

Animal Model

Development of Spontaneous Mammary Tumors in BALB/c *p53* Heterozygous Mice

A Model for Li-Fraumeni Syndrome

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Breast cancer is the most frequent tumor type among women in the United States and in individuals with Li-Fraumeni syndrome. The *p53* tumor suppressor gene is altered in a large proportion of both spontaneous breast malignancies and Li-Fraumeni breast cancers. This suggests that loss of *p53* can accelerate breast tumorigenesis, yet *p53*-deficient mice rarely develop mammary tumors. To evaluate the effect of *p53* loss on mammary tumor formation, the *p53*^{null} allele was back-crossed onto the BALB/c genetic background. Median survival was 15.4 weeks for BALB/c-*p53*^{-/-} mice compared to 54 weeks for BALB/c-*p53*^{+/-} mice. Sarcomas and lymphomas were the most frequent tumor types in BALB/c-*p53*^{-/-} mice, whereas 55% of the female BALB/c-*p53*^{+/-} mice developed mammary carcinomas. The mammary tumors were highly aneuploid, frequently lost the remaining wild-type *p53* allele, but rarely lost *BRC1*. Although mammary tumors were rarely detected in BALB/c-*p53*^{-/-} female mice, when glands from BALB/c-*p53*^{-/-} mice were transplanted into wild-type BALB/c hosts, 75% developed mammary tumors. The high rate of mammary tumor development in the BALB/c background, but not C57Bl/6 or 129/Sv, suggests a genetic predisposition toward mammary tumorigenesis. Therefore, the BALB/c-*p53*^{+/-} mice provide a unique model for the study of breast cancer in Li-Fraumeni syndrome. These results demonstrate the

critical role that the *p53* tumor suppressor gene plays in preventing tumorigenesis in the mammary gland. (Am J Pathol 2000, 157:2151–2159)

The *p53* tumor suppressor gene plays a complex and critical role in maintaining genome integrity. In cells with damaged DNA, *p53* mediates the decision to arrest cells to allow for DNA repair or eliminate the cell by apoptotic pathways. Mutations in the *p53* tumor suppressor gene (*TP53*) are the most common genetic abnormality being found in >50% of all human cancers, emphasizing the importance of *p53* function for suppression of tumors.¹ Germline mutations in the *p53* tumor suppressor gene are associated with Li-Fraumeni syndrome in which early-onset breast cancer is the most common cancer affecting women with Li-Fraumeni syndrome.^{2–4} Although this suggests that the loss of *p53* is a critical event in the progression of breast tumorigenesis, *p53*-deficient mice rarely developed mammary tumors.⁵ Instead, mice lacking *p53* died prematurely from a variety of other tumors.⁶ The lack of mammary tumor formation in mice deficient for *p53* suggested that loss of *p53* alone was not sufficient for tumor development in the mammary gland or that its role as a tumor suppressor in this tissue was not essential.

Different strains of inbred mice have been shown to differ in their susceptibility to mammary tumorigenesis.⁷ Female BALB/c mice were shown to be sensitive to radiation-induced mammary tumor development, whereas C57Bl/6 mice were resistant.^{7,8} This difference was correlated with an increase in chromosomal instability in

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BALB/c mice compared to the C57Bl/6 mice rather than variations in hormonal levels.⁷ In the analysis of families with Li-Fraumeni syndrome, it was shown that some women, in particular families, developed breast cancers more frequently than women in other families,⁹ suggesting the presence of modifier loci that may also be involved in the predisposition to mammary tumor formation. Similarly, lack of mammary tumorigenesis in *p53*-deficient mice could have been because of the resistance of the genetic backgrounds.

Therefore, the *p53*^{null} allele was transferred to the BALB/c genetic background to determine whether this altered the tumor spectrum. BALB/c-*p53*^{-/-} mice displayed a similar tumor incidence and spectrum to that observed in other backgrounds with a predominance of lymphomas and hemangiosarcomas. An abnormal mammary phenotype, ranging from stromal alterations to preneoplastic changes in the mammary epithelium, was observed in the majority of the female BALB/c-*p53*^{-/-} mice. In contrast, female BALB/c-*p53*^{+/-} mice showed a prevalence of mammary carcinomas with a latency of 8 to 14 months. These data reveal a genetic predisposition to mammary tumor development in BALB/c mice, which is accelerated upon loss of *p53*. Furthermore, the prevalence of mammary tumors in BALB/c-*p53*^{+/-} mice provides a model that more accurately reflects the tumor spectrum in individuals with Li-Fraumeni syndrome.

Materials and Methods

Mice and Tissues

C57Bl/6x129/Sv *p53*-deficient mice (generous gift from Tyler Jacks, Cambridge, MA) were mated with BALB/c mice. The *p53*^{null} allele from these mice was backcrossed for nine generations onto the BALB/cMed strain. Individuals were genotyped by multiplexed PCR as described previously.^{10,11} Mice were monitored weekly for 18 months and sacrificed when overt tumor development was detected or when signs of morbidity were evident. A total of 44 BALB/c-*p53*^{-/-} mice (eight male, 36 female) and 45 BALB/c-*p53*^{+/-} mice (seven male, 38 female) underwent necropsy. Five wild-type mice were sampled from age-matched animals in our colony ($n = 50$). Tumor tissues as well as the fourth inguinal mammary glands were excised and fixed for 8 to 12 hours in 10% neutral-buffered formalin. Tissues were stored in 70% ethanol before embedding in paraffin. Tissues were sectioned at a thickness of 4 μ m and were stained with hematoxylin and eosin for evaluation by light microscopy. Additional samples of tumor tissue were snap-frozen and stored in liquid nitrogen.

