

# Chromosome instability contributes to loss of heterozygosity in mice lacking p53

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**The p53 tumor suppressor protein participates in multiple cellular processes including cell cycle checkpoints and programmed cell death. In cell lines, loss of p53 function is associated with increased genetic instability including aneuploidy, gene amplification, and point mutation. Although similar genetic instability often accompanies the progression of malignancy in tumors, its role in tumor initiation in normal cells is not clear. To study whether or not loss of p53 leads to genetic instability in normal cells *in vivo*, we have examined mechanisms of loss of heterozygosity (LOH) at the *Aprt* (adenine phosphoribosyltransferase) and flanking loci in normal fibroblasts and T lymphocytes of p53-deficient mice. Somatic cell variants that arose *in vivo* as a consequence of genetic or epigenetic alterations abolishing *Aprt* function were selected and expanded *in vitro* by virtue of their resistance to 2,6-diaminopurine (DAP). We observed that p53 null mice produced about three times as many DAP-resistant fibroblast colonies than wild-type mice, but the frequency of DAP-resistant T lymphocyte colonies was not significantly changed. Mitotic recombination, but not point mutation, partly accounted for the increase in the frequency of DAP-resistant fibroblasts. Most significantly, chromosome loss/duplication and interstitial deletion, which were extremely rare events in the wild-type mice, represented a significant proportion of LOH events in both fibroblasts and T lymphocytes of p53 null mice. Also, increased interstitial deletion was observed in fibroblasts of p53 heterozygous mice. These data suggest that increased genetic variation, including chromosome instability, starts at the initiation stage of tumorigenesis when functional p53 is absent or reduced.**

**T**he tumor suppressor gene *TP53* plays a key role in the regulation of cell cycle progression and apoptosis (1, 2). Its action also has been implicated in nucleotide excision repair (3), base excision repair (4), and recombinational repair (5, 6). These cellular functions of p53 can presumably prevent the accumulation of cells harboring deleterious genomic alterations. Consistent with this proposition, cells in culture that lack p53 exhibit increased genomic instability, evidenced by increased aneuploidy (7–9), gene amplification (10, 11), point mutation (12), and homologous recombination (13). An elevated frequency of loss of heterozygosity (LOH) at the *TK* locus was also observed in a human lymphoblastoid cell line with mutant *TP53* (14, 15).

Mutation or loss of p53 appears to be a late event in colorectal cancer (16), but an early event in skin carcinogenesis (17). The manner by which loss of p53 function can exert a deleterious effect on the cell can range from induction of abnormal cellular proliferation, failure of apoptosis, accumulation of genetic alterations, or enhanced angiogenesis at late stages of malignant progression (18).

One approach to understanding the role of p53 mutations in the initiation of carcinogenesis is the evaluation of genetic instability in pathologically normal tissues of p53<sup>-/-</sup> mice. By using the *lacI* transgene as a reporter, two groups have characterized point mutations in *lacI* in multiple tissues of p53<sup>-/-</sup> mice. No change in either the frequency or the spectrum of point mutations was detected (19, 20), raising questions about the proposed role of p53 in the maintenance of genomic integrity.

However, the *lacI* reporter strategy used in these studies has the following shortcomings. First, the *lacI* transgene may behave differently than endogenous genes (21). Second, rodent cells are less efficient in global nucleotide excision repair than human cells (22); thus *lacI* may not be a sensitive reporter of the changes, if any, in p53<sup>-/-</sup> mice. Third, it cannot detect genetic alterations at the chromosomal level, such as chromosome loss/duplication, mitotic recombination, and large deletions. This latter shortcoming is underscored by the observation that pro-B cells and thymocytes from p53<sup>-/-</sup> *scid* mice displayed a significant degree of aneuploidy during the premalignant phase (23), indicating that certain types of genetic instability may start to accumulate at an early stage of carcinogenesis in the absence of p53.

We have used the *Aprt* (adenine phosphoribosyltransferase) gene as a reporter of *in vivo* LOH in human T cells (24) and mouse fibroblasts (25). In both cases, we were able to distinguish between multiple pathways of LOH and to demonstrate that mitotic recombination accounted for 70–80% of the *Aprt* LOH events analyzed. Chromosome loss/duplication and deletion were extremely rare.

To study whether or not the loss of p53 may affect genomic stability at both gene and chromosomal levels in normal tissues, 129 × C3H *Aprt*<sup>+/-</sup> mice were crossed into a p53-deficient background, and the frequency and mechanistic spectrum of the 2,6-diaminopurine (DAP)-resistant variants of skin fibroblasts and T lymphocytes were characterized. We observed an increased level of chromosomal abnormalities, including chromosome loss/duplication and interstitial deletion, in both cell types. Because fibroblasts and T-cells are known to exhibit an elevated predisposition to sarcomas and lymphomas, respectively, in p53-deficient mice (26, 27), increased chromosome instability likely plays a role in the initiation of carcinogenesis in these cells.

