[CANCER RESEARCH 57, 2550–2554, July 1, 1997]

Advances in Brief

p53-dependent DNA Damage-induced Apoptosis Requires Fas/APO-1-independent Activation of CPP32β[1]

Ephraim J. Fuchs, Karen A. McKenna, and Atul Bedi[2]

Johns Hopkins Oncology Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287

Abstract

In many cell types, the p53 tumor suppressor protein is required for the induction of apoptosis by DNA-damaging chemotherapy or radiation. Therefore, identification of the molecular determinants of p53-dependent cell death may aid in the design of effective therapies of p53-deficient cancers. We investigated whether p53-dependent apoptosis requires activation of CPP32β (caspase 3), a cysteine protease that has been found to mediate apoptosis in response to ligation of the Fas molecule or to granzyme B, a component of CTL lytic granules. Irradiation-induced apoptosis was associated with p53-dependent activation of CPP32β-related proteolysis, and normal thymocytes were protected from irradiation by Acetyl-Asp-Glu-Val-Asp-CHO (Ac-DEVD-CHO), a specific inhibitor of CPP32β. We next examined whether the Fas system is required for p53-dependent apoptosis and whether stimuli that induce activation of CPP32β induce apoptosis in p53-deficient cells. Thymocytes or activated T cells from Fas-deficient mice were resistant to apoptosis induced by ligation of Fas or CD3, respectively, but remained normally susceptible to irradiation. Thymocytes from p53-deficient mice, although resistant to DNA damage, remained sensitive to CPP32β-mediated apoptosis induced by ligation of Fas or CD3, or by exposure to cytotoxic T cells. These results demonstrate that DNA damage-induced apoptosis of T cells requires p53-mediated activation of CPP32β by a mechanism independent of Fas/FasL interactions and suggest that immunological or molecular methods of activating CPP32β may be effective at inducing apoptosis in p53-deficient cancers that are resistant to conventional chemotherapy or irradiation.

Introduction

DNA-damaging drugs or irradiation kill tumor cells by the induction of a genetically determined suicide program called apoptosis (1, 2). In cells such as thymocytes or resting T cells, the p53 tumor suppressor protein has been found to be a critical mediator of apoptosis in response to DNA damage (1). Thus, loss of p53 function, a frequent occurrence in human cancers, may be intimately associated with the resistance of a tumor to chemotherapy or irradiation (3–9). The design of antineoplastic strategies to overcome such resistance requires the elucidation of the fundamental molecular mechanisms involved in p53-dependent apoptosis. A common, if not universal, feature of apoptosis is the activation of one or more members of a family of caspases, evolutionarily conserved cysteine proteases that include ICE[3] (caspase 1) and CPP32β (Yama/apopain/caspase 3; Refs. 10 and 11). The requirement of these proteases in vertebrate cell death has been demonstrated for apoptosis induced by ligation of Fas/Apo1/CD95 (12, 13). Binding of the FasL to the Fas receptor transduces an apoptotic signal that requires the sequential activation of at least three ICE-related proteases, including FLICE, ICE, and CPP32β (12, 14). Recent studies have also suggested that the Fas molecule may be involved in chemotherapy-induced apoptosis, given that doxorubicin-induced cytotoxicity of a human T-cell leukemia line is inhibited by blocking Fas-mediated signaling with $F(ab')_2$ anti-Fas antibody fragments (15). These studies raise the possibility that p53-dependent, DNA damage-induced apoptosis requires signals through the Fas molecule. However, there is conflicting evidence regarding the involvement of ICE/CPP32β-related proteolysis and the Fas system in p53-dependent apoptosis. Although p53 has been reported to activate transcription of the *Fas* gene (16), thymocytes from ICE-deficient mice are resistant to anti-Fas antibody but remain susceptible to p53-dependent, irradiation-induced apoptosis (17). Moreover, the involvement of CPP32β in the mechanism by which p53 augments DNA damage-induced apoptosis has not been explored.

We investigated whether p53-dependent apoptosis requires CPP32β activation or the Fas system by examining the effects of ionizing radiation and anti-Fas antibodies on the viability of thymocytes and peripheral T cells from mice deficient in p53, cell surface Fas, or neither molecule. Our results demonstrate that, in thymocytes and T cells, p53 induction (by DNA damage) and Fas ligation initiate independent pathways leading to the activation of CPP32β, which in both cases is required for the induction of apoptosis. We also find that CTL-derived granzyme B can overcome resistance to apoptosis mediated by deficiency of either p53 or Fas. These results show that molecular or immunological methods of CPP32β activation may be used to bypass p53 deficiency and induce death in cells that are resistant to conventional genotoxic anticancer agents.

Materials and Methods

Irradiation-induced Apoptosis. Thymocytes and splenocytes were isolated from homozygous p53-deficient mice [B6-Trp53^{tm1Tyj} ($p53^{-/-}$); The Jackson Laboratory, Bar Harbor, ME; Ref. 6], mice with *Fas* gene mutations at the *lpr* locus (lpr; The Jackson Laboratory) and their corresponding normal controls of the same sex and genetic background [B6 (wt $p53^{+/+}$; National Cancer Institute, NIH, Frederick, MD) and MRL/MpJ+ (wt Fas; The Jackson Laboratory)], as described previously (6). Cells were resuspended at a concentration of 5×10^5 cells/ml in EHAA medium (Biofluids, Rockville, MD) containing 10% FCS, 5×10^{-5} M 2-mercaptoethanol, glutamine, and antibiotics (complete medium) and maintained at 37°C in a 5% CO_2 humidified atmosphere. Following exposure to graded doses of ionizing radiation (0–10 Gy) with a ^{137}Cs dual source γ-cell irradiator (Atomic Energy Commission, Ottawa, Ontario, Canada), cells were assessed for viability by trypan blue dye exclusion. The percentage viability was normalized to the corresponding viability in untreated cells derived from the same animal. Values represent the average viability (±SE) from four independent experiments; each experiment compared cells pooled from two p53-deficient or Fas-deficient animals and their corresponding wt controls. The induction of apoptosis was confirmed by oligonucleosomal DNA fragmentation assays (9).

Received 3/24/97; accepted 5/15/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was funded in part by a grant from the Alexander and Margaret Stewart Trust, Grant K08AI01249 from the National Institute of Allergy and Infectious Diseases (to E. J. F.), and Grant 1 R29CA71660-01A1 from the National Cancer Institute (to A. B). A. B. is a recipient of a Passano Physician Scientist award, a Jose Carreras American Society of Hematology Scholar award, and grants from the American Cancer Society and the Wendy Will Case Cancer Fund.

[2] To whom requests for reprints should be addressed, at 3-127 Johns Hopkins Oncology Center, 600 North Wolfe Street, Baltimore, MD 21287. Phone: (410) 955-8797; Fax: (410) 955-1969.

[3] The abbreviations used are: ICE, interleukin 1β-converting enzyme CTL; FasL, Fas ligand; lpr, MRL/MpJ-*Fas*^{lpr}; wt, wild type; Ac-DEVD-CHO, Acetyl-Asp-Glu-Val-Asp-aldehyde; PARP, poly(ADP-ribose) polymerase; B6 mice, C57BL/6NCr mice.

Anti-Fas Antibody-mediated Apoptosis. Thymocytes (5×10^5/ml) from $p53^{-/-}$ or lpr mice and their corresponding normal controls were treated with 1 μg/ml anti-Fas antibody (Jo2; PharMingen, San Diego, CA), as described (18). Fas-treated cells and untreated controls were harvested after 12 and 24 h and assessed for viability as described above. Values represent the mean viability (±SE) from three independent experiments; each experiment compared cells pooled from two p53-deficient or Fas-deficient animals and their corresponding wt controls.

CD3-mediated Apoptosis of Activated T Cells (Activation-induced Cell Death). Splenocytes from B6, $p53^{-/-}$, and Fas-deficient mice were cultured (2×10^6 cells/ml) in 75-cm^2 flasks containing 50 ml of Iscove's modified Dulbecco's medium (BioWhittaker, Inc., Walkersville, MD) with 10% FCS and 2 μg/ml concanavalin A (Sigma Chemical Co., St. Louis, MO). After 48 h, cells were washed twice and then added at 10^6 cells/ml in Iscove's modified Dulbecco's medium and 10% FCS supplemented with recombinant human interleukin-2 (50 units/ml; Chiron, Emeryville, CA) to plates precoated overnight with 10 μg/ml anti-CD3 (2C11; gift of Dr. Jeff Bluestone, Ben May Institute, Chicago, IL). CD3-induced cell death was measured after 24 h by trypan blue dye exclusion.

Inhibition of Thymocyte Apoptosis by CPP32β-related Protease Inhibitors. Thymocytes from B6 mice (5×10^5/ml) were preincubated for 3 h with or without the CPP32β-related protease inhibitor, Ac-DEVD-CHO (300 μM; BACHEM, Inc., Torrance, CA), or without the inhibitor, and then treated with graded doses of ionizing radiation (0–10 Gy) or anti-Fas antibody (1 μg/ml). The cells were maintained in the absence or presence of the inhibitor and assessed for viability and apoptosis as described above.

Immunoblot Analyses of Proteolytic Cleavage of PARP. Thymocytes were isolated 8 h after exposure to 0, 5, or 10 Gy irradiation, washed once in PBS, and lysed by addition of 100 μl ice-cold lysis buffer [6 M guanidine hydrochloride, 250 mM Tris-HCl (pH 8.5), 10 mM EDTA, 1% 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin]. The lysates were centrifuged at 15,000 × *g* for 10 min at 4°C, and total protein was quantified by bicinchoninic assay (Pierce Chemical Co., Rockford, IL). Samples containing 50 μg of protein were subjected to 10% SDS-PAGE, transferred to nitrocellulose, and probed with a goat polyclonal antibody against an epitope corresponding to amino acids 1–20 at the NH_2 terminus of PARP of mouse origin (A-20; Santa Cruz Biotechnology, Santa Cruz, CA). Immunoblots were developed with an enhanced chemiluminescence kit (Amersham Corp., Arlington Heights, IL).

Induction and Assays of Activated CTLs. CTLs were generated by culturing replicates of 4 million responder spleen cells and 2 million irradiated (30 Gy) spleen stimulators in 2 ml of complete medium. After 5 days of culture, replicate wells were pooled, and killing of target cells was assessed at various responder:target ratios by the JAM test (19), a sensitive assay for target cell DNA fragmentation. Target cells were prepared by culturing 2 million splenocytes for 48 h in 2 ml of complete medium containing 2 μg/ml concanavalin A and adding 0.5 μCi [^{3}H]thymidine for the last 16 h of culture. Strains of origin of the responder, stimulator, and target cells are indicated in the figure legends.

Results

Irradiation-induced Apoptosis Requires p53-dependent Activation of CPP32β. Thymocytes and resting splenic lymphocytes undergo p53-dependent apoptosis in response to DNA damage (6–8). We confirmed the requirement of p53 in DNA damage-induced apoptosis of thymocytes by demonstrating the relative resistance of cells from homozygous $p53^{-/-}$ mice compared to $p53^{+/+}$ controls to graded doses of irradiation (Fig. 1, *A* and *B*). The role of CPP32β in p53-dependent apoptosis was then assessed by incubating $p53^{+/+}$ thymocytes with Ac-DEVD-CHO, a specific tetrapeptide inhibitor of CPP32β, prior to and after irradiation. Ac-DEVD-CHO protected $p53^{+/+}$ thymocytes from radiation-induced apoptosis (Fig. 1, *A* and *C*), indicating that p53-dependent apoptosis requires activation of CPP32β. The p53-dependent activation of CPP32β was also confirmed by examining the irradiation-induced cleavage of PARP, a known endogenous substrate for CPP32β (20, 21). CPP32β activation results in cleavage of PARP (M_r 116,000) into a M_r 85,000 COOH-terminal fragment and a M_r 24,000 NH_2-terminal fragment. Irradiation-induced apoptosis of $p53^{+/+}$ thymocytes was accompanied by PARP cleavage, as demonstrated by loss of the M_r 116,000 protein by Western blot analyses using an antibody directed against the NH_2 terminus (amino acids 1–20) of mouse PARP (Fig. 1*D*). PARP cleavage was not observed following irradiation of $p53^{-/-}$ thymocytes, indicating that functional p53 is required for the enzymatic activation of CPP32β following DNA damage (Fig. 1*D*).

p53-dependent Apoptosis Does Not Require Fas Ligation. A previous report has shown that doxorubicin treatment of a human T-cell leukemia line induces FasL expression and apoptosis through a Fas-FasL interaction (15). Because doxorubicin induces p53 and because Fas ligation activates CPP32β, it is possible that p53-dependent apoptosis of normal thymocytes or T cells requires a signal through cell surface Fas. However, thymocytes and splenocytes from Fas-deficient lpr mice and their normal Fas^+ (MRL/MpJ+) counterparts were equally susceptible to irradiation-induced apoptosis (Fig. 2, *A* and *B*). These data indicate that p53-dependent activation of CPP32β and apoptosis in response to irradiation does not require signaling through the Fas molecule.

p53-deficient Cells Remain Susceptible to Fas-induced Apoptosis. The preceding results suggest that p53 induction (by DNA damage) and Fas ligation initiate distinct signaling pathways, each of which result ultimately in the activation of CPP32β and apoptosis. These findings raise the possibility that p53-deficient cells that are resistant to genotoxic agents may be induced to undergo apoptosis by activating CPP32β through Fas-mediated signal transduction. To test this hypothesis, we examined the effect of Fas-mediated activation of CPP32β on the sensitivity of thymocytes from p53-deficient ($p53^{-/-}$), Fas-deficient (lpr), and normal wt mice to ligation of Fas with an anti-Fas antibody (Jo2). Although thymocytes from lpr mice were resistant to anti-Fas antibody-mediated apoptosis, p53-deficient and normal thymocytes were equally susceptible to Fas-induced death (Fig. 3*A*). We next tested the sensitivity of peripheral T cells from $p53^{-/-}$, Fas-deficient, and normal mice to activation-induced cell death, a form of apoptosis that results from treating activated T cells with antibodies to the CD3 complex, and which has been demonstrated to be dependent upon signals through T-cell surface Fas molecules (22–24). Whereas activated T cells from lpr mice were resistant to anti-CD3-induced death, p53-deficient cells were normally susceptible to activation-induced apoptosis (Fig. 3*B*). Therefore, p53-deficient cells remain susceptible to Fas-dependent apoptosis induced by anti-Fas antibody or CD3 ligation.

Neither p53 Deficiency Nor Fas Deficiency Confers Protection against Apoptosis Induced by CTLs. Activated CTLs induce rapid DNA fragmentation in target cells primarily through the action of granzyme B, a serine protease that shares substrate specificity with ICE family proteases and which directly cleaves and activates CPP32β (25). Unlike normal CTLs, which can induce target cell apoptosis within minutes after contact, activated CTLs from mice deficient in granzyme B induce little if any target cell DNA fragmentation after as much as 4 h of coculture (26), thereby demonstrating the critical role of granzyme B in rapid target cell apoptosis. Because CPP32β activation is responsible for granzyme B-mediated cytotoxicity exerted by CTLs, we tested the hypothesis that activated alloreactive CTLs can efficiently induce apoptosis of p53-deficient and Fas-deficient targets. Normal, p53-deficient, and Fas-deficient target cells were equally susceptible to rapid (≤2 h) induction of apoptosis by activated, alloreactive CTLs (Fig. 4, *A* and *B*). These results demonstrate that, akin to Fas ligation, CTL-induced activation of CPP32β by granzyme B is another effective mechanism for inducing apoptosis in p53-deficient cells.

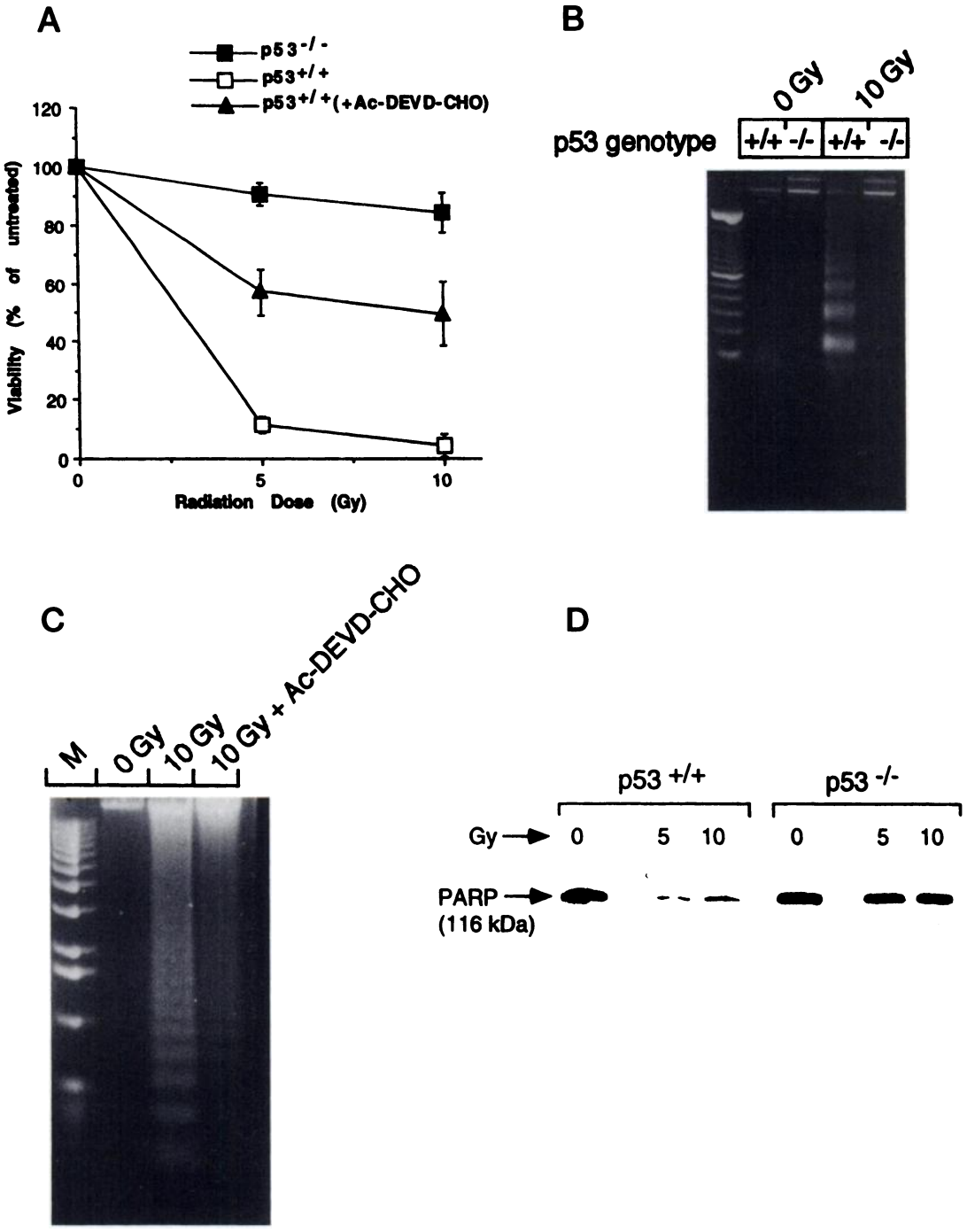

Fig. 1. Irradiation-induced apoptosis requires p53-dependent activation of CPP32β. *A*, thymocytes from homozygous p53-deficient ($p53^{-/-}$) mice and $p53^{+/+}$ controls of the same genetic background (B6) were exposed to graded doses of ionizing radiation (0–10 Gy) in the presence or absence of the CPP32β inhibitor, AcDEVD-CHO, and assessed for viability at the various times indicated, as described. The percentage viability was normalized to the corresponding viability in untreated cells derived from the same animal. Values represent the average viability (*bars*, SE) from four independent experiments; each experiment compared cells pooled from two p53-deficient animals and their corresponding wt controls. *B* and *C*, DNA was extracted from $p53^{+/+}$ and $p53^{-/-}$ thymocytes 8 h after exposure to 0 or 10 Gy irradiation in the presence or absence of Ac-DEVD-CHO and subjected to oligonucleosomal DNA fragmentation assays, as described. *D*, total protein, extracted from thymocytes 8 h after exposure to 0, 5, or 10 Gy irradiation, was subjected to gel electrophoresis, transferred to nitrocellulose, and probed with an antibody against an epitope contained within the NH_2 terminus of mouse PARP.

Discussion

The success of genotoxic anticancer therapy is determined by the effective activation of apoptosis in tumor cells in response to the induced DNA damage. Genotypic alterations in human cancers that interfere with DNA damage-induced apoptosis confer resistance to diverse chemotherapeutic agents or irradiation. Previous studies have demonstrated that the *p53* tumor suppressor gene is an important component of DNA damage-induced apoptosis of diverse primary and tumor cell types. The identification of the critical molecular determinants of p53-dependent apoptosis should provide fundamental insights into the mechanism by which p53-deficient human cancers evade cell death by genotoxic anticancer therapy. In this study, we have demonstrated that irradiation-induced apoptosis requires p53-dependent activation of CPP32β, a member of the caspase family of

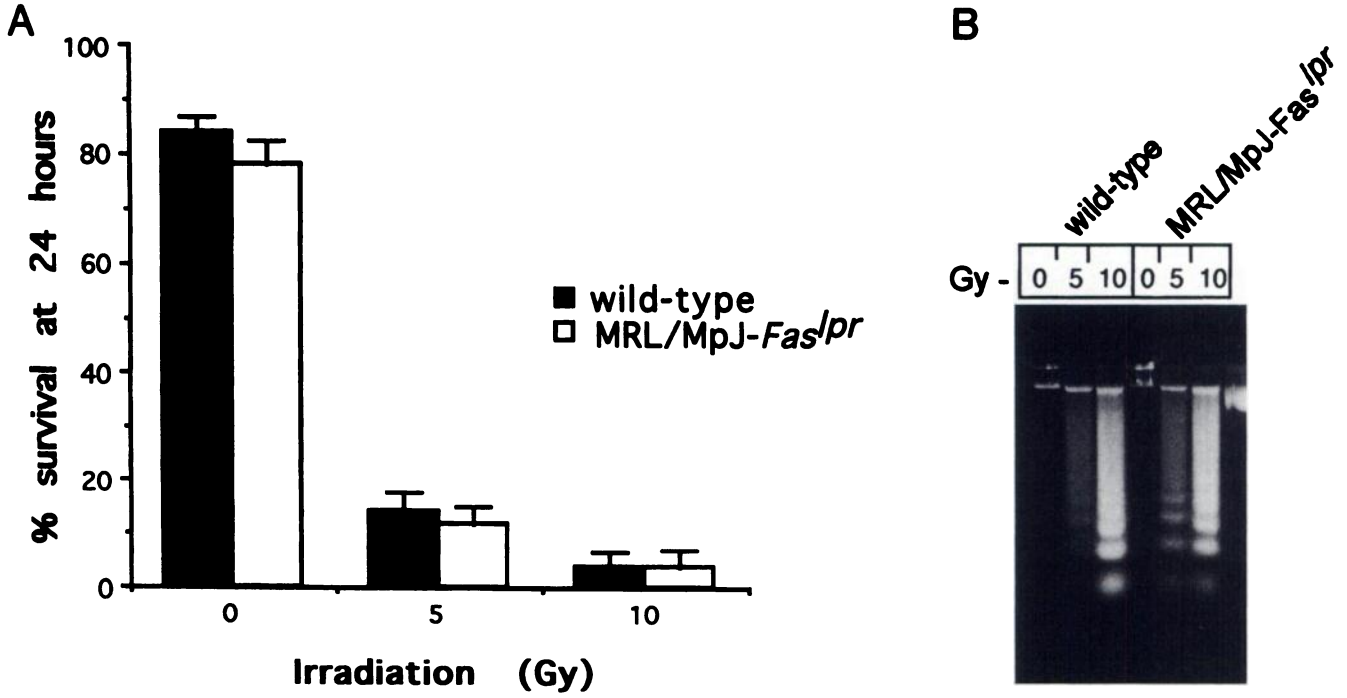

Fig. 2. p53-dependent apoptosis does not require the Fas-FasL system. *A*, thymocytes from Fas-deficient lpr mice and wt (MRL/MpJ+) controls were exposed to graded doses of ionizing radiation (0–10 Gy) and assessed for viability after 24 h, as described. The percentage viability was normalized to the corresponding viability in untreated cells derived from the same animal. Values represent the average viability (*bars*, SE) from four independent experiments; each experiment compared cells pooled from two Fas-deficient animals and their corresponding wt controls. *B*, DNA was extracted from wt (MRL/MpJ+) and Fas-deficient (lpr) thymocytes 8 h after exposure to 0, 5, or 10 Gy of irradiation and subjected to oligonucleosomal DNA fragmentation assays, as described.

cysteine proteases that are evolutionarily conserved determinants of cell death. These data suggest that the inability of p53-deficient cells to activate CPP32β in response to DNA damage may be responsible for their resistance to genotoxic anticancer agents. We have also shown that p53-dependent activation of CPP32β during DNA damage-induced apoptosis is independent of the Fas-FasL system. A corollary of this finding is that p53-independent mechanisms of CPP32β activation may induce apoptosis in chemotherapy- or radiation-resistant, p53-deficient tumors. In support of this premise, we have demonstrated that Fas or CTL (granzyme B)-mediated activation

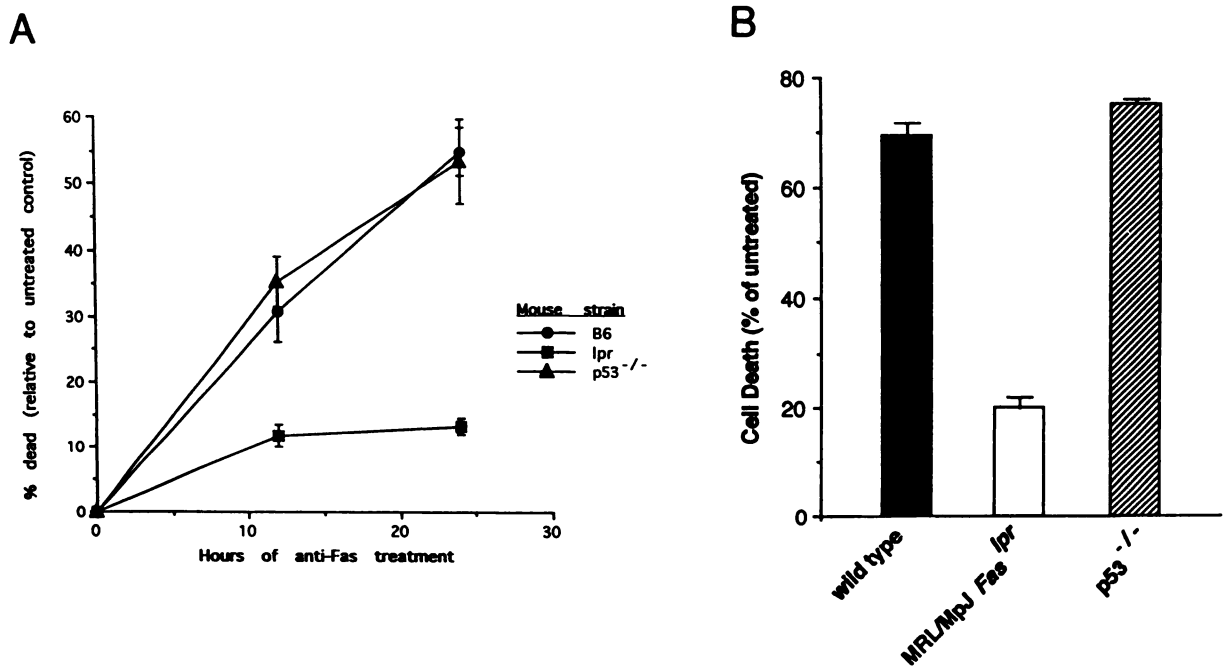

Fig. 3. p53-deficient cells remain susceptible to Fas-mediated apoptosis. *A*, thymocytes from Fas-deficient mice (lpr), p53-deficient mice (B6-Trp53^{tm1Tyj}), and normal wt mice (B6) were incubated at 37°C for 24 h with or without 1 μg/ml anti-Fas antibody, and the percentage of dead cells was assayed, as described, to determine the kinetics of anti-Fas-mediated death of thymocytes of each genotype. Values represent the average viability (*bars*, SE) from three independent experiments; each experiment compared cells pooled from two p53-deficient or Fas-deficient animals and their corresponding wt controls. *B*, splenocytes from B6, p53$^{-/-}$, and Fas-deficient mice were activated for 48 h, washed twice, and then added to plates precoated with anti-CD3. CD3-induced cell death was measured after 24 h by trypan blue dye exclusion. Data shown are means; *bars*, SE.

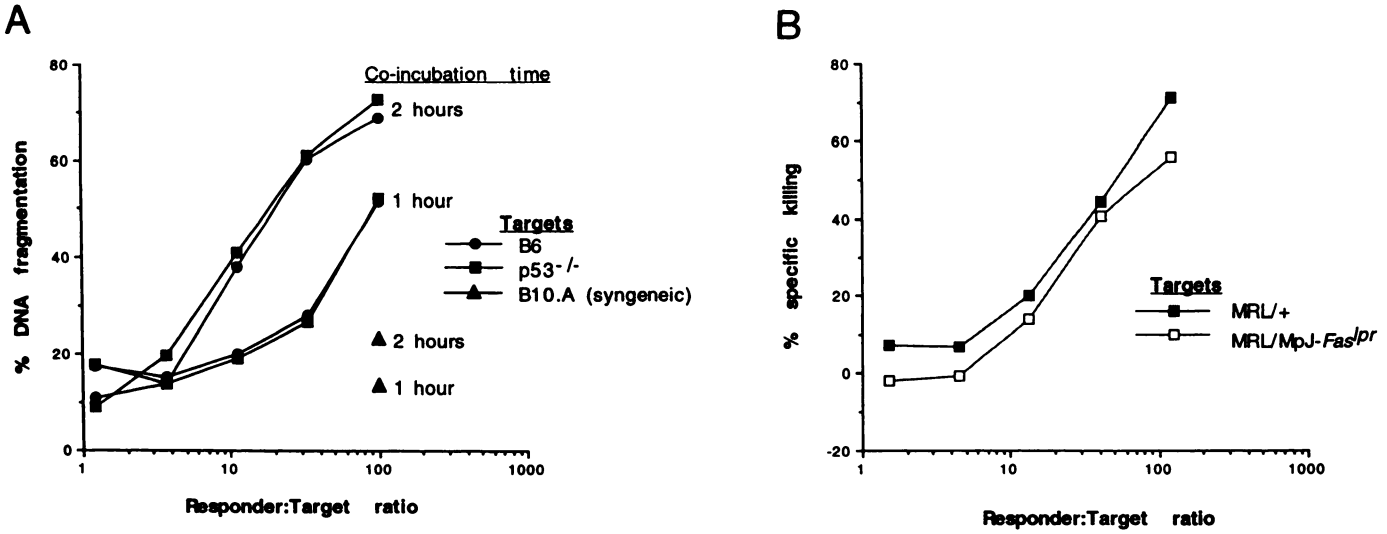

Fig. 4. Neither p53 deficiency nor Fas deficiency confers protection against apoptosis induced by CTLs. *A*, normal and p53-deficient cells are equally susceptible to apoptosis induced by activated, alloreactive CTLs. CTLs specific for H-2^b alloantigens were generated by culturing B10.A H-2^a responder spleen cells with irradiated B6 (H-2^b) stimulators. Five days later, responder CTLs were harvested and tested for killing of 10^5 B6, $p53^{-/-}$ (B6-$Trp53^{tm1Tyj}$; H-2^b), or B10.A targets after 1 or 2 h of coculture, as indicated. *B*, normal and Fas-deficient cells are equally susceptible to apoptosis induced by activated, alloreactive CTLs. CTLs specific for H-2^k alloantigens were generated by culturing B6 responder spleen cells with irradiated MRL/+ (H-2^k) stimulators. Five days later, CTLs were tested for killing of MRL/+ or Fas-deficient lpr targets after 2 h of coculture.

of CPP32β is indeed effective in inducing apoptosis of p53-deficient cells. These insights provide a biological rationale for experimental therapeutic strategies based on the biochemical or immunological activation of CPP32β to eliminate p53-deficient cancers that resist conventional genotoxic anticancer agents.

References

1. Fisher, D. E. Apoptosis in cancer therapy: crossing the threshold. Cell, *78:* 539–542, 1994.
2. Thompson, C. B. Apoptosis in the pathogenesis and treatment of disease. Science (Washington DC), *267:* 1456–1462, 1995.
3. Kinzler, K. W., and Vogelstein, B. Cancer therapy meets p53. N. Engl. J. Med., *331:* 49–50, 1994.
4. Kerr, J. F., Wyllie, A. H., and Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer, *26:* 239–257, 1972.
5. Lowe, S. W., Ruley, H. E., Jacks, T., and Housman, D. E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell, *74:* 957–967, 1993.
6. Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, M. L., and Wyllie, A. H. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature (Lond.), *362:* 849–852, 1993.
7. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature (Lond.), *362:* 847–849, 1993.
8. Strasser, A., Harris, A. W., Jacks, T., and Cory, S. DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell, *79:* 329–339, 1994.
9. Yao, S. L., Akhtar, A. J., McKenna, K. A., Bedi, G. C., Sidransky, D., Mabry, M., Ravi, R., Collector, M. I., Jones, R. J., Sharkis, S. J., Fuchs, E. J., and Bedi, A. Selective radiosensitization of p53-deficient cells by caffeine-mediated activation of p34cdc2 kinase. Nat. Med., *2:* 1140–1143, 1996.
10. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazebnik, Y. A., *et al.* Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature (Lond.), *376:* 37–43, 1995.
11. Martin, S. J., and Green, D. R. Protease activation during apoptosis: death by a thousand cuts? Cell, *82:* 349–352, 1995.
12. Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., Orourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J. D., Zhang, M., Gentz, R., Mann, M., Krammer, P. H., Peter, M. E., and Dixit, V. M. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signalling complex. Cell, *85:* 817–827, 1996.
13. Enari, M., Talanian, R. V., Wong, W. W., and Nagata, S. Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis. Nature (Lond.), *380:* 723–726, 1996.
14. Nagata, S., and Golstein, P. The Fas death factor. Science (Washington DC), *267:* 1449–1456, 1995.
15. Friesen, C., Herr, I., Krammer, P. H., and Debatin, K. M. Involvement of the CD95 (APO-1/FAS) receptor/ligand system in drug-induced apoptosis in leukemia cells. Nat. Med., *2:* 574–577, 1996.
16. Owen-Schaub, L. B., Zhang, W., Cusack, J. C., Angelo, L. S., Santee, S. M., Fujiwara, T., Roth, J. A., Deisseroth, A. B., Zhang, W. W., Kruzel, E., *et al.* Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. Mol. Cell. Biol., *15:* 3032–3040, 1995.
17. Kuida, K., Lippke, J. A., Ku, G., Harding, M. W., Livingston, D. J., Su, M. S., and Flavell, R. A. Altered cytokine export and apoptosis in mice deficient in interleukin-1 β converting enzyme. Science (Washington DC), *267:* 2000–2003, 1995.
18. Ogasawara, J., Suda, T., and Nagata, S. Selective apoptosis of $CD4^+CD8^+$ thymocytes by the anti-Fas antibody. J. Exp. Med., *181:* 485–491, 1995.
19. Matzinger, P. The JAM test: a simple assay for DNA fragmentation and cell death. J. Immunol. Methods, *145:* 185–192, 1991.
20. Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G., and Earnshaw, W. C. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. Nature (Lond.), *371:* 346–347, 1994.
21. Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E., and Poirier, G. G. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res., *53:* 3976–3985, 1993.
22. Ju, S. T., Panka, D. J., Cui, H., Ettinger, R., el-Khatib, M., Sherr, D. H., Stanger, B. Z., and Marshak-Rothstein, A. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. Nature (Lond.), *373:* 444–448, 1995.
23. Dhein, J., Walczak, H., Baumler, C., Debatin, K. M., and Krammer, P. H. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). Nature (Lond.), *373:* 438–441, 1995.
24. Brunner, T., Mogil, R. J., LaFace, D., Yoo, N. J., Mahboubi, A., Echeverri, F., Martin, S. J., Force, W. R., Lynch, D. H., Ware, C. F., *et al.* Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature (Lond.), *373:* 441–444, 1995.
25. Darmon, A. J., Nicholson, D. W., and Bleackley, R. C. Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. Nature (Lond.), *377:* 446–448, 1995.
26. Heusel, J. W., Wesselschmidt, R. L., Shresta, S., Russell, J. H., and Ley, T. J. Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells. Cell, *76:* 977–987, 1994.

p53-dependent DNA Damage-induced Apoptosis Requires Fas/APO-1-independent Activation of CPP32 β

Ephraim J. Fuchs, Karen A. McKenna and Atul Bedi

Cancer Res 1997;57:2550-2554.