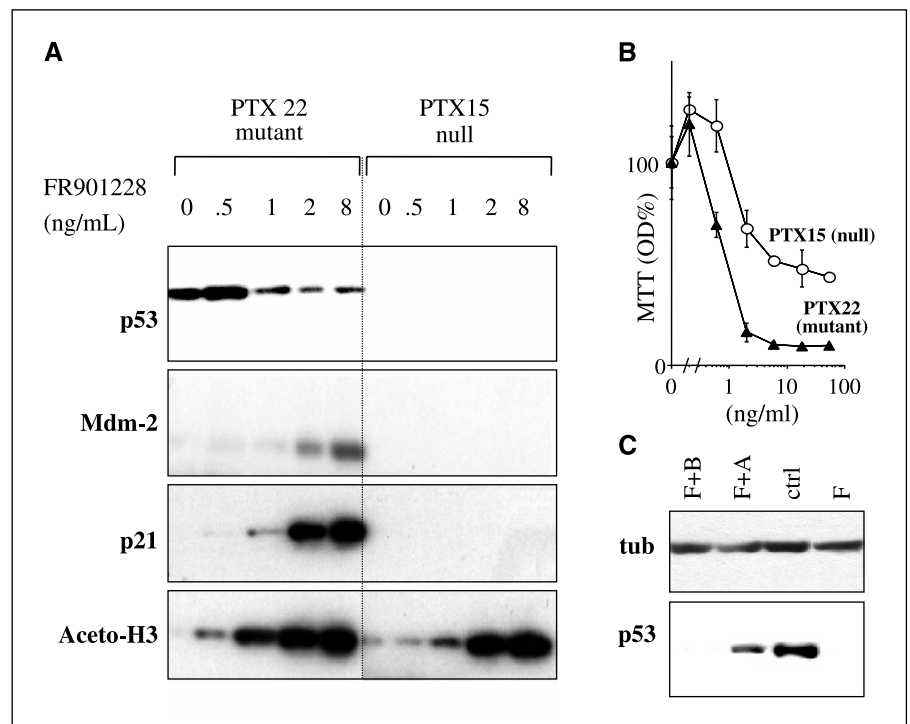


Figure 5. Comparison of parental cells with mutant-p53 and p53-null subclones. *A–B*, parental cells with wt p53 [A2780 (1A9)], a clone with undetectable p53 [PTX15, pseudo-null p53], and a clone with mutant p53 [PTX22] were treated with FR901228 (*A*) and trichostatin A (*B*), respectively. Cytotoxicity was measured by MTT assay after 3 days, as described in Materials and Methods.

Figure 6. PTX22 cells and PTX15 cells were treated with the indicated concentrations of FR901228. **A**, after 1 day, p53, Mdm-2, p21, and acetylated histone H3 were measured by immunoblot. **B**, MTT test was done after 3 days as described in Materials and Methods. **C**, PTX22 cells were treated with 10 ng/mL FR901228 alone (**F**), or with 10 μ mol/L nutlin-3a (**F + A**) or its inactive analogue nutlin-3b (**F + B**), or left untreated for 36 hours. Immunoblot for p53 and tubulin was done as described in Materials and Methods.



broader perspective, this shows how certain levels of selectivity may be achieved by targeting a non-cancer-specific target, such as histone deacetylases, in the presence of cancer-specific alteration, such as p53 mutation.

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