## Materials and Methods

**Mice.** p53<sup>+/-</sup> mice in either 129/Sv and N5C3H/HeOuJ background were purchased from The Jackson Laboratory (27). p53<sup>+/-</sup> mice of 129/Sv strain were crossed to 129/Sv *Aprt*<sup>+/-</sup> to generate p53<sup>+/-</sup> *Aprt*<sup>+/-</sup> double heterozygotes. The N5C3H/HeOuJ p53<sup>+/-</sup> mice were backcrossed to C3H/HeJ for three generations. The N8C3H/HeJ p53<sup>+/-</sup> mice were crossed to 129/Sv p53<sup>+/-</sup> *Aprt*<sup>+/-</sup> to generate (129 × C3H)F<sub>1</sub> hybrids that are *Aprt*<sup>+/-</sup> and p53<sup>+/+</sup>, p53<sup>+/-</sup>, or p53<sup>-/-</sup>. Some p53<sup>+/+</sup> and

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Abbreviations: LOH, loss of heterozygosity; *Aprt*, adenine phosphoribosyltransferase; DAP, 2,6-diaminopurine; p53, p53 tumor suppressor gene; CFE, colony-forming efficiency; SSR, simple sequence repeat; FISH, fluorescence *in situ* hybridization; cM, centimorgan; DSB, double strand break.

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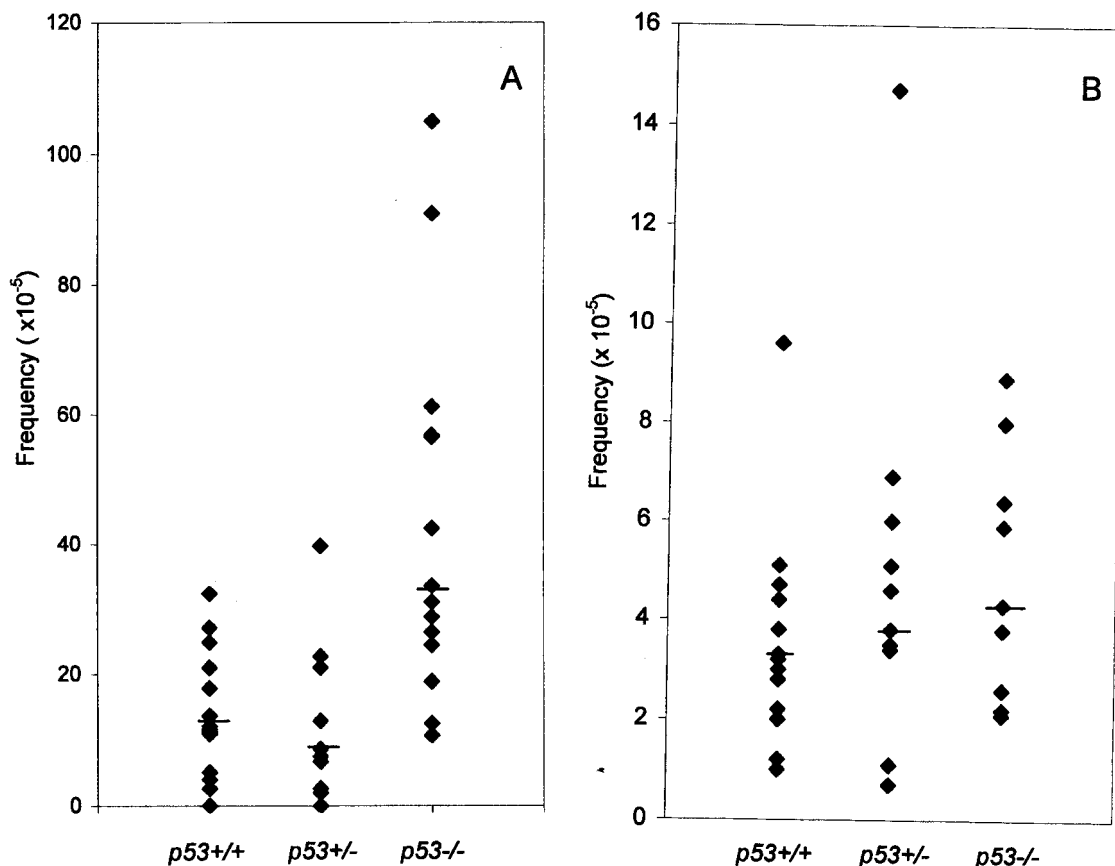


Fig. 1. Frequency of DAP<sup>+</sup> variants in *p53*<sup>+/+</sup>, *p53*<sup>+/-</sup>, and *p53*<sup>-/-</sup> mice; with fibroblasts (A), each point represents one ear; with T cells (B), each point represents one spleen. The bars indicate the median values.

*p53*<sup>+/-</sup> mice were also obtained from the crosses between 129/Sv *p53*<sup>+/-</sup> *Aprt*<sup>+/-</sup>, and C3H/HeJ mice.

**Cell Culture.** Single skin fibroblasts were prepared from each ear as described (25). They were seeded at the density of  $8 \times 10^5$  per 100-mm dish in selection medium (DAP, 50  $\mu$ g/ml). At the same time,  $1 \times 10^4$  cells were plated in drug-free medium with feeder cells to estimate colony-forming efficiency (CFE). The feeder cells were primary skin cells irradiated with 10,000 rads of gamma rays. The selection medium was changed every 4 days. The dishes for estimation of CFE, which were incubated without change of medium, were scored at day 12. At days 13–15, DAP-resistant colonies were counted and isolated from dishes containing selection medium.

The preparation and culture of spleen T lymphocytes were as reported (28). To recover DAP-resistant variants, primed lymphocytes were seeded at  $2 \times 10^4$  per well in 96-well plates containing growth medium and DAP (50  $\mu$ g/ml). