Although the phage-like appearance of the virus was surprising, marine viruses are highly diverse, and morphologies traditionally characteristic of phages cannot be assumed to infect only prokaryotes (*20*, *21*). Our experimental work indicates that the observed virus is the lysing agent of *A. anophagefferens*.

For *A. anophagefferens*, this study has established that viral control is an important consideration to be evaluated relative to the recurrence of blooms and their sudden dissipation. Viral lysis of *A. anophagefferens* during bloom periods may also release cellular products into the marine environment. This release would increase the concentration of dissolved and particulate organic matter (*22*) and possibly dimethylsulfide and acrylic acid (*23*–*26*), chemical compounds that might be associated with the chronic toxicity of bivalve starvation.

Another consideration in the population dynamics of *A. anophagefferens* is the inactivation of virus by environmental factors, thus allowing for blooms. For example, high concentrations of iron promote "brown tide" blooms in Long Island bays by stimulating growth of *A. anophagefferens* (*8*). Solid clay minerals and silts, iron oxides, and metal coagulants temporarily bind free virus particles (*27*–*29*), possibly precipitating and removing the potential for viral infection from the water column and effectively removing viral control on population densities.

REFERENCES AND NOTES

1. J. McN. Sieburth, P. W. Johnson, P. E. Hargraves, *J. Phycol.* **24**, 416 (1988).
2. E. M. Cosper *et al.*, in *Novel Phytoplankton Blooms*, E. M. Cosper, V. M. Bricelj, E. J. Carpenter, Eds. (Springer-Verlag, New York, 1989), pp. 317–340.
3. C. S. Yensch, D. A. Phinney, L. P. Shapiro, in (*2*), pp. 77–83.
4. V. M. Bricelj and S. H. Kuenstner, in (*2*), pp. 491–509.
5. G. A. Tracey *et al.*, *J. Shellfish Res.* **7**, 671 (1988).
6. T. J. Smayda and T. A. Villareal, in (*2*), pp. 159–187.
7. E. M. Cosper, C. Lee, E. J. Carpenter, in *Toxic Marine Phytoplankton*, E. Graneli, B. Sundstrm, L. Elder, D. M. Anderson, Eds. (Elsevier, New York, 1990), pp. 17–28.
8. E. M. Cosper, R. T. Garry, A. J. Milligan, M. H. Doall, in *Toxic Phytoplankton Blooms in the Seas*, T. J. Smayda and Y. Shimizu, Eds. (Elsevier, New York, 1993), pp. 667–673.
9. M. E. C. Vieira, E. M. Cosper, E. J. Beltrami, *ICES Mar. Sci. Symp.* **195**, 223 (1992).
10. C. A. Suttle, A. M. Chan, M. T. Cottrell, *Appl. Environ. Microbiol.* **57**, 721 (1991).
11. F. M. M. Morel, J. G. Rueter, D. M. Anderson, R. R. L. Guillard, *J. Phycol.* **15**, 135 (1979).
12. K. L. D. Milligan, unpublished data.
13. L. M. Proctor, thesis, University of New York at Stony Brook (1991).
14. K. G. Porter and Y. S. Feig, *Limnol. Oceanogr.* **25**, 943 (1980).
15. E. M. Cosper *et al.*, *Estuaries* **10**, 284 (1987).
16. L. M. Proctor and J. A. Fuhrman, *Nature* **343**, 60 (1990).
17. C. A. Suttle, A. M. Chan, M. T. Cottrell, *ibid.* **347**, 467 (1990).
18. E. L. Martin and R. Benson, in *Selected Papers in Phycology II*, J. R. Rosowski and B. C. Parker, Eds. (Phycological Society of America, Lawrence, KS, 1982), pp. 793–798.
19. J. A. Mayer and F. J. R. Taylor, *Nature* **281**, 299 (1979).
20. J. A. Fuhrman and C. A. Suttle, *Oceanography* **6**, 51 (1993).
21. J. L. Van Etten, L. C. Lane, R. H. Meints, *Microbiol. Rev.* **55**, 586 (1991).
22. L. M. Proctor and J. A. Fuhrman, *Mar. Ecol. Prog. Ser.* **69**, 133 (1991).
23. M. D. Keller, W. K. Bellows, R. R. L. Guillard, in (*7*), pp. 101–115.
24. A. Vairavamurthy, M. O. Andreae, R. L. Iverson, *Limnol. Oceanogr.* **36**, 59 (1985).
25. G. Tracey, R. Steele, L. Wright, in (*7*), pp. 233–237.
26. R. J. Charlson, J. E. Lovelock, M. O. Andreae, S. G. Warren, *Nature* **326**, 655 (1987).
27. R. B. Kapuscinski and R. Mitchell, *Water Res.* **14**, 363 (1980).
28. G. Bitton, *ibid.* **8**, 227 (1974).
29. E. Lund and V. Ronne, *ibid.* **7**, 863 (1973).

