[CANCER RESEARCH 64, 5140–5147, August 1, 2004]

Loss of Heterozygosity Occurs via Mitotic Recombination in *Trp53*$^{+/-}$ Mice and Associates with Mammary Tumor Susceptibility of the BALB/c Strain

Anneke C. Blackburn,[1] S. Christine McLary,[1] Rizwan Naeem,[2] Jason Luszcz,[2] David W. Stockton,[4] Lawrence A. Donehower,[5] Mansoor Mohammed,[6] John B. Mailhes,[7] Tamar Soferr,[1] Stephen P. Naber,[3] Christopher N. Otis,[3] and D. Joseph Jerry[1]

[1]Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts; Departments of [2]Cytogenetics and [3]Pathology, Baystate Medical Center, Springfield, Massachusetts; [4]Department of Molecular and Human Genetics and [5]Division of Molecular Virology, Baylor College of Medicine, Houston, Texas; [6]Spectral Genomics, Houston, Texas; and [7]Department of Obstetrics and Gynecology, Louisiana State University Health Sciences Center, Shreveport, Louisiana

ABSTRACT

Loss of heterozygosity (LOH) occurs commonly in cancers causing disruption of tumor suppressor genes and promoting tumor progression. BALB/c-*Trp53*$^{+/-}$ mice are a model of Li-Fraumeni syndrome, exhibiting a high frequency of mammary tumors and other tumor types seen in patients. However, the frequency of mammary tumors and LOH differs among strains of *Trp53*$^{+/-}$ mice, with mammary tumors occurring only on a BALB/c genetic background and showing a high frequency of LOH, whereas *Trp53*$^{+/-}$ mice on a 129/Sv or (C57BL/6 × 129/Sv) mixed background have a very low frequency of mammary tumors and show LOH for *Trp53* in only ~50% of tumors. We have performed studies on tumors from *Trp53*$^{+/-}$ mice of several genetic backgrounds to examine the mechanism of LOH in BALB/c-*Trp53*$^{+/-}$ mammary tumors. By Southern blotting, 96% (24 of 25) of BALB/c-*Trp53*$^{+/-}$ mammary tumors displayed LOH for *Trp53*. Karyotype analysis indicated that cells lacking one copy of chromosome 11 were present in all five mammary tumors analyzed but were not always the dominant population. Comparative genomic hybridization analysis of these five tumors indicated either loss or retention of the entire chromosome 11. Thus chromosome loss or deletions within chromosome 11 do not account for the LOH observed by Southern blotting. Simple sequence length polymorphism analysis of (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ mammary tumors showed that LOH occurred over multiple loci and that a combination of maternal and paternal alleles were retained, indicating that mitotic recombination is the most likely mechanism of LOH. Nonmammary tumors of BALB/c mice also showed a high frequency of LOH (22 of 26, 85%) indicating it was not a mammary tumor specific phenomenon but rather a feature of the BALB/c strain. In (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ mice LOH was observed in 93% (13 of 14) of tumors, indicating that the high frequency of LOH was a dominant genetic trait. Thus the high frequency of LOH for *Trp53* in BALB/c-*Trp53*$^{+/-}$ mammary tumors occurs via mitotic recombination and is a dominant genetic trait that associates with the occurrence of mammary tumors in (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ mice. These results further implicate double-strand DNA break repair machinery as important contributors to mammary tumorigenesis.

Received 11/3/03; revised 4/23/04; accepted 5/28/04.

Grant support: NIH Grants CA66670 and CA87531 (D. J. Jerry), Massachusetts Department of Public Health Grant 43088PP1017, and Department of Defense Breast Cancer Program Award DAMD17-00-1-0632 administered by the United States Army Medical Research Acquisition Activity. This material is based on work supported by the Cooperative State Research Extension, Education Services, United States Department of Agriculture, Massachusetts Agricultural Experiment Station, and Department of Veterinary and Animal Sciences under projects MAS00821 and NC-1010. A. C. Blackburn was supported by Department of Defense Breast Cancer Program Award DAMD17-01-1-0315, administered by United States Army Medical Research Acquisition Activity, and subsequently by Fellowship 179842 awarded by the National Health and Medical Research Council of Australia.

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.

Note: A. C. Blackburn is currently in John Curtin School of Medical Research, Australian National University, Canberra, Australia. R. Naeem is currently in Baylor College of Medicine, Houston, Texas. S. P. Naber is currently in Tufts-New England Medical Center, Boston, Massachusetts.

Requests for reprints: D. Joseph Jerry, Department of Veterinary and Animal Sciences, Paige Laboratory, University of Massachusetts, Amherst, MA 01003-6410. Phone: (413) 545-5335; Fax: (413) 545-6326; E-mail: jjerry@vasci.umass.edu.

INTRODUCTION

Loss of heterozygosity (LOH) plays an important role in carcinogenesis in both sporadic and familial cancers. LOH is a means by which mutations, germline or sporadic, in tumor suppressor genes can become homozygous leading to tumor predisposition in the affected cells (1, 2). Mutations in the p53 tumor suppressor gene are commonly observed in human cancers and occur together with LOH at the *TP53* locus (3, 4). Although the p53 tumor suppressor gene (*TP53* in humans or *Trp53* in mice) is critical for inhibiting tumor development in many tissues, the breast epithelium appears particularly dependent on proper p53 function. This is evident from the high frequency of mutations in *TP53* in sporadic human breast cancers (5) and the high frequency of breast cancer in Li-Fraumeni syndrome patients (LFS), of whom approximately half carry mutations in one allele of *TP53* (6). Even in the context of mutations in the breast cancer susceptibility genes, *BRCA1* and *BRCA2*, high rates of p53 mutation are found (7, 8) and inactivation of p53 is frequent in the development of mammary tumors in *Brca1* or *Brca2* conditional mutant mice (9, 10). Thus p53 mutation and LOH appear to play particularly prominent roles in the development of breast cancer.

Mice heterozygous for the *Trp53* null allele develop a spectrum of tumors similar to LFS patients (11, 12). However, only when the *Trp53* null allele was backcrossed onto the BALB/c genetic background was the high frequency of mammary tumors seen in LFS patients recapitulated (13). Therefore BALB/c-*Trp53*$^{+/-}$ mice serve as a unique model for breast cancer in LFS. The BALB/c susceptibility to *Trp53*$^{+/-}$ mammary tumors has both dominant and recessive genetic components, as determined by breeding with the C57BL/6 strain (14). Female BALB/c-*Trp53*$^{+/-}$ mice developed mammary tumors at a frequency of 55% and a latency of 8–14 months, with the majority being adenocarcinomas that exhibit karyotypic instability and are often aneuploid (13). Mammary tumors arising in BALB/c-*Trp53*$^{+/-}$ mice exhibited a high frequency of loss of the wild-type *Trp53* allele (13), whereas other tumor types in other strains displayed lower frequencies of LOH (15). Examination of the *TP53* locus in LFS tumors also revealed frequent loss of the wild-type allele (16). The mechanism by which the wild-type *Trp53* allele is lost in BALB/c-*Trp53*$^{+/-}$ mammary tumors and the extent of chromosomal loss around *Trp53* are unknown.

Various mechanisms can result in LOH that may or may not lead to changes in gene copy number, thus affecting our ability to detect LOH. Loss of an entire chromosome by missegregation will lead to LOH along the entire chromosome length, and unless accompanied by reduplication, this loss will result in only one copy of that chromosome being present. Recombination between homologous chromosomes rather than sister chromatids during mitosis (mitotic recombination, MR) will result in LOH occurring over the distance between the recombination break points, with multiple rounds of this over numerous cell divisions producing a mosaic effect of retained heterozygosity and LOH at different loci within the one chromosome,

although maintaining two copies of each gene. Deletion events, perhaps occurring as a result of nonhomologous end joining (NHEJ) of double-strand DNA breaks, will result in LOH and a reduction in gene dosage for loci within the deleted region.