Ploidy Analysis

Single cell suspensions were obtained by digesting five 50- μ m paraffin sections in pepsin. The suspensions were filtered, stained with propidium iodide, and analyzed using a FACScan flow cytometer. A total of 20,000 events were collected from each sample as described previous-

ly.¹² DNA histograms were produced using ModFit LT software (Verity Software House, Topsham, ME).

Cytogenetic Analysis

Fresh tumor tissue was removed at necropsy, rinsed with Dulbecco's modified Eagle's medium (Life Technologies, Inc., Grand Island, NY) to remove cellular debris, minced, and digested for 3 hours in 10 μ g/ml of collagenase III (Sigma, St. Louis, MO). The cell suspension was washed with 5% adult bovine serum in phosphate-buffered saline and cultured in a 100-mm dish in Dulbecco's modified Eagle's medium/F12 media supplemented with sodium bicarbonate, HEPES buffer, 2% adult bovine serum, insulin (10 μ g/ml), and epidermal growth factor (5 ng/ml) until 60% confluent. The actively dividing culture was treated with colcemid (Life Technologies, Inc.) for 18 hours, then harvested by trypsin digestion. Cells were lysed with 0.068 mol/L KCl and fixed with a 3:1 methanol and glacial acetic acid solution. Interphase and metaphase nuclei were trypsinized and stained with Giemsa (BDH Chemicals, Poole, UK) for visualization.

Southern Blot Analysis

Fresh-frozen tumor samples taken at the time of necropsy were homogenized in 100 mmol/L Tris, 5 mmol/L ethylenediaminetetraacetic acid, 0.2% sodium dodecyl sulfate, 200 mmol/L NaCl, and digested with 100 μ g/ml proteinase K. Genomic DNA was extracted and purified with phenol/chloroform (1:1; v/v). Ten micrograms of DNA were digested with *Eco*RI and *Stu*I, then separated on a 0.7% agarose gel. The DNA was transferred to a nylon membrane, then hybridized to a genomic clone (probe B, a generous gift from Tyler Jacks) spanning the region from exon 7 to exon 9 of the *p53* gene.¹¹ The blot was stripped and hybridized sequentially with probes for *BRCA1* (exon 11, provided by Roger Wiseman, Research Triangle Park, Durham, NC), and β -casein cDNA (exons 1 to 9). Southern blot was performed in duplicate and quantitation was performed using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). Hybridization values for *p53* and *BRCA1* were normalized to β -casein to control for loading variation. Hybridization values were compared to the genomic DNA of corresponding genotypes isolated from tail biopsies. Allelic loss was scored if the hybridization value of the tumor was less than or equal to 50% of the value obtained from *p53*^{+/-} tail DNA.

Mammary Transplants

Whole gland transplants were performed by surgically removing the fourth inguinal mammary glands from mature (>8weeks) BALB/c-*p53*^{-/-} ($n = 16$) or BALB/c-*p53*^{+/-} ($n = 8$) females and suturing them onto the abdominal fascia of age-matched BALB/c wild-type recipients. Reconstituted mammary gland transplants were prepared with modifications of previous procedures.¹³ The fourth inguinal mammary glands from 21- to 24-day-old BALB/c-*p53*^{+/-} females were cleared of

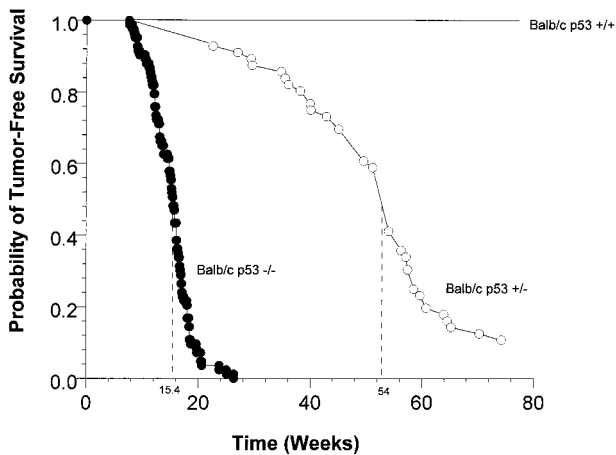


Figure 1. Survival curves of BALB/c *p53*-deficient mice. The probability of tumor-free survival of BALB/c-*p53*^{-/-} mice ($n = 85$; **closed circles**), and BALB/c-*p53*^{+/-} mice ($n = 56$; **open circles**) was monitored for 80 weeks. No wild-type animals died during this study ($n = 5$). **Dashed lines** represent the median time at which 50% of the animals had developed tumors.

mammary epithelium. Single ducts from mature BALB/c-*p53*^{-/-} donors were dissected and transplanted into the cleared fat pads from BALB/c-*p53*^{+/-} recipients ($n = 14$). Two weeks after the initial surgery, the reconstituted glands were removed and then transplanted onto the abdominal fascia of age matched BALB/c wild-type females. All transplant recipients were palpated weekly for tumor formation and sacrificed when tumor masses were detected. Tissues were processed and sectioned for histological evaluation as above.

Results

Tumor Incidence and Survival Rate of BALB/c-*p53*-Deficient Mice

Mice deficient for *p53* showed heightened tumor susceptibility compared to wild-type animals. BALB/c-*p53*^{-/-} mice developed tumors earlier and at higher frequency than did BALB/c-*p53*^{+/-} mice. All of the BALB/c-*p53*^{-/-} mice had developed tumors or died by 26 weeks of age with a median of 15.4 weeks (Figure 1). Among the BALB/c-*p53*^{+/-} mice, 50% developed tumors by 54 weeks and only 10% of the animals were tumor-free throughout the duration of the study. No wild-type animals developed tumors within the 80-week period of observation.

Tumor Spectrum and Mammary Phenotype in BALB/c-*p53*^{-/-} Mice

There were four predominant cancers detected in BALB/c-*p53*^{-/-} mice (Figure 2A). Multiple tumor types were often present in individual mice. Lymphomas were the most frequent tumor type, affecting 53% of the BALB/c-*p53*^{-/-} mice and involved primarily the thymus, lymph nodes, or other secondary lymphoid tissues. Hemangio-

sarcomas were the second most frequently detected tumor, affecting 39% of the animals. These tumors usually were present in the subcutaneous and soft tissues on the limbs or head, or in the mammary glands. Although the focus of the study was on primary tumors, metastases were detected in the lung and liver in several cases. Only one mammary carcinoma developed in the BALB/c-*p53*^{-/-} mice.

Previous studies have shown that mating of *p53* heterozygous animals yielded a reduced number of *p53*-null female offspring because of exencephaly.¹⁴ Similarly, BALB/c-*p53*^{-/-} female offspring were not born at the expected Mendelian ratios indicating that there were gestational defects resulting in embryonic lethality. BALB/c-*p53*^{-/-} females were primarily normal, but microscopic lesions were detected in 78% of the mammary glands. These included sarcomas, epithelial hyperplasia at 2 to 5 months of age, and alterations in stromal morphology. Stromal abnormalities and sarcomas were the predominant mammary lesions present in 68% of the BALB/c-*p53*^{-/-} female mice. The stromal changes were characterized by adipocytes with microvesicular fat droplets and a hypercellularity of the stroma (Figure 2C, ii). Periductal stromal tissue was thickened and many of the mammary ducts were markedly dilated with attenuated epithelial lining (Figure 2C, iii). In addition to the morphological changes observed in the mammary glands of nulliparous BALB/c-*p53*^{-/-} mice, abnormal stromal and glandular architecture were also seen in glands of pregnant, lactating, and postinvoluting mice (data not shown).

Tumor Spectrum and Mammary Phenotype in BALB/c-*p53* Heterozygous Mice

Although BALB/c-*p53*^{+/-} mice developed tumors frequently, there was a delay in their appearance and a difference in the tumor spectrum compared to the BALB/c-*p53*^{-/-} mice. Hemangiosarcomas, lymphomas, and osteosarcomas commonly found in some other strains of *p53*-heterozygous mice^{6,11} were less frequent in BALB/c-*p53*^{+/-} mice, but still accounted for 36% of all of the tumors (Figure 3A).

Although mammary carcinomas were rare in BALB/c-*p53*^{-/-} mice, they were the most prevalent tumor type in BALB/c-*p53*^{+/-} mice, accounting for 42% of the tumors (Figure 3A). All of the female heterozygous mice developed mammary abnormalities, which were either overt mammary carcinomas (55%) or hyperplasias (45%) (Figure 3B). Mammary tumors originated in both the inguinal and thoracic glands with a latency of 8 to 14 months. These tumors were generally adenoacanthomas or acinar-type adenocarcinomas (Figure 3C) with occasional poorly differentiated carcinomas identified. One animal developed two separate primary mammary tumors in different glands that were of different histological types. Many of the mammary carcinomas had infiltrating growth patterns, however metastases were not detected. Mammary carcinomas were present in nulliparous animals

