[CANCER RESEARCH 61, 6577–6582, September 1, 2001]

Impact of Ionizing Radiation and Genetic Background on Mammary Tumorigenesis in p53-deficient Mice[1]

Michael G. Backlund, Scott L. Trasti, Dana C. Backlund, Victoria L. Cressman, Virginia Godfrey, and Beverly H. Koller[2]

Curriculum in Genetics and Molecular Biology [M. G. B.], Division of Laboratory Animal Medicine [S. L. T.], and Departments of Medicine [D. C. B., V. L. C., B. H. K.] and Pathology [V. G.], University of North Carolina, Chapel Hill, North Carolina 27599

ABSTRACT

Loss of p53 function is known to compromise cell cycle regulation, induction of apoptosis, and DNA damage repair and can facilitate neoplastic transformation of cells. Mutations in the *p53* gene are identified frequently in breast carcinomas. Li-Fraumeni patients inheriting a mutant *p53* allele have an increased risk for developing tumors including breast cancer. Although mouse lines carrying mutations in the *p53* gene have been generated, they die primarily of lymphoma and thus to date provide a limited model for the study of this disease and the role of p53 in nonfamilial breast cancer. An increasing body of literature suggests that the incidence of various tumors is determined largely by the genetic background on which mutations are studied. In addition, population studies and studies in animals suggest that environmental factors, together with genetic factors, determine overall risk for development of specific types of tumors. We therefore examined the impact of genetic background together with exposure to ionizing radiation on the development of tumors, particularly mammary tumors, in p53-deficient animals. We report here that modifier alleles present in the BALB/c strain increase the incidence of hemangiosarcomas [15 of 53 (28.3%); $P = 0.0007$] in $p53^{-/-}$ mice above rates reported previously in $p53^{-/-}$ mice on a mixed background as compared to the incidence observed in DBA/$p53^{-/-}$ mice. However, no increase in the frequency of mammary tumors is seen in these mice or in $p53^{-/-}$ DBA/2 animals, nor was an increase in mammary tumors observed in the DBA/2 $p53^{+/-}$ mice, even after exposure to 5 Gy of whole-body ionizing radiation. In contrast, a significant increase in the incidence of mammary tumors was observed in similarly treated BALB/c $p53^{+/-}$ mice (37.3% *versus* 6.8%; $P = 0.0007$). This was accompanied by a comparable decrease in the incidence of lymphomas. These results show that environmental agents together with genetic factors can increase the frequency and decrease the latency of mammary tumors, leading to an incidence similar to that observed in Li-Fraumeni syndrome. Furthermore, it suggests that the risk of development of a particular type of tumor by individuals deficient in p53 after exposure to damaging agents can be influenced by modifier alleles.

INTRODUCTION

The *p53* tumor suppressor gene encodes a regulatory transcription factor that has been shown to be involved in cell cycle regulation, induction of apoptosis, and in protecting the genome from DNA damage (1–7). Mutations in *p53* are often found in spontaneous human neoplasms, including breast cancer. In fact, analysis of DNA from tumor tissue from sporadic breast cancers in women indicates the presence of a mutant *p53* allele in 40–60% of the tumors (8). Further support for the importance of this gene in the pathogenesis of breast tumors comes from the observation that ~24% of women that carry a mutant copy of *p53* (Li-Fraumeni syndrome) develop breast cancer (9). With the development of technologies that allow rapid introduction of specific mutations into the mouse, it became possible to generate mice that carried germ-line mutations in p53 (10, 11). It was anticipated that these animals would provide a model system to study the mechanism by which this gene contributes to initiation and progression of tumor growth. However, although mice that are homozygous or heterozygous for mutant *p53* alleles display a diverse spectrum of tumors, these tumors are primarily lymphomas, and only a small number of mammary tumors were observed on examination of both $p53^{+/-}$ and $p53^{-/-}$ animals (~2%; Refs. 12 and 13).

A number of possible reasons for the lack of mammary tumors in p53-deficient mice have been suggested. For example, the transformation of mammary epithelial cells might depend on the accumulation of numerous mutations or an appropriate mammary gland stimulus in addition to loss of p53 function (14). In comparison, thymic lymphocytes appear to be very sensitive to loss of individual tumor suppressor genes such as *p53*, suggesting that only a limited numbers of additional mutations are required for malignant transformation. For this reason, animals deficient in p53 may die of lymphomas before sufficient time has elapsed for accumulation of mutations and transformation of mammary epithelial cells. In the human population, exposure to DNA-damaging agents, such as carcinogens and ionizing radiation, is likely to contribute to the accumulation of such mutations. Consistent with this hypothesis, higher incidences of breast cancer were observed in atomic bomb survivors and in women exposed to high-dose ionizing chest radiation during childhood or puberty (15–17).

Numerous studies have shown that ionizing radiation can increase the frequency and decrease the age at which tumors are observed in mice. This observation is apparent in animals carrying mutations in known tumor suppressor genes and even more so in animals deficient in genes shown to have a role in DNA repair and maintenance of chromosome stability. For example, exposure of neonatal $p53^{-/-}$ mice to ionizing radiation markedly decreases the latency to tumor formation. A similar decrease in tumor latency has been observed in irradiated $p53^{+/-}$ animals (18). This treatment also increased the frequency of malignant lymphomas and decreased the incidence of sarcomas in these animals. In contrast, exposure of $p53^{+/-}$ mice to ionizing radiation did not alter the incidence of mammary tumors, and in fact no mammary tumors were identified on analysis of 33 treated $p53^{+/-}$ animals (18). These findings indicate that exposure to ionizing radiation can decrease tumor latency and alter the frequency with which specific types of tumors arise in p53-deficient animals. These results also suggest that differential exposure of humans and mice to such DNA-damaging agents alone cannot explain the extremely low incidence of mammary tumors in p53-deficient mice as compared to patients with the Li-Fraumeni syndrome.

An additional explanation for the failure of p53-deficient mice to develop mammary tumors may be the increasingly frequent finding that differences in the genetic background on which a tumor suppressor is studied can dramatically alter the tumor spectrum (13, 19). It has been shown that the incidence of teratocarcinomas is elevated in $p53^{-/-}$ mice on the 129/Sv genetic background as compared with $p53^{-/-}$ mice on a mixed C57BL/6-129/Sv background (13). This

Received 4/4/01; accepted 7/3/01.

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 supported by NIH grant CA70490 (to B. H. K.) and the Department of Defense USAMRMC grant DAMD17-97-1-7102 (to V. L. C. and M. G. B.)

[2] To whom requests for reprints should be addressed, at Department of Medicine, CB 7248, Thurston-Bowles Building, UNC-Chapel Hill, Chapel Hill, NC 27599. E-mail: Treawouns@aol.com.

suggests that different strains of mice contain sets of alleles that modify the impact of the loss of p53 in a cell type-specific manner.

In this study, we examined the impact of modifier genes alone and in combination with ionizing radiation on the development of mammary tumors in mice. We compare the formation of mammary tumors in p53-deficient mice of mixed genetic background to the development of mammary tumors in DBA/2 and BALB/c congenic p53-deficient animals. In addition, we determined whether ionizing radiation can alter tumor latency or spectrum in these three p53-deficient populations. These studies show that loss of p53 function in combination with exposure to DNA-damaging agents can result in the formation of mammary tumors. They also show that mice that are p53 deficient and exposed to DNA-damaging agents have an incidence of mammary tumors similar to that observed in Li-Fraumeni syndrome.

MATERIALS AND METHODS

Mice. Mice with a germ-line p53 mutation, $p53^{\Delta exon2-6}$, on a mixed genetic background (129/SvEv × C57BL/6) were obtained from The Jackson Laboratory and backcrossed to both the BALB/cJ and DBA/2J backgrounds to generate female p53-deficient animals for use in survival experiments (11). Those mice heterozygous for the *p53* mutation, between the ages of 4 and 6 weeks, were exposed to 5 Gy of whole-body ionizing radiation from a ^{137}Cs source (1.24 Gy/min) and monitored weekly for the presence of tumors, whereas mice homozygous for the *p53* mutation were monitored for the spontaneous generation of tumors. Mice that developed tumors between 1 and 2 cm, along with moribund mice that did not display visible growths, were euthanized and necropsied. In several instances, animals died without appearing moribund. These animals were also necropsied, but autolysis of the tissue prevent accurate histopathology. Additionally, animals with tissues displaying distinctive phenotypes associated with lymphomas were not fixed for histopathological analysis. All animals were cared for in compliance with Institutional Animal Care and Use Committee regulations.

Genomic DNA was recovered from tail biopsy and mammary tumors, and genotypes for the wild-type and mutant *p53* alleles were determined by both PCR amplification and Southern blot analysis. The wild-type *p53* allele was amplified using PCR primers directed against exon 6 (6.5: 5′-ACAGCGTGGTGGTACCTTAT-3′) and exon 7 (X7: 5′-TATACTCAGAGCCGGCCT-3′), whereas the mutant $p53^{\Delta exon2-6}$ allele was amplified using primers against *neo* (neo: 5′-CTATCAGGACATAGCGTTGG-3′) and X7 (11). Additionally, genomic DNA from tail biopsies was digested with *Stu*I, electrophoresed in a 0.8% agarose gel, and transferred to Hybond-N nylon membrane (Amersham, Arlington Heights, IL). Hybridization was done using a ^{32}P-labeled probe specific for exons 7–9 of the *p53* genomic sequence. This probe was prepared from murine genomic DNA using the primers 5′-CGGCTCTGAGTATACCACCATC-3′ and 5′-CTTTTGCGGGGGAGAGG-3′.

Tumor Analysis/Histopathology. Tumor samples were surgically removed post-mortem and fixed in 10% phosphate-buffered neutral formalin at pH 7.0. Specimens were processed for histology, embedded in paraffin, sectioned at 3 μm, and stained with H&E. Tumors of nonlymphoid origin were classified both by observation of anatomical location and by histological examination of tumor biopsies by a veterinary pathologist.

Statistics. The Kaplan-Meier test was used to calculate the latency to tumor formation, and the log-rank test was used for evaluation of significance. The significance of differences in frequencies between groups and/or tumor types was evaluated by χ^2 analysis. $P < 0.05$ was considered significant.

RESULTS

Impact of the DBA/2 and the BALB/c Genetic Backgrounds on Health and Survival in Mice Homozygous for a Null *p53* Allele. Previous studies have reported the generation of mice homozygous for a null p53 mutation (11, 13). These animals were of mixed genetic background, and the majority of these animals died of malignant lymphomas before 5 months of age. To determine whether the survival and/or type of tumor that developed in p53-deficient mice could be modified by alleles present in BALB/c or DBA/2 genetic background, we initiated breeding strategies designed to generate p53-deficient BALB/c and DBA/2 congenic lines. Mice heterozygous for the mutant *p53* allele on a 129/Sv × C57BL/6 mixed genetic background were crossed to either BALB/c or DBA/2 mice. The heterozygous offspring were designated backcross (BX) 1 and were intercrossed to generate BX1 mice homozygous for the *p53* mutation. In addition, some mice heterozygous for the mutant *p53* allele were again crossed to BALB/c or DBA/2 mice to generated BX2 mice of each lineage. Again, the intercross of BX2 heterozygous mice generated BX2 mice homozygous for the *p53* mutation. This breeding scheme was continued for an additional four generations. With each generation, the percentage of BALB/c alleles in one lineage and DBA/2 alleles in the other lineage is expected to increase. At each generation, ~10 $p53^{-/-}$ mice and $p53^{+/+}$ mice were generated for study. Few differences were observed on comparison of consecutive generations, either in the survival time or the spectrum of tumors that developed in the mice. To increase the power of the analysis, mice were placed into two groups for comparison, mice derived after 1–3 generations or 4–6 generations of consecutive breeding to BALB/c or DBA/2. In all studies, only virgin female mice were included. As described previously, $p53^{-/-}$ female mice were less prevalent in litters at weaning than expected because of the development of anencephaly in female but not male $p53^{-/-}$ embryos. This decreased survival was also observed in the p53-deficient BALB/c and DBA/2 populations. Twenty-eight $p53^{-/-}$ and 29 $p53^{+/+}$ BALB/c and 28 $p53^{-/-}$ DBA/2 and 28 DBA/2 control animals generated from intercrosses of BX1–BX3 $p53^{+/-}$ animals were monitored biweekly for the development of tumors and killed when tumors reached 1 cm or when animals appeared moribund. As shown in Fig. 1*A*, the latency to tumor formation was similar for both BALB/c and DBA/2 BX1–3 $p53^{-/-}$ mice (log-rank test, $\chi^2 = 1.407$, $P = 0.24$). By 119 days of age, ~50% of the p53-deficient BALB/c animals had developed tumors and were moribund. In comparison, 50% of the mice carrying DBA/2 alleles survived for 136 days before developing tumors. Although there was a trend toward a decreased latency to tumor formation in the $p53^{-/-}$ BALB/c mice as compared with the $p53^{-/-}$ DBA/2

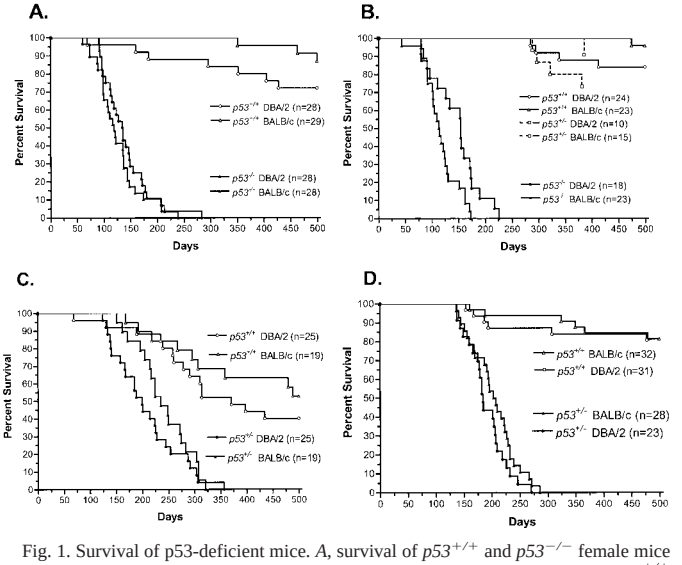

Fig. 1. Survival of p53-deficient mice. *A*, survival of $p53^{+/+}$ and $p53^{-/-}$ female mice on both the BALB/c and DBA/2 genetic backgrounds from BX1–3. *B*, survival of $p53^{+/+}$ and $p53^{-/-}$ female mice on both the BALB/c and DBA/2 genetic backgrounds from generations 4–6 of backcrossing. *C*, survival of $p53^{+/+}$ and $p53^{+/-}$ female mice on both BALB/c and DBA/2 genetic backgrounds after exposure to 5 Gy of whole-body ionizing radiation through 3 generations of backcrossing. *D*, survival of $p53^{+/+}$ and $p53^{+/-}$ female mice on both BALB/c and DBA/2 genetic backgrounds after exposure to 5 Gy of whole-body ionizing radiation from generation 4 to generation 6 of backcrossing.

Table 1 *Tumor spectrum in $p53^{-/-}$, $p53^{+/-}$, and $p53^{+/+}$ mice on BALB/c and DBA/2 genetic backgrounds from backcross 1–6 from a mixed background*

	BALB/c			DBA/2		
	$p53^{+/+}$	$p53^{+/-}$	$p53^{-/-}$	$p53^{+/+}$	$p53^{+/-}$	$p53^{-/-}$
	n	*n*	*n*	*n*	*n*	*n*
Lymphoma (NOS)[a]		2	35 (66.0%)[b]	2		40 (86.9%)
Hemangiosarcoma			15 (28.3%)			1 (2.3%)
Fibrosarcoma			1 (1.9%)			
Histiocytic sarcoma			1 (1.9%)			
Osteosarcoma						1 (2.3%)
Sarcoma (NOS)						1 (2.3%)
Carcinoma of unknown origin			1 (1.9%)			
Mammary gland carcinoma		2	0			0
Unknown					1	
No visible or metastatic tumors at death			6	8		3
Total number of tumors	0	3	53	2	0	43
Total number of mice	52	15	51	52	11	46

[a] NOS, not otherwise specified.
[b] Percentages of tumor types observed among all tumors from a given strain and genotype.

mice, this difference in latency to tumor formation between the two populations was not statistically significant and did not differ significantly from the mean survival time of the original p53-deficient mice of mixed genetic background.

To examine this question further, we studied $p53^{-/-}$ mice generated from heterozygous animals obtained after 4–6 generations of consecutive crosses to either the BALB/c or DBA/2 line (BX4–6). Twenty-three $p53^{-/-}$ and 23 $p53^{+/+}$ BALB/c along with 18 $p53^{-/-}$ and 24 $p53^{+/+}$ DBA/2 animals ranging between BX4–BX6 were monitored for the development of tumors. Increasing the percentage of BALB/c-derived alleles present in the $p53^{-/-}$ mice had only a small effect on the mean survival time of the $p53^{-/-}$ animals (119 to 114 days). In contrast, increasing the number of DBA/2-derived alleles present in the population increased the survival time of the $p53^{-/-}$ mice. Thus, as shown in Fig. 1*B*, 50% of the $p53^{-/-}$ mice generated from BX4–6 on a DBA/2 background survived for >154 days. Additionally, a significant difference in survival was observed on comparison of latency to tumor formation in BALB/c and DBA/2 $p53^{-/-}$ BX4–6 populations (log-rank test, $\chi^2 = 7.680$, $P = 0.006$).

None of the wild-type mice included in this study developed tumors. Tumors that developed in the p53-nullizygous mice were classified both by observation of anatomical location and histological examination by a trained pathologist. Previous studies of $p53^{-/-}$ mice of mixed genetic background report that 72.5% of the mice die after developing lymphomas, primarily of thymic origin (20). In both the BALB/c and the DBA/2 $p53^{-/-}$ populations, this continued to be the most common tumor type observed (Table 1). Mutant *p53* homozygotes on either inbred strain did not show a significantly altered prevalence of lymphomas, and additional histological examination of the liver, spleen, and kidney from these mice did not reveal distinct differences in metastases to other organ systems.

The majority of other tumors observed previously in the $p53^{-/-}$ mice of mixed genetic background also occurred at approximately similar frequencies in the BALB/c and DBA/2 $p53^{-/-}$ populations. One notable exception was the observation of increased numbers of hemangiosarcomas in $p53^{-/-}$ BALB/c animals (Table 1; $\chi^2 = 11.53$; $P = 0.0007$). This tumor type has also been observed in studies of the $p53^{-/-}$ of mixed genetic background (12, 18, 20). The increase in the incidence of these tumors might account, at least in part, for the decreased survival of the BALB/c mice. Similar to the observation of $p53^{-/-}$ mice on a mixed genetic background, mammary tumors were not observed in our $p53^{-/-}$ BALB/c or DBA/2 populations (20).

Impact of the DBA/2 and the BALB/c Genetic Backgrounds on Health and Survival in Mice Heterozygous for a Null *p53* Allele. We next examined the development of tumors in $p53^{+/-}$ BALB/c and DBA/2 congenic mice. All heterozygous animals included in this study were obtained by six generations of backcrossing of the mutation onto either the BALB/c or DBA/2 genetic background. The survival of these mice, similar to the $p53^{+/-}$ mice of mixed genetic background was significantly extended (Fig. 1*B*) as compared with the animals homozygous for the *p53* mutation (18). Of the 15 $p53^{+/-}$ BALB/c mice in this study, 11 continue to be disease free after 300 days. Tumors were observed in all four animals that have died to date, and the location and gross morphology of two of the tumors are consistent with the development of thymic lymphoma, whereas two of the $p53^{+/-}$ BALB/c tumors were classified as mammary tumors. Of the 10 DBA/2 $p53^{+/-}$ mice examined, only a single mouse has died to date, and no tumor was observed upon necropsy.

Tumorigenesis in Irradiated BALB/c or DBA/2J p53-deficient Mice. Consistent with published work (11, 12, 18), our observations show that $p53^{-/-}$ mice display a high incidence of lymphomas. The increased frequency of lymphomas in these mice might obscure the role of p53 and modifying alleles found in BALB/c and DBA/2 in mammary tumorigenesis. Although preliminary studies of $p53^{+/-}$ BALB/c mice suggest that mammary tumors might be observed more frequently in this strain, the latency in tumor formation in these populations makes these studies difficult. Previous studies have shown that exposure of $p53^{+/-}$ mice to ionizing radiation results in a decreased latency to tumor formation (18). We therefore wanted to determine the impact of ionizing radiation on tumor formation, particularly on the development of mammary tumors in $p53^{+/-}$ BALB/c and DBA/2 mice. Nineteen $p53^{+/-}$ and 19 $p53^{+/+}$ BALB/c along with 25 $p53^{+/-}$ and 25 $p53^{+/+}$ DBA/2 female animals between BX1–3 were exposed to a single dose of 5 Gy of whole-body ionizing radiation. Mice were observed biweekly, sacrificed when moribund, and necropsied. Compared with our previous report of unirradiated $p53^{+/-}$ mice (20), the median age of tumor incidence and morbidity decreased in both $p53^{+/-}$ irradiated BALB/c and DBA/2J inbred strain (BX1–3) from 500 days to 236 and 200 days, respectively (Fig. 1*C*). Twenty-eight $p53^{+/-}$ and 32 $p53^{+/+}$ BALB/c along with 23 $p53^{+/-}$ and 31 $p53^{+/+}$ DBA/2 animals ranging between BX4 and BX6 were exposed to a single dose of 5 Gy of whole-body ionizing radiation and monitored for the development of tumors. Comparison of tumor latency in these animals showed an additional decrease in the median age of tumor incidence and morbidity to 207 in BALB/c and 184 days in DBA/2, respectively (Fig. 1*D*).

Table 2 *Tumor spectrum in irradiated $p53^{+/-}$ and $p53^{+/+}$ mice on BALB/c and DBA/2 genetic backgrounds from backcross 1–6 from a mixed background*

	BALB/c		DBA/2	
	$p53^{+/+}$	$p53^{+/-}$	$p53^{+/+}$	$p53^{+/-}$
	n	*n*	*n*	*n*
Lymphoma (NOS)[a]	5	21 (41.2%)[b]	8	36 (81.8%)
Mammary gland carcinoma		19 (37.3%)		3 (6.8%)
Luteoma		2 (3.9%)		
Osteosarcoma	1	3 (5.9%)		
Rhabdomyosarcoma		1 (2.0%)		
Pituitary gland adenocarcinoma		1 (2.0%)		
Adrenocortical adenocarcinoma		1 (2.0%)		
Fibrosarcoma		1 (2.0%)		
Basal cell carcinoma		1 (2.0%)		
Carcinoma (NOS)		1 (2.0%)		
Sarcoma (NOS)				2 (4.5%)
Squamous cell carcinoma				1 (2.3%)
Teratoma				
Number of visible or metastatic tumors at death	5	7	6	6
Total number of tumors	6	51	8	44
Total number of mice	52	46	55	48

[a] NOS, not otherwise specified.
[b] Percentages of tumor types observed among all tumors from a given strain and genotype.

The survival of wild-type mice was also reduced. A reduction in life span after exposure to even low doses of ionizing radiation is consistent with published reports. Surprisingly, this decrease in survival was greater in the $p53^{+/+}$ mice generated from BX1–3 than that observed from BX4–6 in both BALB/c and DBA/2 mice. Of the deaths observed in wild-type controls, 8 of the 55 DBA/2 $p53^{+/+}$ mice died of lymphomas, whereas the cause of death in 6 of the other mice could not be established. Examination of 52 irradiated BALB/c $p53^{+/+}$ mice revealed that 6 control mice developed tumors and 5 mice displayed no visible tumors at death (Table 1).

Tumors arising from irradiated mice heterozygous and wild-type for *p53* on all genetic backgrounds were classified both by observation of anatomical location and by histological examination of tumor biopsies by a trained veterinary pathologist. Previous studies have shown that the most prevalent tumor arising in irradiated $p53^{+/-}$ mice of mixed genetic background is thymic lymphomas, indistinguishable from those observed in the untreated $p53^{-/-}$ mice. Similar to the $p53^{+/-}$ mice of mixed genetic background, 82% of the tumors that developed in the DBA/2 $p53^{+/-}$ population were lymphomas. In comparison, only 41% of the BALB/c $p53^{+/-}$ population developed this type of tumor. Surprisingly, and in contrast to both the DBA/2 and mixed $p53^{+/-}$ irradiated animals, mammary tumors were almost as frequent as lymphomas in the BALB/c population (37%). This difference in the incidence of mammary tumors arising from p53-deficient BALB/c mice from that observed in either $p53^{+/-}$ animals on the mixed or DBA/2 background was statistically significant (Table 2; $\chi^2 = 11.56$; $P = 0.0007$). The change in the relative frequency of mammary tumors and lymphomas in BALB/c mice was observed equally in both the Bx1–3 and the Bx4–6 population.

Characterization of Radiation-induced Mammary Tumors Arising in $p53^{+/-}$ Mice. To determine whether the development of mammary tumors within the BALB/c population was dependent on the loss of p53 function, DNA prepared from mammary tumor tissue was subjected to Southern blot analysis using a p53-specific probe. Analysis of tumor DNA by Southern blot revealed that 13 of 18 (72%) mammary tumors analyzed showed loss of heterozygosity of the wild-type *p53* allele (data not shown). Loss of heterozygosity was also observed in the three mammary tumors arising in the DBA/2 population. The high percentage of loss of the wild-type *p53* allele in mammary carcinomas suggests a correlation between *p53* status and the formation of mammary tumors. Examination of histological sections prepared from the mammary tumors revealed multiple cellular patterns, often within the same tumor. Patterns that were present included acinar, tubular, ductular, solid, comedo, and mixtures of each. The tumors frequently contained areas of necrosis and/or apoptosis. Many tumors had high mitotic indices. In addition, a number of the mammary tumors showed both keratinizing and nonkeratinizing squamous differentiation.

DISCUSSION

In this study, we showed that although modifier genes have only a modest impact on the survival of p53-deficient animals, they can dramatically alter the spectrum of tumors that arises in these animals. Alleles present in the BALB/c genetic background decrease the incidence of lymphomas and increase the susceptibility of $p53^{+/-}$ mice to mammary tumors, particularly after exposure to ionizing radiation. In contrast, no mammary tumors were observed in BALB/c $p53^{-/-}$ mice. Modifiers present in the BALB/c mice did, however, increase the number of hemangiosarcomas that arose in this population of animals. The spectrum of tumors that arose in DBA/2 $p53^{-/-}$ mice was not significantly different from that reported for p53-deficient mice of mixed genetic background. However, modifiers present in the DBA/2 strain extended the mean survival of the $p53^{-/-}$ mice by 3 weeks.

BALB/c mice that are homozygous for the mutant *p53* allele display an increased incidence of hemangiosarcomas as compared with both DBA/2 $p53^{-/-}$ mice (0%) and $p53^{-/-}$ mice of mixed genetic background in our colony (6%; Ref. 20). Previous studies have also noted the development of these tumors in p53-deficient mice (12, 18).

Hemangiosarcomas are among the most rare types of soft tissue sarcomas in humans. They comprise <1% of all sarcomas (21) and are rarely, if ever, found in patients with Li-Fraumeni syndrome. However, a possible role for this tumor suppressor gene in the pathogenesis of hemangiosarcomas was suggested by a study of a cohort of 33 patients diagnosed with this tumor (22). Mutations in *p53* were observed in many of the tumors, although the frequency of observed mutations varied depending largely on the tissue in which the tumor arose (22). Additionally, indirect evidence supporting a role for this pathway in development of these tumors comes from the observation of increased expression of *MDM-2*, a gene whose transcription is regulated largely by p53, in human hemangiosarcomas (23). The failure to observe hemangiosarcomas in $p53^{-/-}$ mice on the DBA/2 background and the high incidence of these tumors in $p53^{-/-}$ BALB/c mice and in $p53^{-/-}$ mice of mixed (129/NIH) background suggest that the formation of these tumors is influenced by modifier alleles. It is possible that the modifier alleles necessary for the formation of these tumors, although present in a number of mouse lines, are infrequent in the human population.

The increase in hemangiosarcomas observed in the BALB/c $p53^{-/-}$ mice is consistent with a slight decrease in the survival of this population when compared with both wild-type and the DBA/2 $p53^{-/-}$ animals. However, this difference in median survival was apparent only on comparison of the BALB/c $p53^{-/-}$ and DBA/2 $p53^{-/-}$ mice generated after at least three generations of backcrossing. Our results suggest that the DBA/2 genetic background carries a modifying allele(s) that can increase survival time of $p53^{-/-}$ mice, but that this modifier allele is likely to be recessive, in linkage disequilibria with the *p53* locus, and/or that multiple modifiers rather than a single modifier contribute to this difference.

As expected, exposure to ionizing radiation reduced the life span of both the BALB/c and the DBA/2 wild-type mice. The primary tumor

type observed in both populations was thymic lymphoma. Exposure of BALB/c and DBA/2 $p53^{+/-}$ mice to ionizing radiation dramatically decreased their life span. This decrease was similar for both congenic lines and did not differ significantly from that observed in our laboratory as well as by other investigators upon exposure of $p53^{+/-}$ mice of mixed genetic background to ionizing radiation (18). However, although only 1 (5.3%; Ref. 20) of the $p53^{+/-}$ mice on a mixed background and 3 (6.8%) of the DBA/2 mice developed mammary tumors, this tumor type comprised 19 of 51 tumors observed in the BALB/c $p53^{+/-}$ mice (37.3%). A corresponding decrease in the frequency of lymphomas was also observed in this population.

Differences in the susceptibility of mouse strains to mammary tumors have long been recognized. These differences have largely been attributed to the fact that a number of mouse lines carried the MMTV[3] and that viral transmission occurred to their offspring through the milk. Although most inbred mouse lines still carry proviral copies of MMTV, in most cases the virus is unable to replicate and is not thought to contribute to the formation of mammary tumors. Although the influences of MMTV in these studies are difficult to ascertain, early studies indicated that DBA/2 mice are particularly susceptible to mammary tumors (24). A high incidence of mammary tumors has also been reported in BALB/c mice; however, analysis of these tumors revealed that the replication of MMTV is defective in this mouse strain (25). In addition, a high incidence of mammary tumors is observed in BALB/c mice exposed to chemical carcinogens (26). These observations suggest that both BALB/c and DBA/2 mice carry modifying alleles that might also lead to an increase in the incidence of mammary tumors in mice carrying mutations in tumor suppressor genes. The increase in the frequency of mammary tumors in the irradiated BALB/c $p53^{+/-}$ population is consistent with this supposition. Of the 51 BALB/c $p53^{+/-}$ tumors arising from animals exposed to ionizing radiation, 19 tumors or 37.3% were classified as mammary tumors. It is also interesting to note that the number of mammary tumors observed in BALB/c $p53^{+/-}$ mice after only 1–3 backcross generations was almost identical to the number observed in the $p53^{+/-}$ mice generated after more than 4 backcross generations. In fact, two of six BX1 generation mice developed mammary tumors, and it would be expected that in these animals only a single copy of the BALB/c alleles would be present. Although the numbers of mice examined at each generation were small, these results suggest that at least some of the modifiers that contribute to the susceptibility of these mice to mammary tumor formation act in a dominant fashion. More surprising perhaps was the lack of an increase in the incidence of mammary tumors in the DBA/2 mice. Only 3 of 44 tumors (6.8%) from irradiated DBA/2 $p53^{+/-}$ animals were classified as mammary tumors. A possible explanation for this difference in the incidence of mammary tumors in DBA mice in previous studies and our findings is that alleles placing mice at risk for MMTV-initiated mammary tumors do not necessarily increase the risk for tumors initiated by other events, such as loss of p53 function and/or exposure to environmental agents.

Initial examination of untreated $p53^{+/-}$ BALB/c mice suggests that modifier genes present in the BALB/c genetic background alone are sufficient to increase the incidence of mammary tumors in p53-deficient mice. Four tumors have been examined to date in this population, and two of them are mammary tumors. These results are further supported by the report published during the preparation of the manuscript (27). These investigators reported a high incidence of mammary tumors in untreated $p53^{+/-}$ BALB/c mice. Similar to the tumors that we have observed in the untreated $p53^{+/-}$ BALB/c mice, these tumors appear late in life; however, the incidence of the mammary tumors is similar to the incidence in our irradiated $p53^{+/-}$ BALB/c animals. Additional support for the presence of modifiers in the BALB/c background that make the mammary epithelial cells particularly sensitive to malignant transformation, especially upon loss of p53, comes from transplantation studies. A high incidence of mammary tumors was observed after transplantation of $p53^{-/-}$ BALB/c mammary epithelial cells into the cleared fat pads of isogenic mice as compared with control studies carried out with wild-type cells (27). Unlike treatment of $p53^{+/-}$ BALB/c mice with ionizing radiation, the incidence of mammary tumors in BALB/c recipient mice from these transplantation experiments did not significantly increase after exposure of these mice to chemical carcinogens (28). This contrasts with the results of our study in which we have shown that the median latency to mammary tumor formation is decreased by ~6 months in $p53^{+/-}$ BALB/c mice exposed to ionizing radiation. This may suggest that repair mechanisms for DNA damage induced by radiation are particularly sensitive to a deficiency in p53, whereas for the most part, the rate of repair of chemical-induced lesions is not altered by loss of p53.

A previous report has shown that radiation-induced chromosomal aberrations from BALB/c mammary epithelial cells persisted for up to 28 population doublings, whereas chromosomal aberrations from similarly treated C57BL/6 cells were repaired within six population doublings (29). This suggests an inherent deficit in the ability of BALB/c epithelial cells to repair specific types of DNA damage, particularly those induced by ionizing radiation. A possible explanation for the decreased repair in the BALB/c cells was suggested by a recent report showing that BALB/c cells have decreased levels and activity of DNA-PKcs protein (30), an enzyme involved in nonhomologous end-joining and DNA double-strand break repair. An increased incidence of these types of damage would be expected after exposure to radiation. The observation of mammary tumors in $p53^{+/-}$ BALB/c but not in $p53^{+/-}$ DBA/2 mice and the decrease in the latency of tumor formation after exposure to ionizing radiation are consistent with a model in which: (*a*) accumulation of DNA lesions, such as double-strand breaks, contribute to mammary tumorigenesis; (*b*) the frequency of these lesions is increased by exposure to ionizing radiation; (*c*) these lesions accumulate more frequently in BALB/c mice because of deficits in repair pathways; and (*d*) p53 is important in protecting cells from accumulation of these lesions.

In summary, the development of mammary tumors in p53-deficient mice is influenced by modifier alleles. Alleles present in the BALB/c background increased the incidence of mammary tumors in the absence of normal *p53* expression. The latency of tumor formation can be altered in p53-deficient BALB/c mice by exposure to ionizing radiation. In comparison, ionizing radiation decreases the latency to development of lymphomas but not mammary tumors in $p53^{+/-}$ DBA/2 mice. The identification of these genes should contribute significantly to our understanding of factors that put individuals at risk for developing breast cancer. In addition, the development of a model in which mammary tumors develop in mice with the first year of life should be valuable in future studies of both the pathogenesis and treatment of this disease.

ACKNOWLEDGMENTS

We thank K. Burns and T. Bartolotta for assistance with histology and D. T. Moore for assistance with statistical analysis.

REFERENCES

1. Levine, A. J. p53, the Cellular Gatekeeper for Growth and Division. Cell, *88:* 323–331, 1997.

[3] The abbreviation used is: MMTV, mouse mammary tumor virus.

2. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.
3. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J., Jr. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, *71:* 587–597, 1992.
4. Lowe, S. W., Scmitt, E. S., Osbrone, B. A., and Jacks, T. *p53* is required for radiation-induced apoptosis in mouse thymocytes. Nature (Lond.), *362:* 847–849, 1993.
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. Lowe, S. W., Jacks, T., Housman, D. E., and Ruley, H. E. Abrogation of oncogene-associated apoptosis allows transformation of *p53*-deficient cells. PNAS, *91:* 2026–2030, 1994.
7. Symonds, H., Krall, L., Reminton, L., Saenz-Robles, M., Lowe, S., Jacks, T., and van Dyke, T. p53-dependent apoptosis suppresses tumour growth and progression *in vivo*. Cells, *78:* 703–711, 2001.
8. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. Mutations in the *p53* tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res., *54:* 4855–4878, 1994.
9. Kleihues, P., Schauble, B., zur Hausen, A., Esteve, J., and Ohgaki, H. Tumors associated with *p53* germline mutations: a synopsis of 91 families. Am. J. Pathol., *150:* 1–13, 1997.
10. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature (Lond.), *356:* 215–221, 1992.
11. Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S. Bronson, R. T., and Weinberg, R. A. Tumor spectrum analysis in *p53*-mutant mice. Curr. Biol., *4:* 1–7, 1994.
12. Harvey, M., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., Bradley, A., and Donehower, L. A. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. Nat. Genet., *5:* 225–229, 1993.
13. Harvey, M., McArthur, M. J., Mongomery, C. A., Bradley, A., and Donehower, L. A. Genetic background alters the spectrum of tumors that develop in p53-deficient mice. FASEB J., *7:* 938–943, 1993.
14. Donehower, L. A., Godley, L. A., Aldaz, M., Pyle, R., Shi, Y-P., Pinkel, D., Gray, J., Bradley, A., Medina, D., and Varmus, H. E. Deficiency of p53 accelerates mammary tumorigenesis in *Wnt-1* transgenic mice and promotes chromosomal instability. Genes Dev., *9:* 882–895, 1995.
15. Tokunaga, M., Norman J. E., Jr., Asano, M., Tokuoka, S., Ezaki, H., Nishimori, I., and Tsuji, Y. Malignant breast tumors among atomic bomb survivors, Hiroshima and Nagasaki, 1950–74. J. Natl. Cancer Inst., *62:* 1347–1359, 1979.
16. Wolden, S. L., Lamborn, K. R., Cleary, S. P., Tate, D. J., and Donaldson, S. S. Second cancers following pediatric Hodgkin's disease. J. Clin. Oncol., *16:* 536–544, 1998.
17. Howe, G. R., and McLaughlin, J. Breast cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian fluoroscopy cohort study and a comparison with breast cancer mortality in the atomic bomb survivors study. Radia. Res., *145:* 694–707, 1996.
18. Kemp, C. J., Wheldon, T., and Balmain, A. p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. Nat. Genet., *8:* 66–69, 1994.
19. Su, L. K. Effect of genetic background on tumor formation in APC-Min mice. Science (Wash. DC), *256:* 668–670, 1992.
20. Cressman, V. L., Backlund, D. C., Hicks, E. M., Gowen, L. C., Godfrey, V., and Koller, B. H. Mammary tumor formation in p53- and BRCA1-deficient mice. Cell Growth Differ., *10:* 1–10, 1999.
21. Enzinger, F. M., and Weiss, S. W. Malignant vascular tumors in soft tissue tumors. *In:* Soft Tissue Tumors, pp. 641–677. St. Louis: Mosby, 1995.
22. Naka, N., Tomita, Y., Nakanishi, H., Araki, N., Hongyo, T., Ochi, T., and Aozasa, T. Mutations of *p53* tumor-suppressor gene in angiosarcoma. Int. J. Cancer, *71:* 952–955, 1997.
23. Zietz, C., Rossle, M., Haas, C., Sendelhofert, A., Hirschmann, A., Sturzl, M., and Lohrs, U. MDM-2 oncoprotein overexpression, *p53* gene mutation, and VEGF up-regulation. Am. J. Pathol., *153:* 1425–1433, 1998.
24. Hoag, W. G. Spontaneous cancer in mice. Ann. NY Acad. Sci., *108:* 805–831, 1963.
25. McGrath, C. M., Prass, W. A., Maloney, T. M., and Jones, R. F. Induction of endogenous mammary tumor virus expression during hormonal induction of mammary adenoacanthomas and carcinomas of BALB/c female mice. J. Natl. Cancer Inst., *67:* 841–852, 1981.
26. Medina, D. Mammary tumorigenesis in chemical carcinogen-treated mice, incidence in BALB/c and C57BL mice. J. Natl. Cancer Inst., *53:* 213–217, 1974.
27. Kuperwasser, C., Hurlbut, G. D., Kittrell, F. S., Dickinson, E. S., Laucirica, R., Medina, D., Naber, S. P., and Jerry, D. J. Development of spontaneous mammary tumors in BALB/c *p53* heterozygous mice: A model for Li-Fraumeni syndrome. Am. J. Pathol., *157:* 2151–2159, 2000.
28. Jerry, D. J., Kittrell, F. S., Kuperwasser, C., Laucirica, R., Dickinson, E. S., Bonilla, P. J., Butel, J. S., and Medina, D. A mammary-specific model demonstrates the role of the *p53* tumor suppressor gene in tumor development. Oncogene, *19:* 1052–1058, 2000.
29. Ponnaiya, B., Cornforth, M. N., and Ullrich, R. L. Radiation-induced chromosomal instability in BALB/c and C57BL/6 mice: the difference is as clear as black and white. Radiat. Res., *147:* 121–125, 1997.
30. Okayasu, R., Suetomi, K., Yu, Y., Silver, A., Bedford, J. S., Cox, R., and Ullrich, R. L. A deficiency in DNA repair and DNA-PK_{CS} expression in the radiosensitive BALB/c mouse. Cancer Res., *60:* 4342–4345, 2000.