In this study, *Trp53*$^{+/-}$ mice of BALB/c and mixed genetic background were used to compare frequencies of LOH at *Trp53* in mammary and nonmammary tumors and examine the mechanisms leading to LOH. Karyotype analysis was used to detect large chromosomal alterations, whereas comparative genomic hybridization (CGH) microarray analysis was used to detect changes in copy number in regions as small as a few megabases. Simple sequence length polymorphism (SSLP) analysis together with Southern blotting was used to determine the identity of the alleles present in tumors. Together these analyses provided insights into the mutagenesis processes leading to LOH in the mouse mammary gland.

MATERIALS AND METHODS

Mice. BALB/c-*Trp53*$^{+/-}$ mice were generated previously (17) by backcrossing (C57BL/6 × 129/Sv) *Trp53*$^{-/-}$ mice onto the BALB/cMed strain for 11 generations. F1 intercross mice were *Trp53*$^{+/-}$ offspring of inbred C57BL/6J-*Trp53*$^{+/+}$ female and BALB/cMed-*Trp53*$^{-/-}$ male mice. N2 backcross mice were the offspring of (C57BL/6J × BALB/cMed) F1-*Trp53*$^{+/+}$ females × BALB/cMed-*Trp53*$^{-/-}$ males. Ninety-seven virgin female BALB/c-*Trp53*$^{+/-}$, 19 virgin female (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ study mice, and 224 virgin female [(C57BL/6 × BALB/c) × BALB/c] N2-*Trp53*$^{+/-}$ study mice were monitored weekly for tumor development or morbidity and were palpated for mammary tumors. The survival from and the occurrence of mammary and other tumors in these mouse populations has been described previously (14).

Tumors from male and female *Trp53*$^{+/-}$ mice of mixed [C57BL/6 × 129/Sv] background analyzed for LOH are those that have been described previously (15, 18). Kaplan-Meier plots of survival (n = 96 females, n = 113 males) were analyzed by the log-rank test (Mantel-Cox) for significant differences.

Isolation of Genomic DNA. Genomic DNA for Southern blotting, PCR, and comparative genomic hybridization were extracted from tumors and normal tail tissues of *Trp53*$^{+/-}$ mice. The tissues were snap frozen in liquid nitrogen at the time of necropsy, later minced and digested overnight with 100 μg/ml proteinase K in 100 mM Tris, 5 mM EDTA, 0.2% SDS, and 200 mM NaCl. Genomic DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1).

***Trp53* Genotyping and LOH.** The *Trp53*$^{-/-}$ males used for generating the *Trp53*$^{+/-}$ mice were genotyped by multiplex PCR as described previously (11, 17). LOH in tumors at the *Trp53* locus was determined by Southern blotting as described previously (13). Briefly, genomic DNA was digested with *Stu*I and *Eco*R1, blotted and hybridized with a probe spanning exon 7 to exon 9 of the *Trp53* gene. The intensity of the wild-type and null bands was quantitated using a phosphorimager (Cyclone; Packard Bioscience, Boston MA) and the OptiQuant software package. The ratio of wild-type:null band hybridization values was calculated. Loss of the wild-type allele was defined as wild-type:null <0.5. Statistical significance for the frequency of LOH was determined by Fisher's exact test.

Genotyping at SSLP Loci. The normal and tumor-derived DNA samples from F1- and N2-study mice were genotyped using fluorescently labeled PCR primers that amplify five SSLP markers on chromosome 11 (Applied Biosystems, Foster City, CA and Research Genetics, Huntsville, AL). Reaction volumes of 7.5 μl were used containing 1.5 μl of sample DNA at 20 ng/μl, 1.5 μl of locus specific primer mix at 4 μM concentration each, 4.5 μl of TrueAllele PCR premix (Applied Biosystems). Amplifications were performed on a tetrad thermocycler (MJ Research, Waltham, MA) with an initial melt at 95°C for 12 min, followed by 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 1 min, then a final hold of 72°C for 7 min. All amplifications included positive control DNA from the parental inbred strains as well as a negative control where sterile water was substituted for the template DNA. Diluted PCR product (1.5 μl) was combined with a mixture containing 1 μl of deionized formamide, 0.5 μl of loading buffer (50 mg/ml blue dextran, 25 mM EDTA), 0.5 μl commercial size standard Genescan500 or Genescan400HD (Applied Biosystems), heated at 95°C for 5 min, and cooled on ice. Then 1.5 μl of the mixture was loaded in a 5% denaturing polyacrylamide gel and electrophoresed for 2.5 h on an ABI PRISM 377 DNA Analyzer to determine the sizes of the PCR products. GeneScan version 3.1 was used to analyze the raw data, to identify and to determine the size of each DNA fragment on the gel. Genotyper version 2.5 was used for analysis of the experimental fragments at each locus to assign the genotypes (Applied Biosystems). The areas of the allele peaks were determined and the ratio calculated. LOH was defined as called when the ratio of peak areas was ≥2-fold the value obtained for corresponding heterozygous normal tail DNA.

Comparative Genomic Hybridization. CGH was performed using 3 megabase resolution genomic DNA microarray slides, the Mouse SpectralChip Microarray, from Spectral Genomics (Houston, TX). Mouse tumor DNA and reference tail genomic DNA were digested with *Eco*R1 for 16 h at 37°C and repurified by Clean and Concentrator (Zymo Research, Orange, CA). The tail and tumor DNAs were labeled with Cy3 and Cy5 by Invitrogen's BioPrime random labeling kit, making the majority of the probe between 100–500 bp in size. The Cy3-labeled reference DNA and Cy5-labeled test DNA samples were combined with 50 μg of blocking DNA for repeat sequences. This mix was precipitated with ethanol, rinsed in 70% ethanol and air-dried. The same procedure was repeated with the Cy5-labeled tail and Cy3-labeled tumor DNAs. The pellets were dissolved in 10 μl of distilled water and mixed with 30 μg of hybridization solution (50% formamide, 10% dextran sulfate in 2 × SSC). The labeled DNAs were denatured at 72°C for 10 min followed by incubation at 37°C for 30 min to block repetitive sequences. Oppositely labeled DNA mixes (Cy3-labeled test and Cy5-labeled reference DNA, Cy3-labeled reference and Cy5-labeled test DNA) were added onto duplicate microarray slides. Hybridization as per the Spectral Genomics protocol was overnight at 37°C. Slides were washed at room temperature 2 × SSC for 3–5 min, then washed at 50°C for 20 min with shaking in 50% formamide/2 × SSC. The wash step was repeated with prewarmed (50°C) 0.1%NP40/2 × SSC for 20 min and with 0.2 × SSC for 10 min at 5°C. The microarrays were briefly rinsed with distilled water at room temperature for 3–5 s and immediately centrifuged for 3 min at 500 × g for drying. Hybridized microarray slides were scanned with GenePix 4000B scanner (Axon Ins. Inc., Union City, CA), and the data obtained were analyzed using the Spectralware 1.0 software (Spectral Genomics). SpectralWare was used to normalize the Cy5:Cy3 intensity ratios for each slide such that the summed Cy5 signal equals the summed Cy3 signal. The normalized Cy5:Cy3 intensity ratios were computed for each of the two slides and plotted together for each chromosome. Gains in DNA copy number at a particular locus are observed as the simultaneous deviation of the ratio plots from a modal value of 1, with the blue ratio plot showing a positive deviation (upward) whereas the red ratio plot shows a negative deviation at the same locus (downward). Conversely, DNA copy number losses show the opposite pattern.