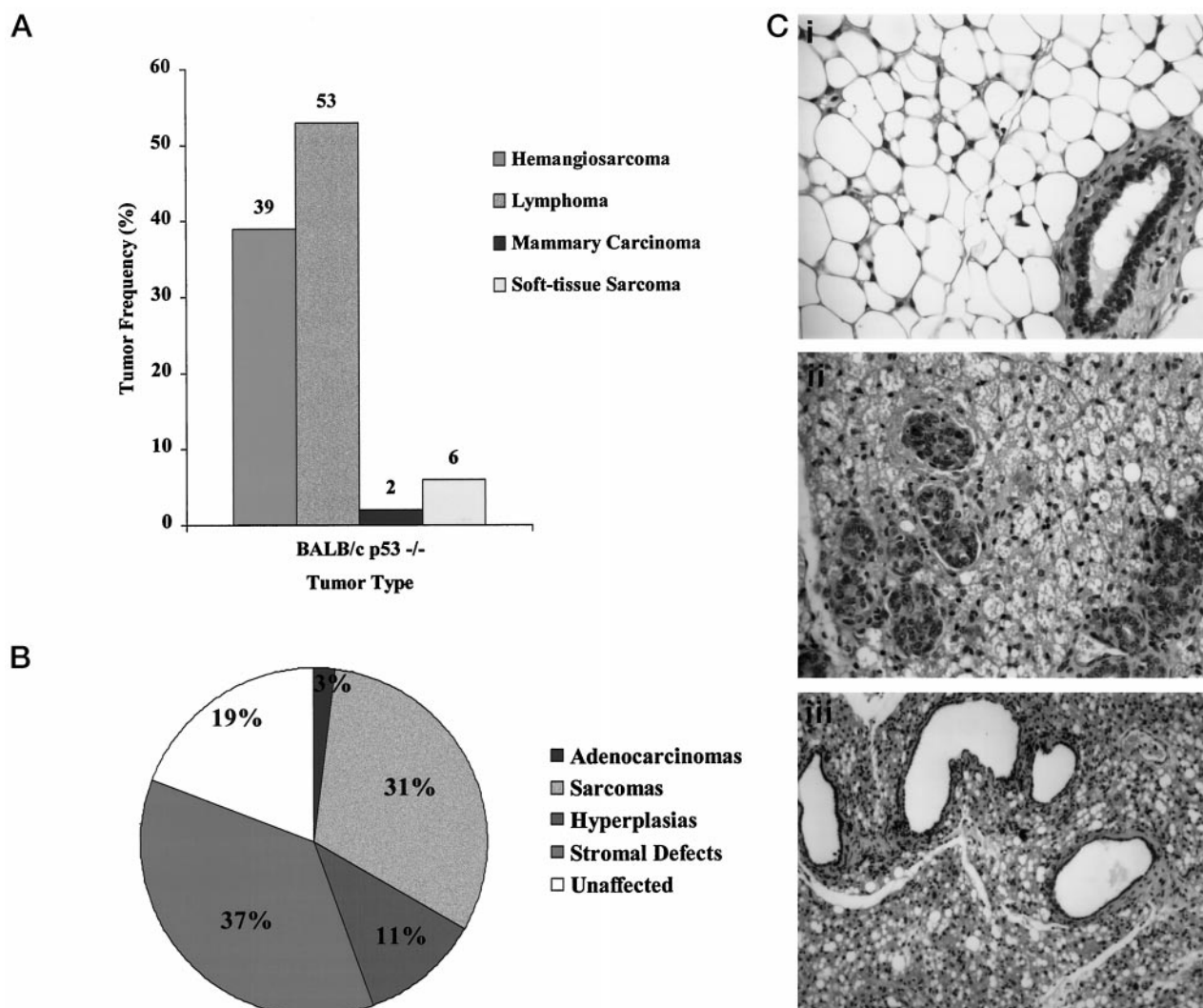


Figure 2. Tumor distribution and mammary abnormalities in BALB/c-*p53*^{-/-} mice. **A:** Tumor spectrum from BALB/c-*p53*^{-/-} mice. Frequency histogram of tumor types observed in both male and female mice (*n* = 44). **B:** Mammary phenotype of female BALB/c-*p53*^{-/-} mice. Relative frequency of abnormalities detected in the inguinal or thoracic gland from nulliparous and breeder females (*n* = 36). **C:** H&E-stained sections of mammary tissue from BALB/c-*p53*^{-/-} females. The normal murine mammary gland architecture is characterized by large adipocytes in the stroma surrounding a sparse population of ducts (**i**, ×40 objective). Typical stromal changes observed in BALB/c-*p53*^{-/-} females (**ii** and **iii**). Stroma characterized by microvesicular adipocytes and proliferation of mammary ducts (**ii**, ×40 objective). Hypercellular stroma and dilated ducts with attenuation of the mammary epithelium (**iii**, ×20 objective).

and breeder females. Epithelial hyperplasia was detected in all of the female BALB/c-*p53*^{+/-} mice that did not develop mammary tumors (Figure 3C, iv) and was also present in the ductal epithelium of those mice that developed mammary carcinomas.

Frequent Loss of *p53* but Not *BRCA1* in Mammary Tumors from BALB/c-*p53*^{+/-} Mice

Flow cytometry was performed to determine whether the tumors contained a population of aneuploid cells (Figure 4A). Seventy-one percent (10 of 14) of the mammary tumors analyzed exhibited rates of aneuploidy ranging from 17 to 89%. Tumors that maintained a diploid state had relatively high S-phase fractions ranging from 5 to 21%. Cytogenetic analysis was performed to examine the types of chromosomal abnormalities present in these

tumors (Figure 4B). Chromosomal translocations, strand breaks, and aberrant mitotic exchanges were common abnormalities associated with mammary tumors from BALB/c-*p53*^{+/-} mice.

Because of the high degree of aneuploidy and chromosomal abnormalities detected in the mammary tumors, Southern blot analysis was performed on mammary carcinomas to analyze the status of the wild-type *p53* allele in the BALB/c-*p53*^{+/-} mice (Figure 4C). In all seven of the mammary tumors analyzed, there was partial or complete loss of the wild-type allele. In contrast, the wild-type *p53* allele was retained in a prepuccial adenoma and a salivary gland carcinoma from BALB/c-*p53*^{+/-} mice. Tail DNA wild-type, *p53*^{+/-}, and *p53*^{-/-} animals were used as positive controls for DNA levels. There was no correlation between ploidy status of the mammary carcinoma and loss of heterozygosity.

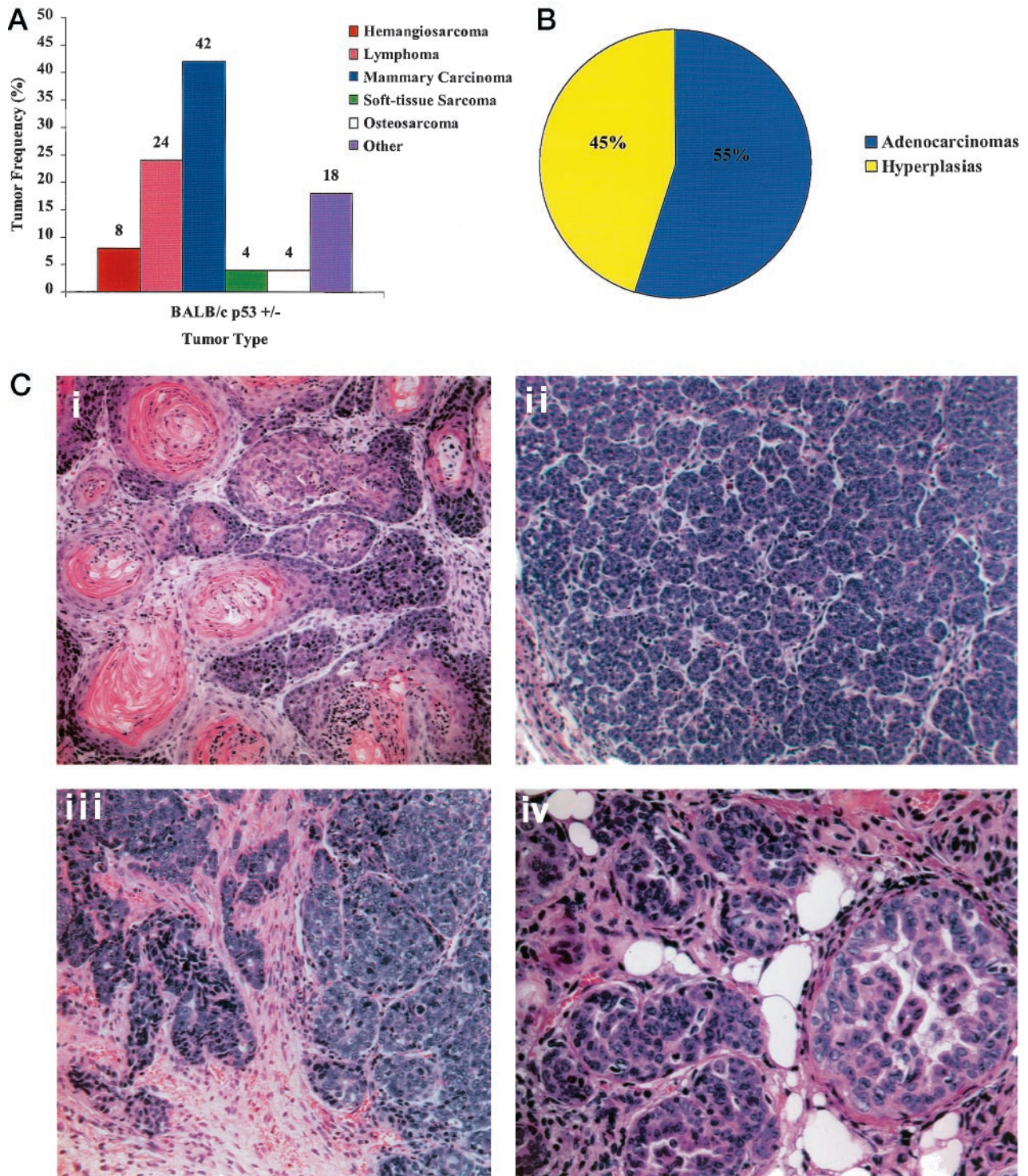


Figure 3. Tumor distribution and mammary abnormalities in BALB/c-*p53*^{+/-} mice. **A:** Tumor spectrum from BALB/c-*p53*^{+/-} mice. Frequency histogram of tumor types observed in both male and female mice ($n = 45$). **B:** Mammary phenotype of female BALB/c-*p53*^{+/-} mice. Relative frequency of abnormalities detected in the inguinal or thoracic mammary glands from nulliparous and breeder females ($n = 38$). Mammary hyperplasia was observed either alone or in association with tumors in all glands analyzed. **C:** H&E-stained sections of mammary tumor tissue from BALB/c-*p53*^{+/-} females ($\times 20$ objective). A typical adenoacanthoma characterized by keratin formation (**i**) and an adenocarcinoma with small acinar structures (**ii**). H&E section of a mammary carcinoma infiltrating adjacent stroma. (**iii**, $\times 20$ objective). Ductal hyperplasia commonly seen in BALB/c-*p53*^{+/-} female mice (**iv**, $\times 40$ objective).

Because the genes for *BRCA1* and *p53* lie 21 centimorgans apart on chromosome 11 in mice, it was possible that *BRCA1* may also have been lost in the mammary tumors of BALB/c-*p53*^{+/-} mice. Therefore,

Southern blot analysis for *BRCA1* was performed on those mammary carcinomas that were analyzed for *p53* loss of heterozygosity (Figure 4C, bottom). In all of the mammary tumors analyzed, little or no loss of *BRCA1* was

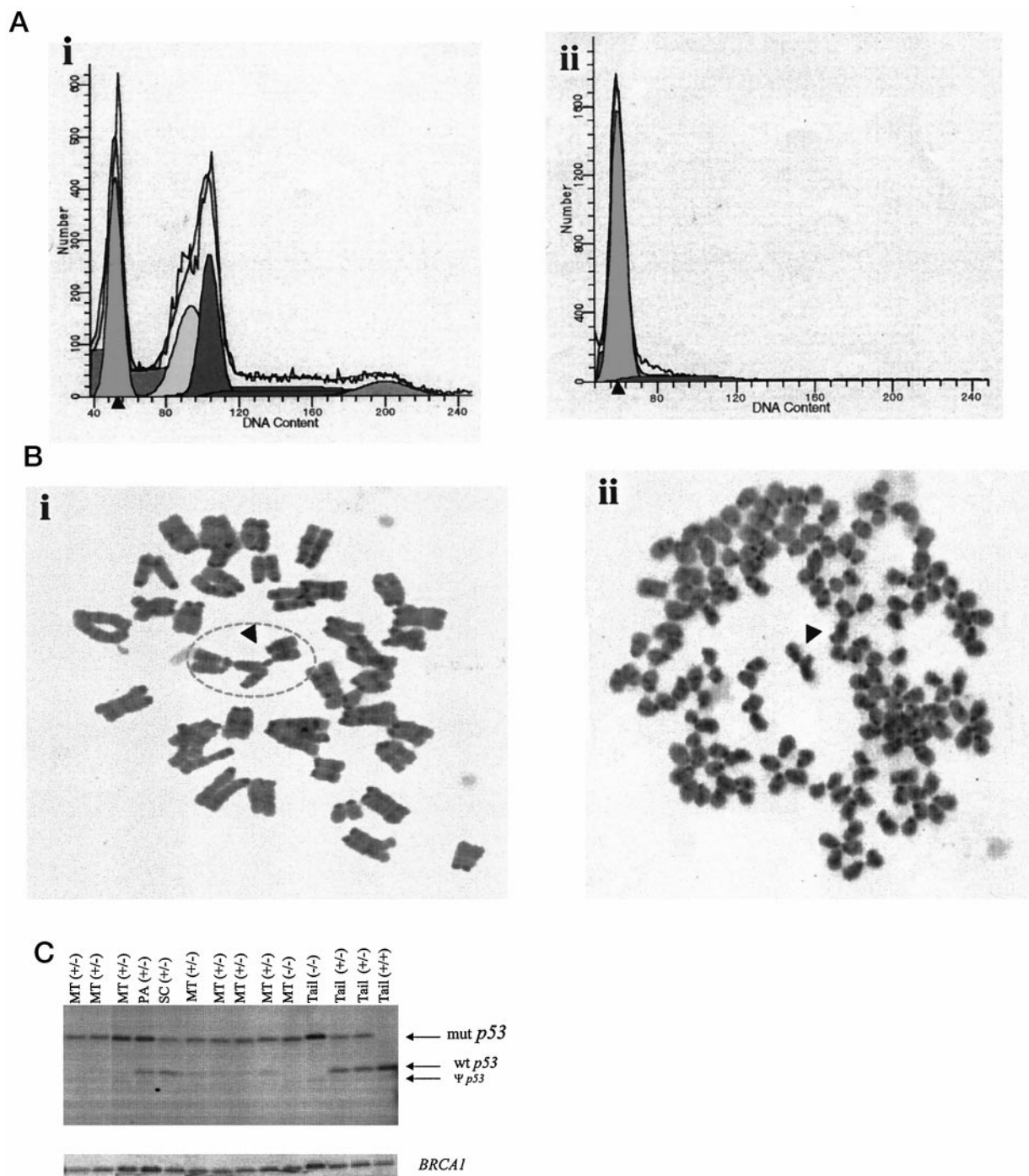


Figure 4. Analysis of DNA from BALB/c-*p53*^{+/-} mammary carcinomas. **A:** Representative DNA histograms determined by FACS analysis from mammary tumors (*n* = 14). Seventy-one percent of the tumors were aneuploid with DNA content >40 (**i**), and the remaining 29% of the tumor were diploid (**ii**). The solid line represents the distribution of cells. Subpopulations of cells were calculated using the ModFit LT software and are represented by the shaded peaks. **B:** Representative Giemsa-stained (original magnification, ×100) metaphase spreads of mammary carcinomas from BALB/c-*p53*^{+/-} females. Typical abnormalities such as quadriradial chromosomes (**i**) and chromosome breaks commonly found in aneuploid karyotypes (**ii**) marked by arrows, respectively. **C:** Southern blot analysis of *p53* and *BRCA1* in BALB/c-*p53*^{+/-} mammary tumors. Genomic DNA from mammary tumors (MT), a prepuccial adenoma (PA), a salivary gland carcinoma (SC), and tails (tail) were digested with *Eco*RI and *Stu*I. Loss of heterozygosity was determined using a genomic DNA clone spanning exon 7 to exon 9 of the *p53* gene. Three bands were detected in tail DNAs from BALB/c-*p53*^{+/-} mice (tail +/+). These fragments represent the wild-type allele (wt *p53*), the mutant allele (mut *p53*), and the pseudogene (Ψ *p53*). The blot was reprobed with exon 11 of *BRCA1* to determine whether there was loss of *BRCA1* in the mammary tumors from BALB/c-*p53*^{+/-} mammary tumors.

detected compared to tail DNA. The β -casein locus was also analyzed to control for the possibility of random losses because of chromosomal instability. Similar to the *BRCA1* locus, there was no evidence of significant loss of alleles at

the β -casein locus compared to tail DNA (data not shown). Therefore, preferential loss of the wild-type *p53* allele in these mammary tumors was not a random event, but seems to be selected for during tumor progression.

Table 1. Tumor Incidence in BALB/c-*p53*^{-/-} Mammary Gland Transplants

Transplant genotype	n	Accepted (%)	Tumors (%)
Whole-gland transplants*			
+/+ whole gland	8	8 (100)	0 (0)
-/- whole gland	16	12 (75)	9 (75)
Reconstituted gland transplants†			
<i>p53</i> ^{null/wt‡}	14	11 (79)	6 (55)
<i>p53</i> ^{wt/wt‡}	4	100	0 (0)

*Whole glands and reconstituted glands were transplanted into BALB/c wild-type recipients.

†*p53*^{-/-} epithelium was transplanted into wild-type stroma.

‡Wild-type epithelium transplanted into wild-type stroma.

Development of Mammary Tumors from BALB/c-*p53*^{-/-} Epithelium

Because mammary tumors developed in only one of the BALB/c-*p53*^{-/-} mice, it was possible that early mortality masked the development of mammary carcinomas. Therefore, whole glands from BALB/c-*p53*^{-/-} mice were transplanted into wild-type (BALB/c-*p53*^{+/+}) recipients and monitored for tumor formation. Of the whole gland

transplants that were accepted, 75% developed mammary carcinomas (Table 1), which were first observed by 7 months after transplantation (Figure 5A). Several BALB/c-*p53*^{-/-} whole gland transplants failed to engraft in the BALB/c wild-type recipients (25%). No tumors developed in the whole gland transplants derived from wild-type BALB/c mice.

Morphological changes in the mammary stroma were observed frequently in BALB/c-*p53*^{-/-} mice. To deter-

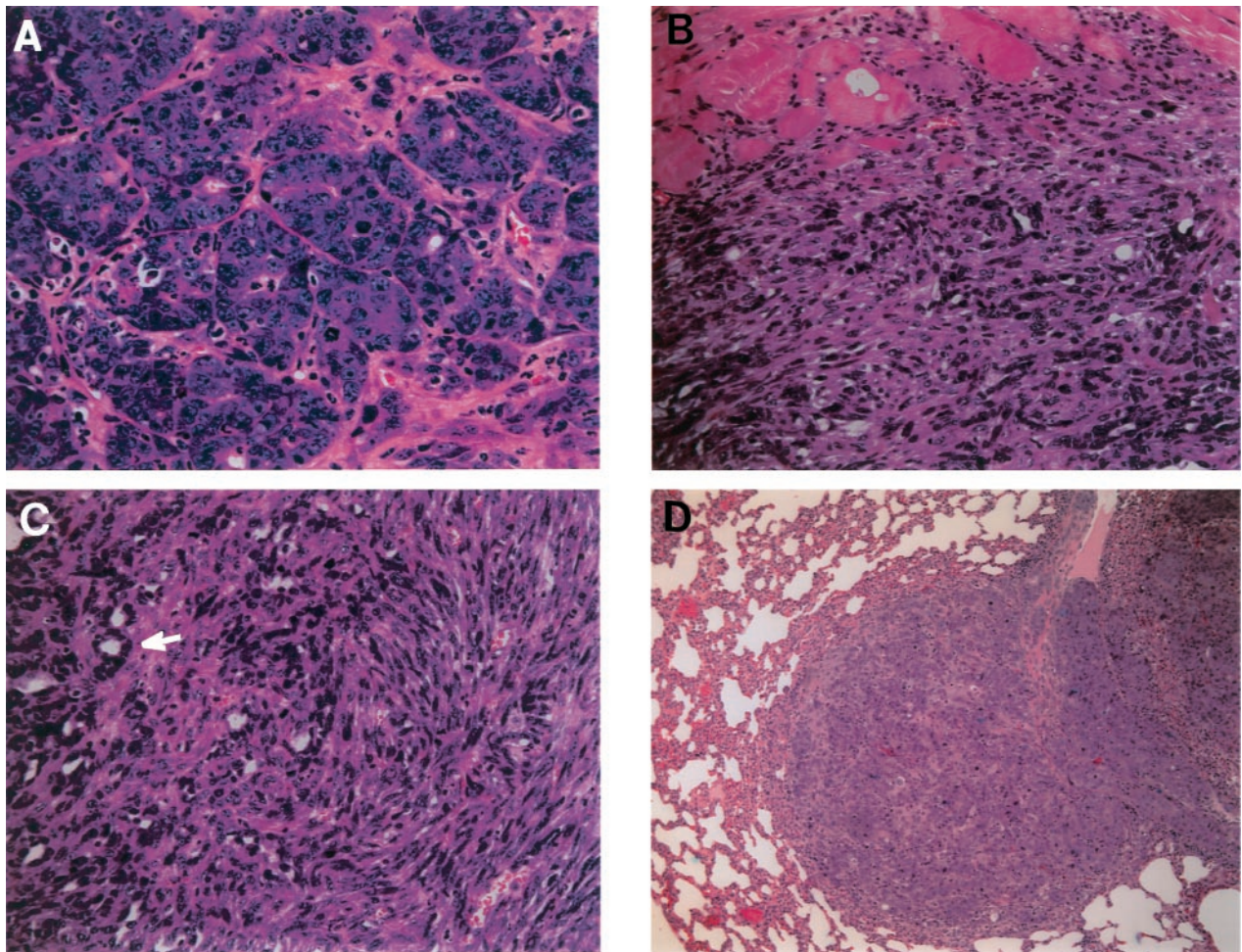


Figure 5. Histology of mammary carcinomas derived from transplants. **A:** The transplantation of a BALB/c-*p53*^{-/-} whole gland into a wild-type recipient yielded mammary tumors as early as 7 months. Typical adenocarcinoma with structures reminiscent of mammary ducts and several mitotic figures (H&E, ×40 objective). **B:** Adenocarcinomas also developed from reconstituted glands consisting of *p53*-deficient epithelium in a wild-type fat pad. A poorly differentiated mammary carcinoma invading the adjacent skeletal muscle (H&E, ×20 objective). **C:** An adenocarcinoma arising from a *p53*^{null/wt} transplant that has both acinar structures (white arrow) and a spindle cell component (×40 objective). **D:** Lung metastasis from a mammary carcinoma derived from a reconstituted gland consisting of *p53*^{-/-} epithelium in a wild-type fat pad (H&E, ×10 objective).

mine whether loss of *p53* in the mammary epithelium was sufficient for the development of mammary carcinomas without the contribution of the *p53*-deficient stroma, reconstituted gland transplants were performed. Reconstituted mammary glands composed of either *p53*^{-/-} epithelium and *p53*^{+/+} stroma (*p53*^{null/wt}) or *p53*^{+/+} epithelium and *p53*^{+/+} stroma (*p53*^{w/w}) were transplanted into wild-type BALB/c hosts and monitored for tumor formation. Of the *p53*^{null/wt}-reconstituted glands that were accepted, 55% developed tumors (Table 1). Many of these tumors were histologically similar to the tumors derived from the BALB/c-*p53*^{-/-} whole-gland transplants. Mammary tumors from *p53*^{null/wt}-reconstituted glands often exhibited sarcomatoid differentiation, invasive components, and distant metastases (Figure 5, A–C). The *p53*^{w/w} transplants, which consisted of wild-type epithelium and stroma, did not develop tumors. Although the number of tumors that developed in the whole-gland transplants was greater than in *p53*^{null/wt}-reconstituted gland transplants, there was no statistical difference in latency or frequency between the transplant experiments ($P > 0.05$). Similar to the BALB/c-*p53*^{-/-} whole gland transplants, 21% of the *p53*^{null/wt}-reconstituted gland transplants failed to engraft. This seemed to be dependent on the lack of *p53* in the epithelium because all of the *p53*^{w/w} transplants were accepted and is consistent with recent experiments with transplants of *p53*-deficient mammary epithelium.¹³

Discussion

Given the large number of breast cancers with *p53* mutations and the prevalence of breast tumors in women with Li-Fraumeni syndrome,^{15–17} it was surprising that the *p53*-deficient mice rarely developed mammary tumors.¹⁸ Therefore, we sought to examine the effects of *p53* loss on the BALB/c strain that has been widely used for the study of the mammary gland and has been shown to be more susceptible to induction of mammary tumors.⁷ BALB/c-*p53*^{-/-} female mice did not develop mammary carcinomas, but stromal changes were frequently observed in the mammary gland. Early mortality because of lymphomas was likely to have precluded the development of mammary tumors. This is supported by the development of mammary carcinomas when *p53*-deficient mammary glands were transplanted into wild-type hosts (Table 1). Furthermore, transplantation of *p53*-deficient epithelium into wild-type fat pads also produced mammary carcinomas, suggesting that *p53* loss in the mammary epithelium is a critical step in tumorigenesis (Table 1).¹³ The histology of mammary tumors observed in the BALB/c-*p53*-deficient mammary tissue is typical of spontaneous mammary tumors in mice¹⁹ and does not exhibit unique histological features that have been observed in other genetically engineered mice.²⁰ Therefore, this knockout model of mammary tumorigenesis seems to reflect the acceleration of sporadic mammary tumors and their progression that is found in otherwise genetically normal mice.

In humans, Li-Fraumeni syndrome is an inherited predisposition to cancer development and more than half of affected families carry germline mutations in the *p53* tumor suppressor gene.² Tumors typically associated with this syndrome are early-onset breast carcinomas, osteosarcomas, soft-tissue sarcomas, brain tumors, and leukemias.^{2,3,15–17} The BALB/c-*p53*^{+/+} mice developed mammary carcinomas and other malignancies in a pattern and frequency similar to that reported for the Li-Fraumeni syndrome in humans (Figure 3A).^{2–4,11,16} The breast cancers that arise in women from Li-Fraumeni families typically develop at mid-life, are highly aneuploid, and frequently lose the remaining wild-type *p53* allele.^{9,17,18} Similarly, the mammary tumors in BALB/c-*p53*^{+/+} mice developed near mid-life (latency of 8 to 14 months), were highly aneuploid, and frequently lost the wild-type *p53* allele (Figure 4). This frequent loss of the wild-type allele in mammary carcinomas from the BALB/c-*p53*^{+/+} mice differs from the situation in lymphomas where more than half of lymphomas that developed in *p53*-heterozygous mice retained the wild-type allele.²¹ Hence, loss of the wild-type *p53* allele may be required for tumor development in the mammary epithelium, but not in other tissues.

The loss of *BRCA1* has been linked to heightened risk of breast cancer, yet mutations in this tumor suppressor gene are infrequent in sporadic breast cancers. In *BRCA1* conditional knockout mice, where mammary tumors developed after 10 months, three out of four mammary tumors had also lost *p53*²² suggesting that loss of both genes may contribute to tumorigenesis. However, none of the mammary carcinomas from BALB/c-*p53*^{+/+} mice lost *BRCA1*, supporting the concept that loss of *p53* alone is a central and perhaps early event for initiation and progression of mammary tumors. Given that the *p53* and *BRCA1* genes lie 21 centimorgans apart on chromosome 11, it was surprising that loss of *BRCA1* was not seen in some of the tumors if genetic losses occurred randomly. Although the exact mechanism by which *p53* was deleted cannot be determined by this analysis, these data suggest that the tumor suppressive effects of *p53* occur independent of *BRCA1*, and that loss of *BRCA1* was not required for the development of mammary tumors.

The high rate of mammary tumor development in the BALB/c *p53*-deficient mice compared to other *p53*-knockout strains is intriguing. Differences in tumor spectrum were reported between C57Bl/6x129/Sv, which primarily developed lymphomas, and pure 129/Sv, which readily developed testicular tumors.¹⁸ It was suggested that the difference in tumor spectrums was related to the difference in strain-specific tumor susceptibilities. The 129/Sv mice have a relatively high incidence of teratocarcinomas²³ whereas the C57Bl/6 do not. Although the normal BALB/c inbred strain has a low incidence of spontaneous mammary tumors, it has been demonstrated that the BALB/c mammary epithelium is susceptible to genetic instability and radiation-induced mammary tumorigenesis.^{7,8,24} A pivotal role of *p53* in determining susceptibility of the mammary epithelium to tumor development is suggested by recent experiments demonstrating that *p53* function is compromised in the normal BALB/c mammary

epithelium.²⁵ The heightened susceptibility of BALB/c mice to mammary tumor formation supports the existence of genetic modifiers that interact with p53-deficiency epistatically and predisposes to tissue-specific tumorigenesis. The BALB/c strain possesses a variant allele for *Cdkn2a* (p16/INK4a, p19/ARF)²⁶ that increases susceptibility of the BALB/c strain to plasmacytomas. P19/ARF-deficient mice are predisposed to tumor development, yet mammary carcinomas are rare.²⁷ This suggests that other unidentified modifier loci may be responsible for the mammary tumor phenotype in BALB/c-p53^{+/-} mice. Identification of the genetic modifier gene(s) responsible for the heightened development of mammary carcinomas in BALB/c mice will be of particular importance because it may explain the variation in susceptibility to tumor development and tumor spectrum in different individuals with the same germline mutation in p53.

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