30. We thank D. Colflesh and G. Rudomen (University Microscopy Imaging Center, Health Sciences Center, State University of New York, Stony Brook) for help with electron microscopy. Also, we thank M. Doall for technical support and J. Ben-Porath for technical assistance. Supported by New York Seagrant Institute to E.M.C. (NOAA grant NA 90AA-D-SG-078).

29 March 1994; accepted 9 September 1994

p53 Status and the Efficacy of Cancer Therapy in Vivo

Scott W. Lowe, Stephan Bodis, Andrea McClatchey, Lee Remington, H. Earl Ruley, David E. Fisher, David E. Housman, Tyler Jacks*

The therapeutic responsiveness of genetically defined tumors expressing or devoid of the *p53* tumor suppressor gene was compared in immunocompromised mice. Tumors expressing the *p53* gene contained a high proportion of apoptotic cells and typically regressed after treatment with gamma radiation or adriamycin. In contrast, *p53*-deficient tumors treated with the same regimens continued to enlarge and contained few apoptotic cells. Acquired mutations in *p53* were associated with both treatment resistance and relapse in *p53*-expressing tumors. These results establish that defects in apoptosis, here caused by the inactivation of *p53*, can produce treatment-resistant tumors and suggest that *p53* status may be an important determinant of tumor response to therapy.

Although the tumor-specific action of most anticancer agents has been attributed to their debilitating effects on actively proliferating cells, an increasing body of evidence suggests that anticancer agents instead induce apoptosis [reviewed in (*1*, *2*)]. This view has profound implications for understanding the therapeutic response of human tumors. First, events subsequent to the interaction between anticancer agents and their primary intracellular targets may have a substantial effect on tumor response. Second, factors that increase the propensity for apoptosis may determine the therapeutic index whereby anticancer agents selectively destroy tumor cells. Finally, because apoptosis requires a genetic program, mutations in apoptotic pathways could produce drug-resistant tumors.

Studies suggest that the *p53* tumor suppressor gene is an essential component of the apoptotic program induced by anticancer agents in oncogenically transformed cells (*3*, *4*). To determine whether *p53* influences tumor responsiveness in vivo, we developed a transplantable fibrosarcoma model in which tumors differed primarily in their *p53* status. Embryonic fibroblasts transformed by coexpression of the adenovirus early region 1A (E1A) and activated *ras* oncogenes form tumors when subcutaneously injected into nude mice regardless of their *p53* status, but $p53^{+/+}$ cells are highly sensitive to apoptosis in vitro (*5*). In agreement with earlier studies, oncogenically transformed $p53^{+/+}$ cells formed fewer tumors and with a longer latency than $p53^{-/-}$ cells. The $p53^{-/-}$ cells produced tumors at all injected sites with an average latency of 8 ± 4 days, whereas the $p53^{+/+}$ cells produced tumors at $82 \pm 24\%$ ($P < 0.11$, t test) of sites injected, with an average latency of 18 ± 7 days ($P < 0.03$). After reaching a palpable size, tumors derived from both $p53^{+/+}$ and $p53^{-/-}$ cells grew at similar rates.

The *p53* status had a dramatic effect on tumor response to gamma irradiation. Most

S. W. Lowe, A. McClatchey, L. Remington, D. E. Housman, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
S. Bodis, Joint Center for Radiation Therapy and Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.
H. E. Ruley, Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA.
D. E. Fisher, Dana-Farber Cancer Institute and The Children's Hospital, Harvard Medical School, Boston, MA 02115, USA.
T. Jacks, Howard Hughes Medical Institute, Center for Cancer Research, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed.

tumors derived from $p53^{+/+}$ cells responded well to 7 gray [(Gy) absorbed dose of ionizing radiation] (Fig. 1A and Table 1), typically regressing to less than 50% of their pretreatment volume within 5 days. Some tumors disappeared completely, although all but one eventually regrew. The magnitude of the response varied with the clone injected. For example, tumors derived from one *p53*-expressing clone (C8) responded well to 2 Gy, whereas another clone (C10) responded poorly to doses as high as 12 Gy (Table 1). In contrast, tumors derived from $p53^{-/-}$ cells displayed little, if any, response to 7 Gy of gamma radiation. Although some tumors remained the same size for several days, 9 of 10 tumors were significantly larger by 1 week after treatment (Fig. 1B and Table 1). In several instances, gamma irradiation had no effect on tumor growth. Whereas tumors derived from $p53^{+/+}$ cells showed enhanced sensitivity to higher doses of radiation, $p53^{-/-}$ tumors were refractory to levels as high as 12 Gy (Fig. 1, C and D, and Table 1).

The *p53* status also influenced tumor responsiveness to the chemotherapeutic drug adriamycin. Adriamycin treatment induced a rapid regression of tumors derived from $p53^{+/+}$ cells but did not substantially affect the growth of $p53^{-/-}$ tumors (Fig. 1, E and F). Interestingly, the one tumor derived from $p53^{+/+}$ cells that did not respond to adriamycin originated from a clone (C10) that also produced relatively radioresistant tumors (Table 1).

Untreated tumors derived from $p53^{+/+}$ and $p53^{-/-}$ fibroblasts displayed a typical fibrosarcoma histology (Fig. 2, A and B). Both tumor types contained many mitotic figures and some necrotic zones. However, untreated tumors derived from $p53^{+/+}$ cells contained more apoptotic cells compared with *p53*-deficient tumors, as measured by the terminal deoxytransferase-mediated deoxyuridine-triphosphate-biotin nick end labeling (TUNEL) assay (Fig. 2, C and D).

Cell death in regressing tumors had morphological and physiological features of apoptosis. Within 2 days after treatment with gamma radiation or adriamycin, tumors derived from $p53^{+/+}$ cells contained many pyknotic cells that displayed fragmented nuclei (Fig. 2C) and stained with TUNEL (Fig. 2G). The remaining normal cells were often surrounded by large regions of extracellular space (Fig. 2, C and E). By contrast, treated $p53^{-/-}$ tumors contained few regions of pyknosis and cell loss (Fig. 2D), although there was a small increase in the number of TUNEL-positive cells interspersed throughout the tumor (Fig. 2, F and H). These data indicate that the regression of $p53^{+/+}$ tumors was due to apoptosis, and that the $p53^{-/-}$ tumors, which did not regress, were resistant to apoptosis.

All initially responsive tumors derived from $p53^{+/+}$ clones became less responsive to subsequent treatments with gamma radiation, and several became completely resistant (Fig. 3A and Table 2). Moreover, tumors derived from $p53^{+/+}$ clone C10 responded poorly to the initial treatments (Fig. 3B and Tables 1 and 2), even though the injected cells readily underwent apoptosis in vitro (*3*). Because an intact apoptotic program appeared essential for tumor regression, we tested whether acquired resistance to therapy correlated with de novo *p53* mutations.

Mutations in *p53* were detected in both resistant or relapsed tumors at codons mutated in human cancer (Table 2) (*6*). Each of the C10-derived tumors had acquired missense mutations at codon 211. No wild-type sequence was detected in these tumors, suggesting that they also lost the normal *p53* allele. Missense mutations at codon 131 were observed in all recurrent tumors arising from clone C6. In one of these tumors, a second mutation was observed at codon 239 (*7*). In no instance were mutations observed in the injected cell populations (*8*). Nevertheless, the fact that tumors aris-

Table 1. Summary of tumor response to gamma radiation or adriamycin. Mice were treated on day 0 with the indicated dose of gamma radiation (in Gy) or adriamycin (Adr) (*25*). Tumor response is presented as the smallest tumor volume [minimum volume (*mV*)] after treatment, expressed as a percentage of the tumor volume on day 0 (V_0). The tumor volume 7 days after treatment (V_7) is expressed as a percentage of the volume on day 0. Values are the average of two or more experiments. Standard deviations are shown where applicable. NA, not applicable.

Clone	Treatment	*n*	Tumor response		
			Days to reach *mV*	mV/V_0 (%)	V_7/V_0 (%)
			$p53^{+/+}$		
C1	7 Gy	2	6	53	53
	9 Gy	2	7	40	40
	12 Gy	4	7	11 ± 10	10
	Adr	2	3	42	NA
C6	7 Gy	2	3	50	89
	9 Gy	3	4	42 ± 27	67
	12 Gy	2	4	20	37
	Adr	2	6	13	NA
C8	2 Gy	3	8	29 ± 12	64
	5 Gy	3	5	8 ± 13	12
	7 Gy	3	7	16 ± 9	17
	Adr	6	6	19 ± 13	15
C10	5 Gy	2	1	96	133
	7 Gy	2	5	82	117
	12 Gy	2	3	89	95
	Adr	1	0	100	186
			$p53^{-/-}$		
A4	7 Gy	2	0	100	230
	12 Gy	2	0	100	305
	Adr	4	0	100 ± 0	289
A8	7 Gy	4	0	91 ± 13	177
	12 Gy	2	0	100	125
A9	7 Gy	2	1	95	223
	12 Gy	4	0	100 ± 0	171
E6	7 Gy	2	0	100	177
	Adr	4	0	100 ± 0	232

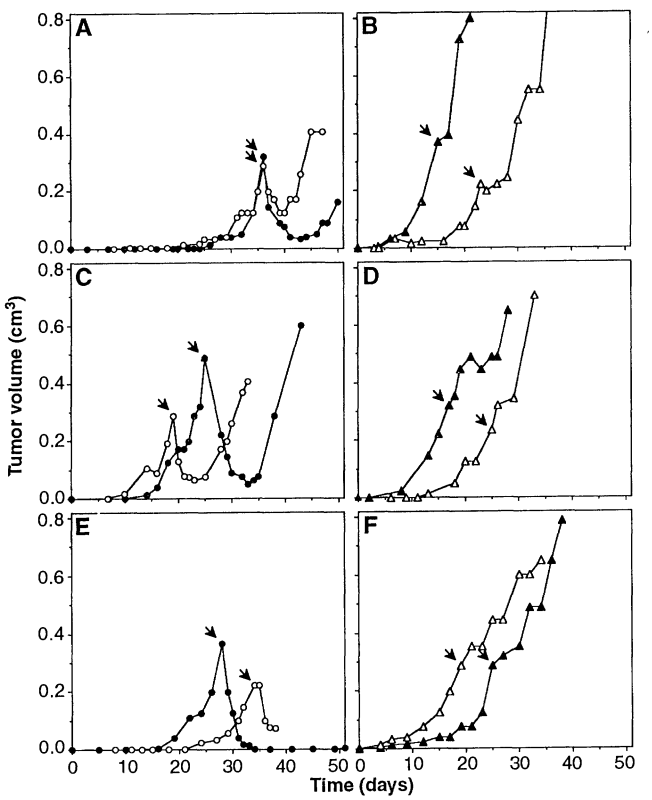

Fig. 1. Response of *p53* and tumor to gamma radiation and adriamycin. Athymic nude mice were injected with embryonic fibroblasts transformed by E1A and an activated *ras* oncogene (T24 H-*ras*), and tumor volumes were monitored (*25*). At the indicated times (arrows), mice with tumors derived from $p53^{+/+}$ (**A**, **C**, and **E**) and $p53^{-/-}$ (**B**, **D**, and **F**) cells were irradiated with either 7 (A and B) or 12 (C and D) Gy or treated with adriamycin (E and F) (*25*).

ing from certain clones harbor the same *p53* mutations suggests that the mutant cells existed as a small percentage of the injected population. By selecting against apoptosis, both tumor growth and cancer therapy may have enriched tumors for cells harboring *p53* mutations.

The *p53* mutations were not detected in several recurrent tumors, including some that demonstrated complete resistance to additional therapy (Table 2). It is possible that some mutations were not detected by our analysis or involved other genes in the pathway. Alternatively, *p53*-independent mechanisms may also produce radioresistant tumors (*9*).

We have proposed that the involvement of *p53* in oncogene-associated apoptosis is a direct mechanism of tumor suppression by *p53* (*5*). In the presence of *p53*, oncogene-expressing cells can form tumors, but cell survival is limited by their increased susceptibility to apoptosis (*10*). As a consequence, selection against *p53* often occurs late in tumor progression. Anticancer agents may simply activate the apoptotic program intrinsic to these sensitized cells. Thus, genetic alterations that accompany malignant transformation can increase the therapeutic index of radiation or chemotherapy. Other mutations may have the opposite effect, leading to tumor resistance.

Overexpression of c-*myc*, *ras*, or *E2F*-1 or

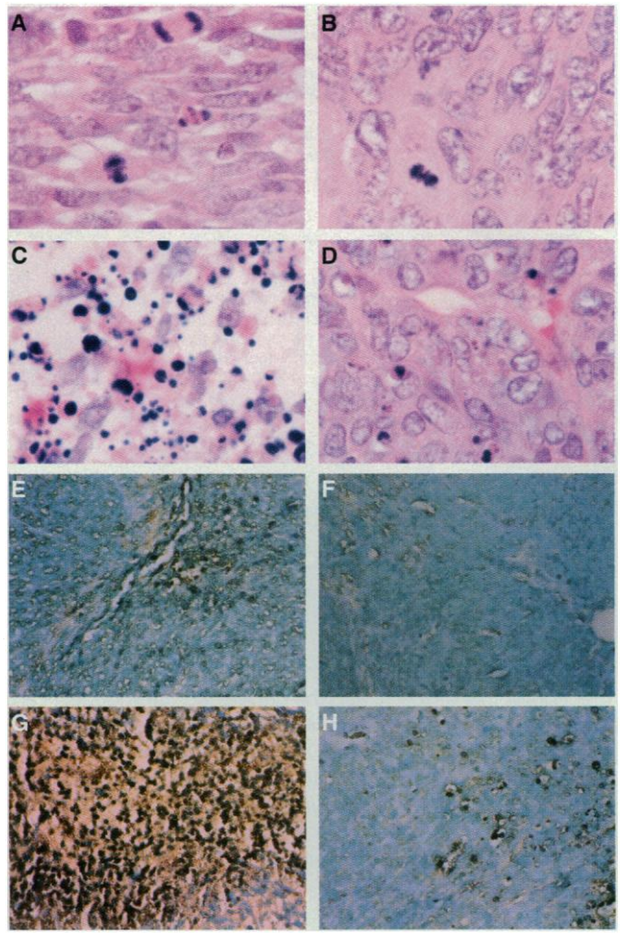

Fig. 2. Histological and TUNEL staining of untreated tumors and tumors recovered 40 to 48 hours after treatment with gamma radiation or adriamycin. Apoptotic cells undergo cell shrinkage, chromatin condensation, and nuclear fragmentation and typically activate an endonuclease that breaks genomic DNA, generating products which can be detected with the terminal TUNEL method (*27*). Hematoxylin-eosin staining of tumor sections from (**A**) an untreated $p53^{+/+}$ tumor, (**B**) an untreated $p53^{-/-}$ tumor, (**C**) a $p53^{+/+}$ tumor, and (**D**) a $p53^{-/-}$ tumor after adriamycin treatment. TUNEL staining of sections from (**E**) an untreated $p53^{+/+}$ tumor, (**F**) an untreated $p53^{-/-}$tumor, (**G**) a $p53^{+/+}$ tumor, and (**H**) a $p53^{-/-}$ tumor after gamma irradiation. (E to H) TUNEL-positive cells were visualized with a horseradish peroxidase–based detection method that produces a dark brown color. Tissue sections were counterstained with methyl green. Magnifications: (A to D), ×1000; (E to H), ×200.

Table 2. Mutations in the *p53* gene in recurrent and resistant tumors. Tumor complementary DNA corresponding to exons 5 to 8 of the *p53* gene were amplified and sequenced (*26*). For each tumor, the treatment or treatments are indicated as the dose of gamma radiation (in Gy) or as adriamycin treatment (A). Tumor response is presented as the smallest tumor volume [minimum volume (*mV*)] after treatment, expressed as a percentage of the tumor volume on day 0 (V_0). The successive responses of tumors receiving multiple treatments corresponds to the order in which the treatments were given. Also shown is the detected nucleotide change, the affected codon in murine *p53* (*24*), the corresponding human codon (*6*), and the predicted amino acid substitution. ND, not detected; NA, not applicable.

Clone	Tumor	Treatments	Tumor response mV/V_0 (%)	Mutational analysis		
				Nucleotide change	Codon murine (human)	Amino acid change
C1	970L	9; 9	32; 74	ND	NA	NA
	970R	9; 9	48; 100	ND	NA	NA
C6	975L	9	35	TTC→TGC	131 (134)	Phe→Cys
	977L	12	21	TTC→TGC	131 (134)	Phe→Cys
	977R	12	19	TTC→TGC	131 (134)	Phe→Cys
				TGC→GGC	239 (242)	Cys→Cly
C8	396L	7	11	ND	NA	NA
	380L	5; 5	0; 41	ND	NA	NA
	912R	2; 2; 2; 2	33; 45; 74; 100	ND	NA	NA
	929L	A; 2; 2; 2; 2	6; 39; 42; 74; 100	ND	NA	NA
C10	934	None	NA	CAC→CGC†	211 (214)	His→Arg
	933R	2 Gy × 7*	78	CAC→CGC†	211 (214)	His→Arg
	915L	7; 7	63; 100	CAC→CGC†	211 (214)	His→Arg

*Treatment consisted of seven daily doses of 2 Gy. †No wild-type sequence detected.

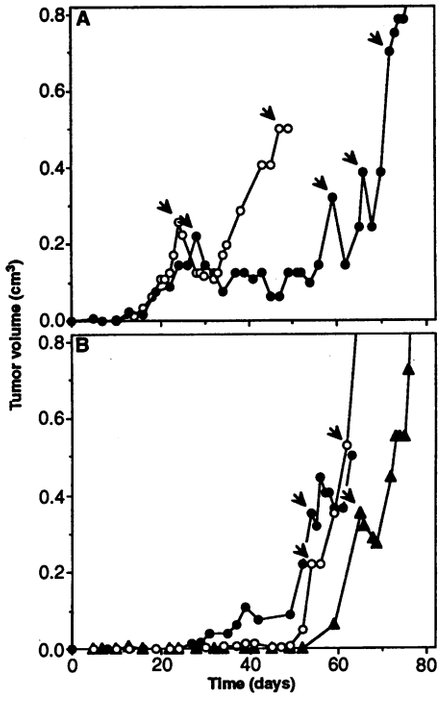

Fig. 3. Acquired resistance of tumors derived from $p53^{+/+}$ cells. Athymic nude mice were injected with transformed embryonic fibroblasts (time 0) and treated as in Fig. 1. (**A**) Mice with tumors derived from clone C1 (open circles) or C8 (closed circles) were treated with multiple doses of gamma radiation (2 Gy per dose and 9 Gy per dose, respectively) at the times indicated by the arrows. (**B**) Tumors arising from $p53^{+/+}$ clone C10 were treated with gamma radiation (7 Gy, triangles; 12 Gy, closed circles) or adriamycin (10 mg/kg, open circles) as indicated (arrows).

loss of *Rb* function can promote spontaneous apoptosis and can enhance apoptosis induced by certain anticancer agents (*4*, *11–13*). In many of these instances, apoptosis involves *p53* (*4*, *12–15*). These observations predict that reintroduction of normal *p53* function into tumors harboring *p53* mutations will enhance apoptosis after radiation or chemotherapy, an approach that has proved successful for cisplatin in a lung carcinoma cell line (*16*).