Karyotypic Analysis of Mammary Tumors. Primary mammary epithelial cell cultures were prepared from mammary tumors arising in BALB/c-*Trp53*$^{+/-}$ mice. Tumor tissue was minced finely with razor blades and digested in mammary epithelial cell media [consisting of DMEM/F12 (Sigma) plus 25 mM HEPES, 1.2g/L NaHCO, 10 μg/ml insulin, 5 ng/ml EGF] supplemented with 2% adult bovine serum and 0.4% collagenase type III (Life Technologies, Inc.) at 37°C for 3–4 h with gentle agitation. The digested cell suspension was then washed several times in PBS, and the finer clumps of cells were plated in flasks with mammary epithelial cell plus 2% adult bovine serum and grown 1–2 nights until a monolayer was formed. Cells were then grown in mammary epithelial cell media plus 5% fetal bovine serum overnight followed by treatment with Colcemid (Life Technologies, Inc.) overnight at 30 ng/ml or 3–5 h at 100 ng/ml. After Colcemid treatment, cells were harvested with trypsin, lysed with 0.068 M KCl hypotonic solution and the nuclei fixed with methanol:acetic acid (3:1). Chromosome spreads were prepared and stained with Giemsa for G-banding. At least 20 cells were scored for each tumor and at least 90 cells for wild-type and *Trp53*$^{+/-}$ control cultures derived from 5 individual 1-year-old mice of each genotype.

Cultures were also grown in chamber wells and checked for epithelial cell content by performing immunohistochemistry for cytokeratin. This confirmed that over 95% of cells in these cultures were epithelial cells.

RESULTS

Tumor Spectrum. The tumor free survival and the spectrum of tumors occurring in the three *Trp53*$^{+/-}$ mouse populations used in this study have been analyzed in detail previously (14). In brief, mammary tumors were the most common tumor type observed in BALB/c-, [(C57BL/6 × BALB/c) × BALB/c] N2-, and (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ mice, although the frequency decreased and latency increased with decreasing BALB/c genetic component, indicating that both dominant and recessive alleles were contributing to the BALB/c mammary tumor susceptibility. The age of overall tumor free survival increased with decreasing BALB/c background. The remainder of the tumor spectrum observed in BALB/c-, F1-, and N2-*Trp53*$^{+/-}$ study mice included the tumor types most commonly reported in *Trp53*$^{+/-}$ mice on other genetic backgrounds, including lymphoma and osteosarcoma. Adrenal gland tumors were also observed as a major tumor type in the N2-*Trp53*$^{+/-}$ mice, a tumor type restricted to BALB/c background (14).

LOH for *Trp53* in Tumors. In the initial report of BALB/c-*Trp53*$^{+/-}$ mammary tumors, 7 of 7 mammary tumors examined showed LOH for *Trp53* wild-type allele (13). To confirm this result, 25 additional mammary tumors from virgin BALB/c-*Trp53*$^{+/-}$ mice were analyzed by Southern blotting for LOH at the *Trp53* locus (Fig. 1). It was found that 22 of 25 showed >50% loss of the wild-type allele and 2 tumors (V05 and V15) showed 35% and 45% loss of wild-type signal, respectively. Histologically, V05 and V15 contained more stromal tissue, which may account for the presence of more wild-type allele in the sample, with one being a papillary ductal hyperplasia and the other being a solid adenocarcinoma but with a significant fibrous stromal component. Interestingly, the remaining tumor (V15) showed complete loss of the null allele, indicating that genetic changes had occurred but not with the usual outcome. Thus loss of the wild-type allele was detected in 24 of 25 (96%) mammary tumors by Southern blotting.

This high frequency of LOH contrasts with previous reports of 50–70% of spontaneous tumors from *Trp53*$^{+\backslash-}$ mice showing LOH for *Trp53* (11, 15, 18, 19). To determine whether this high frequency of LOH was particular to mammary tumors, other tumor types arising in BALB/c-*Trp53*$^{+/-}$ mice were examined for LOH. Lymphomas, sarcomas, and adrenal gland tumors collected from female virgin and breeder BALB/c-*Trp53*$^{+/-}$ mice showed a high frequency of LOH (22 of 26 tumors) that was similar to the mammary tumors ($P = 0.35$; Table 1). Thus the high frequency of LOH is not restricted to mammary tumors.

There are two differences between the mice used in this study and the previous studies reporting lower frequencies of LOH in tumors.

Table 1 *LOH*[a] *for* Trp53 *locus in tumors from* Trp53$^{+/-}$ *mice*

Tumor category	LOH		
	n	%	*P* value
BALB/c, female			
Total BALB/c female	46/51	90	
Mammary	24/25	96	
Nonmammary	22/26	85	0.350[b]
Lymphoma	13/14		
Other	9/12		
129/Sv or (C57BL/6 × 129/Sv)			
Nonmammary	24/56	43	
Female	19/32	59	0.046,[c] 0.002[d]
Male	5/24	21	0.006[e]
(C57BL/6 × BALB/c)-F1, female			
Total [C57BL/6 × BALB/c], female	13/14	93	0.035[e]
Mammary	5/6	83	
Nonmammary	8/8	100	
[(C57BL/6 × BALB/c) × BALB/c]-N2, female			
Mammary			
Young (mean 36.7 wk)	10/12	83	
Old (mean 69.4 wk)	14/14	100	0.203[f]
Total mammary	53/57	93	

[a] LOH, loss of heterozygosity.
[b] *P* values are for comparison with BALB/c mammary.
[c] *P* values are for comparison with BALB/c non-mammary.
[d] *P* values are for comparison with BALB/c female total.
[e] *P* values are for comparison with 129/Sv or (C57BL/6 × 129/Sv) non-mammary female.
[f] *P* values are for comparison with Young.

The previous studies used mice of 129/Sv or mixed (C57BL/6 × 129/Sv) background and examined both males and females, whereas this study used all BALB/c female mice; therefore, both strain and gender could be contributing to the difference in LOH frequency. To determine whether gender affects the frequency of LOH, tumors analyzed previously for LOH (15) were segregated according to gender, and the frequency of LOH was calculated. Tumors from females showed a significantly higher frequency of LOH compared with males, with 59% of tumors from females showing LOH compared with only 21% of male tumors ($P = 0.006$). The frequency of LOH in the female (C57BL/6 × 129/Sv) tumors was still significantly lower than in female BALB/c tumors ($P = 0.046$; Table 1). Thus, both a strain effect and a gender effect contribute to the high frequency of LOH in female BALB/c-*Trp53*$^{+/-}$ tumors.

Loss of the wild-type allele of *Trp53* has been suggested to accelerate tumor formation (15). To determine whether the rate of tumorigenesis correlated with the frequency of LOH, the survival of male and female *Trp53*$^{+/-}$ mice of mixed [C57BL/6 × 129/Sv] background was analyzed (Fig. 2). Female mice were found to have a significantly shorter survival time than their male counterparts, with median survival times of 70 and 80 weeks respectively ($P < 0.0001$).

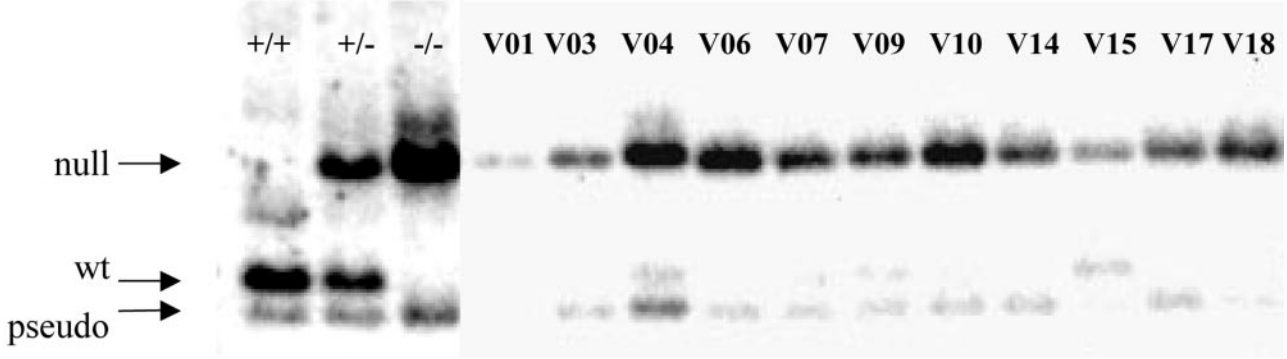

Fig. 1. Southern blotting of BALB/c-*Trp53*$^{+/-}$ mammary tumor DNA for the *Trp53* locus. *Lanes 1–3*, control tail DNA, *Lanes 4–14*, tumor DNA. The majority of tumors show almost complete loss of signal from the wild-type allele. The ratio wild-type:null band for V15 was >0.5, indicating retention of the wild-type allele. A p53 pseudogene also weakly hybridizes with the probe (*pseudo*). *Wt*, wild-type.

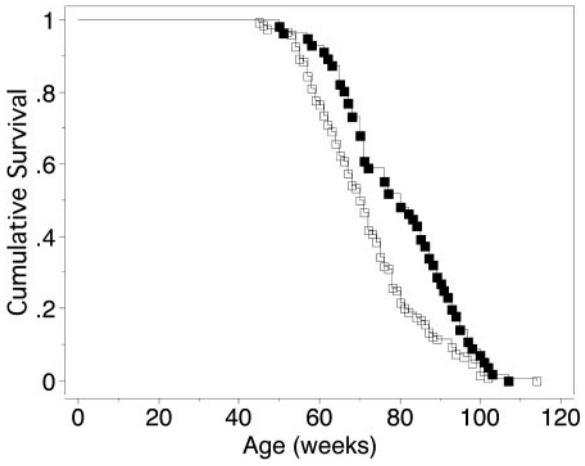

Fig. 2. Kaplan-Meier survival curves for male (■; $n = 113$) and female (□; $n = 96$) *Trp53*$^{+/-}$ mice of mixed (C57BL/6 × 129/Sv) background.

This difference in survival was not accounted for by gender-specific tumors because there were essentially no mammary, ovarian, or uterine cancers in the *Trp53*$^{+/-}$ females included in this analysis. Wild-type females did not show statistically significant differences in survival compared with wild-type males, albeit the numbers of wild-type mice analyzed were low (data not shown).

To determine whether the genetic factors leading to LOH in BALB/c-*Trp53*$^{+/-}$ mice were dominant or recessive, tumors arising in the (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ mice were analyzed for LOH. With the exception of the benign sclerosing adenosis of the mammary gland, all tumors examined showed loss of the wild-type allele (Table 1) giving a frequency of 93%. This was not different from the frequency for all BALB/c-*Trp53*$^{+/-}$ tumors (90%) and was significantly different from the (C57BL/6 × 129/Sv) female frequency (59%, $P = 0.035$) indicating that it was a dominant genetic trait.

To determine whether the frequency of retention of the wild-type allele increased with age, mammary tumors from [(C57BL/6 × BALB/c) × BALB/c] N2-*Trp53*$^{+/-}$ mice (acquired previously in an experiment genetically mapping recessive factors contributing to mammary tumor susceptibility; Ref. 14), were examined. Because of the size of this study population and the mixed genetic background of mice, considerable numbers of mammary tumors from older mice were able to be collected. LOH was analyzed in the earliest 12 (21.4–46.9, mean latency 36.7 weeks) and the latest 14 (66.9–77.6, mean latency 69.4 weeks) occurring mammary tumors. Interestingly, two of the early-onset tumors (21.4 and 40 weeks) retained the wild-type allele, whereas all other mammary tumors, old and young, had lost the wild-type allele (Table 1).

Karyotype Analysis and Comparative Genomic Hybridization of Mammary Tumors. The occurrence of aneuploidy was studied in five mammary tumors arising in BALB/c-*Trp53*$^{+/-}$ mice by karyotyping using short-term culture methods and by CGH microarray analysis.

Karyotype analysis revealed significant genetic instability in each of the tumors with the proportion of diploid cells ranging from 25–70% in the tumors (Table 2) compared with >90% diploid or tetraploid in normal mammary epithelial cells from one-year-old *Trp53*$^{+/-}$ or wild-type females. The remainder of cells in the tumors were either hypodiploid or near-tetraploid. Loss of one copy of chromosome 11 was observed in the hypodiploid population of each tumor (Fig. 3). In contrast, loss of one entire copy of chromosome 11 was detected in only three of five tumors when analyzed by CGH (tumors MTuV04, MTuV14, MTuV17; Table 2). These results are, however, consistent with the karyotype of the dominant cell population within the metaphase samples. Thus, MTuV02, containing 50% diploid cells shows no loss on chromosome 11 by CGH, whereas MTuV14 containing 65% aneuploid cells shows loss of the entire chromosome 11 by CGH (Table 2; Fig. 3). This CGH trace is typical of those obtained from the other tumors. The small difference in the normal and tumor signal in the CGH results, contributed to by the diploid population of cells present in the tumor, is further diluted by the presence of variable numbers of chromosome 11 in the near-tetraploid cell population. Of note, CGH analysis of these tumors rarely found losses or gains at particular clones of the array, but rather, it found loss across the entire chromosome 11 or no loss at all. Thus, deletion of portions of chromosome 11 is not a common feature of these tumors.

Marker chromosomes bearing translocations (Fig. 3) and/or expanded regions of heterogeneously staining segments were also observed in the hypodiploid cells and in the polyploid cells. Therefore, the hypodiploid population appears to be the progenitor of the polyploid population of cells. In other tumors, the polyploid cells also show evidence of further genomic instability with double minutes present that are characteristic of amplified segments of the genome.

In contrast to both the karyotype and CGH results, Southern blotting indicated the unambiguous loss of one allele of *Trp53* in 4 of 5 of these tumors, including MTuV14 (Table 2, Fig. 1). This was especially the case in V02, where the population of hypodiploid cells was only 25% of the total, loss along chromosome 11 was not detectable by CGH (Fig. 3), and yet >90% loss of one *Trp53* allele was detected by Southern blot hybridization (Table 2). This is indicative of LOH occurring by a mechanism other than chromosomal loss and before the development of aneuploidy.

LOH at SSLP Markers. The F1 and N2 mice are heterozygous for C57BL/6 and BALB/c polymorphic markers throughout the genome, allowing more extensive analysis of LOH in tumors arising in these mice. Because the number of F1 mammary tumors available was small, informative N2 mammary tumors were analyzed in addition to all of the F1 tumors. SSLP markers spanning 1.1–37 cM of chromosome 11 were used to analyze 21 tumors from F1- and N2-*Trp53*$^{+/-}$ mice. The genotyping of the normal tail DNA from F1 mice was used to define the haplotype of the paternal chromosome bearing the *Trp53* null allele (Fig. 4*A*). The paternal chromosome 11 carried BALB/c alleles for all markers except D11MIT4 (located at 37 cM). This was expected because the *Trp53* null allele (located at 39 cM) was generated in embryonic stem cells from 129/Sv mice (11). The markers were all informative in the F1 mice, and therefore were used to deduce the haplotypes of tumors arising in these mice. Tumors from F1 mice

Table 2 *Karyotype of mammary tumors from BALB/c*-Trp53$^{+/-}$ *mice*

Sample	Karyotype (% cells) Diploid	Hypo-diploid	Hyper-diploid	Tetraploid[c]	CGH[a] on chr 11	Southern % LOH *Trp53*
Tumors						
MTuV02	**50**[b]	25		25	No loss or gains	>90[d]
MTuV04	31	**38**		31	Loss of entire chr 11, gain on one clone	91
MTuV14	35	**40**		25	Loss of entire chr 11	92
MTuV15	**70**	10		20	No loss or gains	45
MTuV17	25	**50**		25	Loss of entire chr 11	92
Normal						
Wild-type	73	5	4	17		
Trp53$^{+/-}$	73	2	4	19		

[a] CGH, comparative genomic hybridization; chr, chromosome; LOH, loss of heterozygosity.
[b] Numbers in bold indicate the dominant cell population.
[c] In tumors, these cells were near-tetraploid, presumably derived from the hypodiploid cells.
[d] Loss of the null allele.

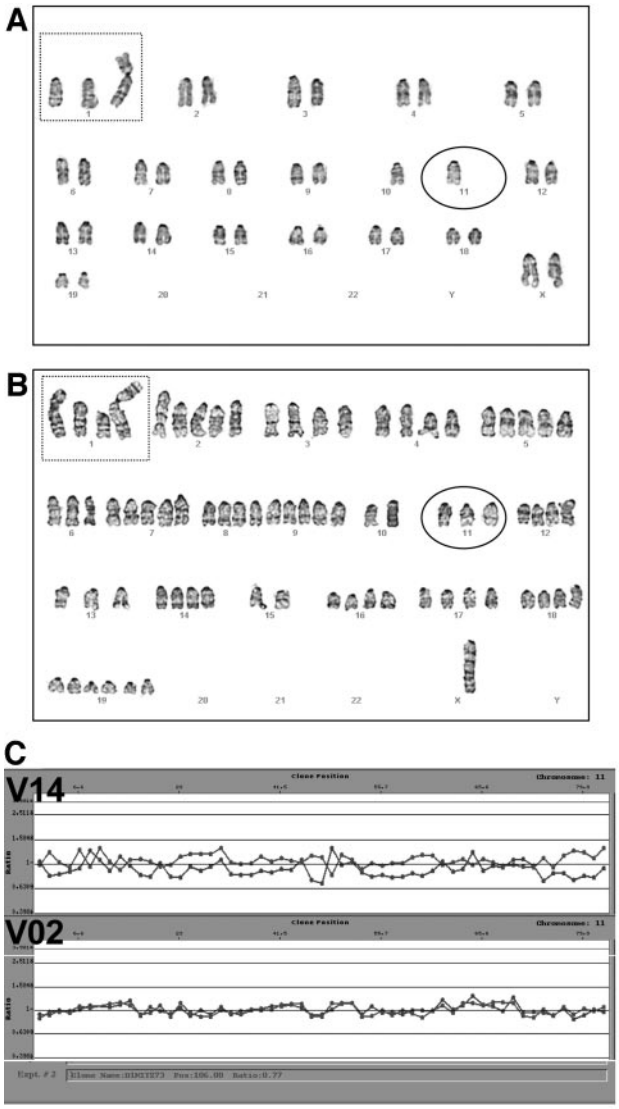

Fig. 3. Analysis of chromosome 11 by karyotyping and comparative genomic hybridization (CGH). *A*, two karyotypes from mammary tumor V02. The hypodiploid cell has only one copy of chromosome 11 (*circle*); however, this was not the dominant population (Table 2). A translocation of chromosome 10 to chromosome 1 (t1:10) has occurred (*box*) providing a "marker chromosome" in the karyotype. *B*, the polyploid cell is near tetraploid. The presence of the t1:10 marker chromosome suggests that the polyploid cells are derived from the hypodiploid population. *C*, CGH ratio plots from mammary tumors showing loss (V14) or no loss (V02) of chromosome 11.

included five mammary adenocarcinomas, one benign sclerosing adenosis of the mammary gland and seven tumors from other tissues. An initial screen of normal tail DNA from 13 N2 mice bearing mammary tumors was performed and the eight mice bearing the most informative polymorphisms were selected for further analysis. LOH and haplotypes of N2 tumor alleles were determined by comparison with normal tail DNA from the same mouse.

Examination of five SSLP markers allowed the tumors to be classified into four groups, as shown in Fig. 4, *B–E*: (*B*) retention of heterozygosity (no LOH) at all markers; (*C*) LOH at all markers with the paternal alleles being retained; (*D*) LOH at all markers with a mixture of maternal and paternal alleles being retained; and (*E*) LOH at some markers and a mixture of maternal and paternal alleles being retained. These classifications are based on the markers analyzed that are assumed to be indicative of the rest of the chromosome. Two tumors were found to have retained heterozygosity at all markers (Fig. 4*B*), consistent with the Southern blotting results for the *Trp53* locus. Where LOH was present by Southern blotting, SSLP analysis demonstrated that LOH was not restricted to the *Trp53* locus but was present at many of the SSLP loci examined in the tumors (Fig. 4, *C–E*). In the majority of tumors that showed LOH at *Trp53*, one or more adjacent loci also exhibited LOH. Thus, LOH around the *Trp53* locus spanned at least 2–22 cM, indicating that small deletions involving just the *Trp53* locus are an unlikely mechanism of loss of the wild-type allele of *Trp53*. This is consistent with the absence of partial chromosome losses in the CGH analysis.

Loss of the wild-type *Trp53* allele by missegregation and chromosome loss would be expected to be accompanied by loss of all of the maternal C57BL/6 alleles, with retention of the paternal BALB/c and 129/Sv alleles of chromosome 11. The allele pattern present in tumors 264Adr, 267Adr, and 268Lym (Fig. 4*C*) is consistent with this process. In mammary tumors 192, 252, 257, and 266, LOH was observed at all loci examined, suggesting that one copy of chromosome 11 was lost from these tumors (Fig. 4*D*). However, a combination of maternal and paternal alleles was retained, indicating that mitotic recombination had occurred during tumorigenesis before loss of one copy of chromosome 11. In the majority (12 of 21) of tumors, a fourth pattern was detected in which LOH was observed at some loci with retention of heterozygosity at other loci (Fig. 4*E*). Where LOH was present, a mixture of maternal and paternal alleles were retained, intermingled with retention of heterozygosity at other loci. These results are most consistent with the occurrence of multiple recombination events in cells retaining two copies of chromosome 11.

Of note, the three tumors that had undergone LOH without recombination (264Adr, 267Adr and 268Lym) were not of mammary origin (Fig. 4*C*). Thus, the number of syntenic regions of homozygosity or heterozygosity detected per tumor, indicative of recombination break points, was compared between mammary tumors ($n = 13$, excluding the benign sclerosing adenosis 259Msa) and other tumor types ($n = 7$). Mammary tumors had 2.92 ± 0.86 regions per tumor compared with 1.86 ± 0.90 regions per tumor in all other tumors ($P = 0.02$), suggesting that recombination is a more common event in mammary tumors than in other tumor types.

DISCUSSION

The initial report on mammary tumors in BALB/c-*Trp53*$^{+/-}$ mice found a high frequency (seven of seven) of LOH for the wild-type *Trp53* allele (13). The current report confirms and expands that finding with the analysis of 57 more spontaneous mammary tumors from both BALB/c and mixed (C57BL/6 × BALB/c) genetic backgrounds using Southern blotting, karyotyping, CGH, and SSLP analysis to examine the mechanism of LOH in these mammary tumors. Southern blotting demonstrated that a high degree of LOH for *Trp53* was found in 93% of mammary tumors (Fig. 1; Table 1). Karyotype analysis indicated that cells lacking one copy of chromosome 11 were present in all five mammary tumors analyzed but were not always the dominant population (Table 2), suggesting that loss of chromosome 11 was not an early event in tumorigenesis and could not account for the high degree of LOH observed by Southern blotting. CGH analysis indicated either loss or retention of the entire chromosome 11, thus eliminating deletions within chromosome 11 as a mechanism of LOH (Fig. 3). SSLP analysis showed that LOH occurred over multiple loci, and that a combination of maternal and paternal alleles were retained, indicating that MR is the most likely mechanism of LOH (Fig. 4). Thus we propose a model for the genetic progression of spontaneous *Trp53*$^{+\backslash-}$ mammary adenocarcinomas whereby loss of the wild-type allele of *Trp53* occurs as a result of MR, which may be followed by missegregation and aneuploidy promoted by the absence of p53.

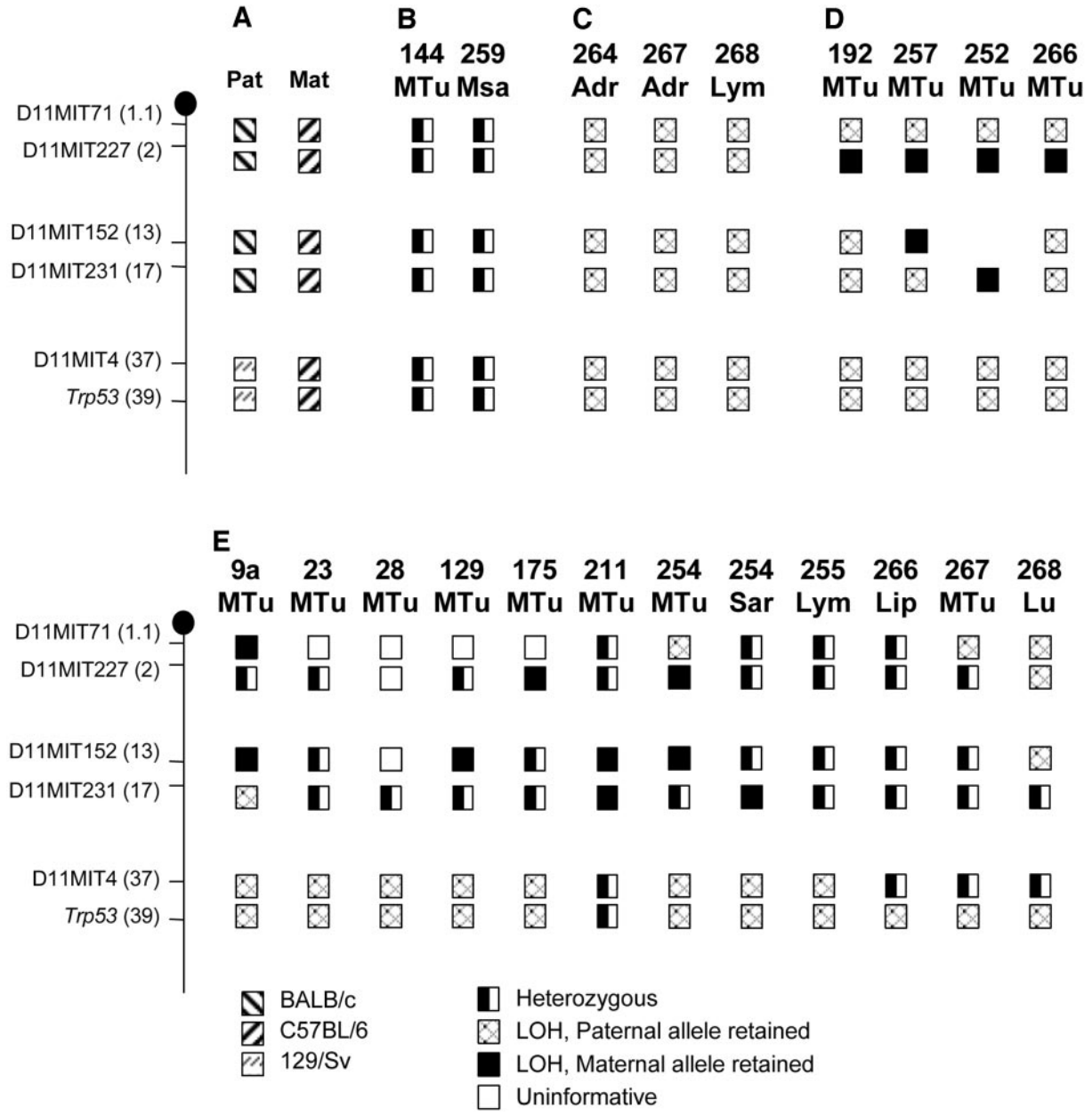

Fig. 4. Patterns of loss of heterozygosity (LOH) on chromosome 11 in spontaneous mammary tumors. *A*, haplotypes of the paternal (*Pat*) *Trp53* null bearing chromosome and maternal (*Mat*) C57BL/6 chromosome of F1 mice are depicted. On the basis of these haplotypes, the parental origin of alleles present in tumors of N2 (9–211) and F1 (252–268) mice was deduced. *B*, tumors showing no LOH and no chromosome loss. *C*, tumors showing LOH at all loci and retention of the paternal haplotype indicating loss of the maternal chromosome. *D*, tumors showing LOH at all loci with retention of some maternal alleles, indicating mitotic recombination occurred followed by chromosome loss. *E*, tumors showing LOH at some loci and retention of a mixture of maternal and paternal alleles. *Adr*, adrenal gland tumor; *Lip*, liposarcoma; *Lu*, lung tumor; *Lym*, lymphoma; *Msa*, benign mammary sclerosing adenosis; *MTu*, mammary tumor; *Sar*, sarcoma.

Mitotic recombination is thought to be responsible for the majority of LOH that occurs spontaneously in normal somatic cells. This is indicated by studies on LOH for the HLA locus in cultured human lymphocytes (20), on the pink-eyed unstable locus in mice (21) and on the *Aprt* locus in human lymphocytes and mouse fibroblasts (22, 23). The gender bias observed in LOH frequency in *Trp53*$^{+/-}$ tumors (59% in females *versus* 21% in males, Table 1) is consistent with LOH occurring by MR as it has been shown in human lymphocytes that MR rates are 2.5-fold higher in females compared with males (20). Numerous studies have reported that wild-type p53 suppresses homologous recombination, measured as intrachromosomal recombination in integrated plasmid substrates with short tracts of homology (24, 25). In this context, repression of recombination may occur independently of other p53 functions such as transcriptional transactivation and cell cycle control (26–28) and *Trp53*$^{+/-}$ mouse fibroblasts had 3-fold the frequency of homologous recombination compared with wild-type cells, indicating a moderate haploinsufficiency for suppression of homologous recombination (28). However, repression of recombination is much more modest, if detectable, in the context of endogenous loci and interchromosomal recombination events (21, 29). Even without elevated rates of recombination, the background rate of recombination in *Trp53*$^{+/-}$ tissues may be amplified because of haploinsufficiency for p53 transcriptional activation, cell cycle arrest and apoptosis (30–32), allowing the accumulation of more recombination events over time compared with wild-type cells. Mitotic recombination frequencies are inhibited by a high degree of chromosomal divergence as exists between some mouse strains (33);

however BALB/c × C57BL/6 hybrid cells were not affected in this manner.

Once the wild-type allele of *Trp53* has been lost, general genomic instability and aneuploidy will occur as is characteristic of p53-deficient tumors (34). Chromosomal missegregation without reduplication could leave the cells with only one copy of chromosome 11 and lead to deficiency in other tumor suppressor genes such as *Brca1* which lies at 60 cM on mouse chromosome 11. However, the inconsistent demonstration of loss of chromosome 11 by CGH in the mammary tumors argues against the existence of a strong selective pressure for cells possessing only one copy of chromosome 11. This is further supported by Southern blotting analysis, which indicated no significant loss of alleles at the *Brca1* locus in BALB/c-*Trp53*$^{+/-}$ mammary tumors (13). These results support the proposed model in which LOH for *Trp53* by MR occurs as an earlier and more significant event in mammary tumorigenesis than loss of chromosome 11 in this mouse model.

A comparison of the LOH results from this study with what is known in LFS patients is favorable for this mouse model being relevant to the human setting. In a detailed study of LOH for *TP53* in LFS tumors, Varley *et al.* (16) found LOH occurring in 44% of tumors. Examination of allelic imbalance along chromosome 17 by microsatellite analysis produced similar findings to the mouse tumors of this study. Although some tumors showed LOH or allelic imbalance at all loci, consistent with loss of an entire chromosome, the majority of tumors showed LOH for only part of the chromosome. Varley *et al.* note that the relative frequency of this pattern of LOH is higher in their LFS tumors than that reported for other tumor types, such as retinoblastoma. This may be because of haploinsufficiency for suppression of MR by p53 in the heterozygous LFS patients. Interestingly, when LOH frequency in LFS breast cancers alone was considered, 11 of 14 or 79% (seven of eight in the Varley *et al.* study and four of six in other published studies; Ref. 16) of tumors show LOH. Thus, LOH for *TP53* occurs at a much higher frequency in breast cancer than in other tumor types. The effect of gender on LOH in LFS cannot be assessed from these reports because the published genders of these LFS patients is incomplete. However, female carriers of p53 mutations have been shown to have a consistently higher cancer risk compared with male carriers (35), even after the exclusion of cases of breast, ovarian, and prostate cancer. This aspect of LFS is recapitulated in the *Trp53*$^{+/-}$ mouse model (Fig. 2) and correlates with the higher frequency of LOH in female mice (Table 1). Hwang *et al.* (35) do not speculate on the mechanism for the sex difference in cancer risk, but it is tempting to suggest that a higher frequency of LOH of *TP53* occurring in female patients contributes to earlier onset cancers than in males, although the influence of estrogen as a tumor promotor generally cannot be excluded.

Analysis of nonmammary tumors arising in BALB/c-*Trp53*$^{+\backslash-}$ mice demonstrated that LOH occurred at a similar high frequency to mammary tumors (Table 1). Thus, rather than being restricted to mammary tumors, the high frequency of LOH is characteristic of the BALB/c strain. One potential contributor to this trait is the hypomorphic BALB/c allele of *Prkdc* (36), the gene encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PK). DNA-PK has been reported to suppress induced and spontaneous homologous recombination (37). DNA-PK is also an essential component of the NHEJ pathway for DNA double-strand break repair (38). A deficiency in NHEJ may increase the proportion of double-strand break repaired by alternate pathways such as recombination. BALB/c mice have been found to have two missense mutations in *Prkdc*, resulting in decreased protein levels, DNA-PK activity and double-strand break joining activity compared with most other mouse strains (36, 39, 40). This deficiency in DNA-PK function may promote MR, contributing to the higher frequency of LOH via MR for *Trp53* observed in the BALB/c strain.

The finding of a comparable frequency of LOH in the F1-*Trp53*$^{+\backslash-}$ tumors indicates that the elevated frequency of LOH in the BALB/c strain is a dominant genetic trait. We have reported previously that the occurrence of BALB/c-*Trp53*$^{+/-}$ mammary tumors also has a dominant genetic component, with 32% of female (C57BL/6 × BALB/c) F1-*Trp53*$^{+/-}$ developing mammary tumors compared with <1% in published reports for C57BL/6 mice (14). Thus, the high frequency of tumor LOH for *Trp53* associates with the occurrence of mammary tumors. Although LOH for *Trp53* occurred equally among tumor types, the number of recombination events that occurred in the tumors was not equal. SSLP analysis of paternal and maternal alleles retained within the tumors indicated that recombination events may be more common in mammary tumors than in other tumor types. This would be consistent with the higher frequency of LOH for p53 in LFS breast cancer compared with other tumor types. Tumor-specific LOH may be the result of several different mechanisms, discussed in Monteiro (41), including tissue-specific differences in recombination rates. Studies on MR in mouse fibroblasts and T-lymphocytes support the notion that MR is regulated in a tissue-specific manner (33, 42). If MR determines the rate of LOH in spontaneous tumors of this model, and mammary tumors undergo higher rates of MR than other tissues, then a further increase because of strain differences in the MR rate may greatly amplify the LOH rate in the mammary gland over other tissues, thereby specifically accelerating mammary tumor development. Thus, we hypothesize that elevated MR rates may contribute to the high susceptibility of BALB/c-*Trp53*$^{+/-}$ mice to mammary tumors compared with other strains.

If reduced DNA-PK activity is contributing to a higher frequency of LOH, this may have a particularly strong impact on the mammary gland because DNA-PK activity is already low in normal mouse (39) and human (43) mammary glands compared with other tissues. *Prkdc* has been suggested as the gene responsible for BALB/c susceptibility to radiation-induced mammary tumors (39). We have demonstrated clearly that the *Prkdc* allele is not a major recessive locus contributing to spontaneous mammary tumor susceptibility in BALB/c-*Trp53*$^{+/-}$ mice (14), but its dominant actions have not been characterized. In humans, a study has recently been published examining breast cancer risk and polymorphisms in five NHEJ genes, *Ku70*, *Ku80*, *DNA-PKcs*, *Ligase IV*, and *XRCC4* (44). In a multigenic analysis, increased risk of developing breast cancer was found in women harboring a greater number of putative high-risk alleles of NHEJ genes, which was stronger and more significant in women thought to have a greater exposure to estrogen (*i.e.*, no full-term pregnancies; Ref. 44). This is consistent with our hypothesis for BALB/c mice, which stated that elevated MR and decreased DNA-PK activity contribute to mammary tumor susceptibility. The shortcoming of this population study is the lack of functional information for the single nucleotide polymorphism alleles examined. It would be valuable to test this hypothesis with polymorphisms of known functional consequence, either in mouse models or in population studies, to confirm the mechanism responsible for the risk association identified in Fu *et al.* (44) and in our study.

Other genes and pathways are potentially involved in the dominant mammary tumor phenotype observed in these mice. However, the finding that recombination may be responsible for loss of the wild-type allele of *Trp53* in spontaneous mammary tumors in *Trp53*$^{+/-}$ mice implicates the recombination machinery in the tumorigenic process of the BALB/c mouse mammary gland. Both *BRCA1* and *BRCA2* have been shown to be involved in repair of double-strand break particularly via recombination (38, 45). Additional studies are required to determine the underlying reason for the high frequency of

LOH and recombination observed in these tumors and the relationship to the breast cancer susceptibility of the BALB/c strain.

ACKNOWLEDGMENTS

We thank Ellen Dickinson and Amy Roberts for technical assistance.

REFERENCES

1. Tischfield JA. Loss of heterozygosity or: how I learned to stop worrying and love mitotic recombination. Am J Hum Genet 1997;61:995–9.
2. Devilee P, Cleton-Jansen AM, Cornelisse CJ. Ever since Knudson. Trends Genet 2001;17:569–73.
3. Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the p53 gene occur in diverse human tumour types. Nature (Lond) 1989;342:705–8.
4. Varley JM, Brammar WJ, Lane DP, et al. Loss of chromosome 17p13 sequences and mutation of p53 in human breast carcinomas. Oncogene 1991;6:413–21.
5. Coles C, Condie A, Chetty U, et al. p53 mutations in breast cancer. Cancer Res 1992;52:5291–8.
6. Varley JM, McGown G, Thorncroft M, et al. Germ-line mutations of TP53 in Li-Fraumeni families: an extended study of 39 families. Cancer Res 1997;57:3245–52.
7. Crook T, Brooks LA, Crossland S, et al. p53 mutation with frequent novel condons but not a mutator phenotype in BRCA1- and BRCA2-associated breast tumours. Oncogene 1998;17:1681–9.
8. Greenblatt MS, Chappuis PO, Bond JP, Hamel N, Foulkes WD. TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: distinctive spectrum and structural distribution. Cancer Res 2001;61:4092–7.
9. Xu X, Wagner KU, Larson D, et al. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. Nat Genet 1999;22:37–43.
10. Jonkers J, Meuwissen R, van der Gulden H, et al. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001;29:418–25.
11. Jacks T, Remington L, Williams BO, et al. Tumor spectrum analysis in p53-mutant mice. Curr Biol 1994;4:1–7.
12. Donehower LA, Harvey M, Vogel H, et al. Effects of genetic background on tumorigenesis in p53-deficient mice. Mol Carcinog 1995;14:16–22.
13. Kuperwasser C, Hurlbut GD, Kittrell FS, et al. Development of mammary tumors in BALB/c p53 heterozygous mice: a model for Li-Fraumeni Syndrome. Am J Pathol 2000;157:2151–9.
14. Blackburn AC, Brown JS, Naber SP, et al. BALB/c alleles for Prkdc and Cdkn2a interact to modify tumor susceptibility in Trp53$^{+/-}$ mice. Cancer Res 2003;63:2364–8.
15. Venkatachalam S, Shi YP, Jones SN, et al. Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. EMBO J 1998;17:4657–67.
16. Varley JM, Thorncroft M, McGown G, et al. A detailed study of loss of heterozygosity on chromosome 17 in tumours from Li-Fraumeni patients carrying a mutation to the TP53 gene. Oncogene 1997;14:865–71.
17. Jerry DJ, Kuperwasser C, Downing SR, et al. Delayed involution in the mammary epithelium in BALB/c-p53^{null} mice. Oncogene 1998;17:2305–12.
18. Harvey M, McArthur MJ, Montgomery CA Jr, et al. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. Nat Genet 1993;5:225–9.
19. Purdie CA, Harrison DJ, Peter A, et al. Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. Oncogene 1994;9:603–9.
20. Holt D, Dreimanis M, Pfeiffer M, et al. Interindividual variation in mitotic recombination. Am J Hum Genet 1999;65:1423–7.
21. Aubrecht J, Secretan MB, Bishop AJ, Schiestl RH. Involvement of p53 in X-ray induced intrachromosomal recombination in mice. Carcinogenesis (Lond) 1999;20:2229–39.
22. Gupta PK, Sahota A, Boyadjiev SA, et al. High frequency in vivo loss of heterozygosity is primarily a consequence of mitotic recombination. Cancer Res 1997;57:1188–93.
23. Shao C, Deng L, Henegariu O, et al. Mitotic recombination produces the majority of recessive fibroblast variants in heterozygous mice. Proc Natl Acad Sci USA 1999;96:9230–5.
24. Mekeel KL, Tang W, Kachnic LA, et al. Inactivation of p53 results in high rates of homologous recombination. Oncogene 1997;14:1847–57.
25. Akyuz N, Boehden GS, Susse S, et al. DNA substrate dependence of p53-mediated regulation of double-strand break repair. Mol Cell Biol 2002;22:6306–17.
26. Saintigny Y, Rouillard D, Chaput B, Soussi T, Lopez BS. Mutant p53 proteins stimulate spontaneous and radiation-induced intrachromosomal homologous recombination independently of the alteration of the transactivation activity and of the G_1 checkpoint. Oncogene 1999;18:3553–63.
27. Willers H, McCarthy EE, Wu B, et al. Dissociation of p53-mediated suppression of homologous recombination from G_1/S cell cycle checkpoint control. Oncogene 2000;19:632–9.
28. Lu X, Lozano G, Donehower LA. Activities of wildtype and mutant p53 in suppression of homologous recombination as measured by a retroviral vector system. Mutat Res 2003;522:69–83.
29. Shao C, Deng L, Henegariu O, et al. Chromosome instability contributes to loss of heterozygosity in mice lacking p53. Proc Natl Acad Sci USA 2000;97:7405–10.
30. Gottlieb E, Haffner R, King A, et al. Transgenic mouse model for studying the transcriptional activity of the p53 protein: age- and tissue-dependent changes in radiation-induced activation during embryogenesis. EMBO J 1997;16:1381–90.
31. Venkatachalam S, Tyner SD, Pickering CR, et al. Is p53 haploinsufficient for tumor suppression? Implications for the p53± mouse model in carcinogenicity testing. Toxicol Pathol 2001;29(Suppl):147–54.
32. Colombel M, Radvanyi F, Blanche M, et al. Androgen suppressed apoptosis is modified in p53 deficient mice. Oncogene 1995;10:1269–74.
33. Shao C, Stambrook PJ, Tischfield JA. Mitotic recombination is suppressed by chromosomal divergence in hybrids of distantly related mouse strains. Nat Genet 2001;28:169–72.
34. Blackburn AC, Jerry DJ. Knock-out and transgenic mice of Trp53: what have we learned about p53 in breast cancer? Breast Cancer Res 2002;4:101–11.
35. Hwang SJ, Lozano G, Amos CI, Strong LC. Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk. Am J Hum Genet 2003;72:975–83.
36. Okayasu R, Suetomi K, Yu Y, et al. A deficiency in DNA repair and DNA-PKcs expression in the radiosensitive BALB/c mouse. Cancer Res 2000;60:4342–5.
37. Allen C, Kurimasa A, Brenneman MA, Chen DJ, Nickoloff JA. DNA-dependent protein kinase suppresses double-strand break-induced and spontaneous homologous recombination. Proc Natl Acad Sci USA 2002;99:3758–63.
38. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet 2001;27:247–54.
39. Yu Y, Okayasu R, Weil MM, et al. Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the Prkdc (DNA-dependent protein kinase catalytic subunit) gene. Cancer Res 2001;61:1820–4.
40. Mori N, Matsumoto Y, Okumoto M, Suzuki N, Yamate J. Variations in Prkdc encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and susceptibility to radiation-induced apoptosis and lymphomagenesis. Oncogene 2001;20:3609–19.
41. Monteiro AN. BRCA1: the enigma of tissue-specific tumor development. Trends Genet 2003;19:312–5.
42. Vrieling H. Mititoc maneuvers in the light. Nat Genet 2001;28:101–201.
43. Moll U, Lau R, Sypes MA, Gupta MM, Anderson CW. DNA-PK, the DNA-activated protein kinase, is differentially expressed in normal and malignant human tissues. Oncogene 1999;18:3114–26.
44. Fu YP, Yu JC, Cheng TC, et al. Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. Cancer Res 2003;63:2440–6.
45. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 2002;108:171–82.