Our results suggest a basis for the association between *p53* mutation and poor patient prognosis [for examples, see (*17–20*)] and are consistent with studies linking *p53* mutation to drug resistance in human tumors. For example, *p53* mutations dramatically reduce the probability that patients with B cell chronic lymphocytic leukemia will enter remission after chemotherapy (*19*). Mutations in *p53* are also associated with tumor relapse in acute lymphoblastic leukemia (*21*), suggesting that attenuated p53 activity confers a selective advantage on tumor cells undergoing cancer therapy. Finally, *p53* mutations are strictly associated with anaplastic Wilms tumors (*20*), an aggressive subtype that is particularly resistant to therapeutic intervention. In these tumors, *p53* loss is accompanied by attenuated apoptosis (*22*). Because *p53* mutations are among the most common alterations observed in human cancer, defects in *p53*-dependent apoptosis may be a significant impediment to successful cancer therapy.

REFERENCES AND NOTES

1. C. Dive and J. A. Hickman, *Br. J. Cancer* **64**, 192 (1991).
2. J. F. R. Kerr, C. M. Winterford, B. V. Harmon, *Cancer* **73**, 2013 (1994).
3. S. W. Lowe, H. E. Ruley, T. Jacks, D. E. Housman, *Cell* **74**, 954 (1993).
4. N. Tanaka *et al.*, *ibid.* **77**, 829 (1994).
5. S. W. Lowe, T. Jacks, D. E. Housman, H. E. Ruley, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2026 (1994).
6. M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, *Science* **253**, 49 (1991).
7. We did not determine whether the two mutations in tumor 977R were in the same or in separate alleles. In human tumors harboring two *p53* mutations, the mutations are typically in separate alleles.
8. S. W. Lowe *et al.*, data not shown.
9. The *mdm-2* oncogene, which can inactivate *p53* and is amplified in many human sarcomas, was not overexpressed in any of the tumors tested.
10. H. Symonds *et al.*, *Cell* **78**, 703 (1994).
11. G. I. Evan *et al.*, *ibid.* **69**, 119 (1992).
12. X. W. Wu and A. J. Levine, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3602 (1994).
13. T. Jacks *et al.*, *Nature* **359**, 295 (1992); E. Y-H. P. Lee *et al.*, *ibid.*, p. 288; A. R. Clarke *et al.*, *ibid.*, p. 328.
14. S. D. Morgenbesser, B. O. Williams, T. Jacks, R. A. DePinho, *Nature* **371**, 72 (1994).
15. J. Lotem and L. Sachs, *Cell Growth Differ.* **4**, 41 (1993).
16. T. Fujiwara *et al.*, *Cancer Res.* **54**, 2287 (1994).
17. S. Thorlacius, A. L. Borresen, J. E. Eyfjord, *ibid.* **53**, 1637 (1993).
18. Y. Horio *et al.*, *ibid.*, p. 1.
19. S. Elrouby *et al.*, *Blood* **82**, 3452 (1993).
20. N. Bardeesy *et al.*, *Nat. Genet.* **7**, 91 (1994).
21. M. H. Hsiao, A. L. Yu, J. Yeargin, D. Ku, M. Haas, *Blood* **83**, 2922 (1994).
22. J. Pelletier, personal communication.
23. M. E. Scheulen, V. Budach, M. Bambery, *Strahlenther. Onkol.* **165**, 529 (1989).
24. B. Bienz, R. Zakut-Houri, D. Givol, M. Oren, *EMBO J.* **3**, 2179 (1984).
25. Minimally passaged $p53^{+/+}$ and $p53^{-/-}$ mouse embryonic fibroblasts transformed by E1A and T24 H-*ras* (*3*, *5*) were detached from tissue culture plates, washed, and resuspended in phosphate-buffered saline (PBS). Cells (2×10^6) were injected into each flank of athymic nude mice 4 to 8 weeks old. Tumor volumes were estimated from caliper measurements of tumor length (L) and width (l) according to the following formula: $(L \times l^2)/2$. In general, tumors were allowed to expand to a volume of 0.15 to 0.5 cm^3 before treatment. Mice receiving ≤ 7 Gy were given a whole body dose in a Gammacell 40 irradiator containing a ^{137}Cs source (~0.8 Gy/min). For higher doses, a lead shield was used to reduce radiation toxicity to the bone marrow and thoracic cavity. Adriamycin was injected intraperitoneally at a dose of 10 mg per kilogram of body weight in PBS; thus, the amount of adriamycin approached the maximum tolerated dose for a single injection (*23*). Higher doses of adriamycin are capable of killing *p53*-deficient cells in vitro (*3*), but the concentrations required for this effect are toxic to the animal.
26. Tumor cells were dispersed with trypsin and cultured in medium containing hygromycin B (50 μg/ml) to select for tumor-derived cells [which contain hygromycin phosphotransferase (*5*)]. RNA was generally isolated from tumor cells maintained for less than 1 week in culture. For tumor 915L, RNA was isolated from frozen tumor tissue. Complementary DNA was prepared by standard methods with a primer directed to sequences within exons 9 or 11 of the murine *p53* gene (*24*). Amplification of *p53* sequences in exons 5 to 8 was accomplished by polymerase chain reaction with primers directed to exons 4 and 9. This region contains the vast majority of *p53* mutations observed in human cancer (*6*). Sequencing was performed with several primers specific to exons 5 to 8.
27. Y. Gavrieli, Y. Sherman, S. A. BenSasson, *J. Cell Biol.* **119**, 493 (1992).

28. We thank M. McCurrach for technical assistance and helpful advice. S.W.L. is an Anna Fuller postdoctoral fellow and S.B. is supported by a fellowship from the Swiss League of Cancer. Supported by grants R01CA40602 (H.E.R), 5R27CA17575 and CA14051 (D.E.H.), a Cancer Center Support (core) grant P30-CA14051 from NIH, and a grant from the General Cinemas Charitable Foundation (T.J.).

13 July 1994; accepted 9 September 1994

Transformation of Lupus-Inducing Drugs to Cytotoxic Products by Activated Neutrophils

Xiaoxia Jiang, Gus Khursigara, Robert L. Rubin*

Drug-induced lupus is a serious side effect of certain medications, but the chemical features that confer this property and the underlying pathogenesis are puzzling. Prototypes of all six therapeutic classes of lupus-inducing drugs were highly cytotoxic only in the presence of activated neutrophils. Removal of extracellular hydrogen peroxide before, but not after, exposure of the drug to activated neutrophils prevented cytotoxicity. Neutrophil-dependent cytotoxicity required the enzymatic action of myeloperoxidase, resulting in the chemical transformation of the drug to a reactive product. The capacity of drugs to serve as myeloperoxidase substrates in vitro was associated with the ability to induce lupus in vivo.

More than 40 different medications can result in unremitting muscle and joint pain and occasionally compromised heart and lung function when used for long-term therapy. Symptoms eventually resolve after the therapy is discontinued. This side effect has been termed drug-induced lupus because it is clinically indistinguishable from the idiopathic disease systemic lupus erythematosus. Both forms of lupus are accompanied by serum autoantibodies to constituents of the cell nucleus. The prototypic drugs associated with a lupus-like side effect include antiarrhythmics such as procainamide and quinidine, the antihypertensive drug hydralazine, psychotropic drugs such as chlorpromazine, antibiotics such as isoniazid, and the antithyroid agent propylthiouracil (*1*). Although some of these drugs are aromatic amines or hydrazines, there is no apparent common denominator of a pharmacologic, therapeutic, or chemical nature that links drugs with the capacity to induce lupus.

Idiosyncratic drug reactions such as drug-induced lupus may depend on metabolic transformation of the ingested agent to a reactive intermediate that displays biological activity different from the parent compound. Transformation to reactive metabolites with a common property could also explain how chemically dissimilar drugs produce the same adverse reaction. Although the liver is considered to be the main site of xenobiotic metabolism (*2*), drug-induced lupus is a systemic autoimmune disease with no clinical evidence of liver involvement (*1*). These considerations suggest three possibilities for the productive exposure of the immune system to labile metabolites: (i) Immunocytes encounter the reactive metabolite in the liver and then migrate to and expand in an immune compartment. (ii) The metabolite translocates from the liver to immune compartments in a form that preserves its activity. (iii) The reactive metabolite is produced locally within an immune compartment where it acts. Local production of the me-

W. M. Keck Autoimmune Disease Center, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed.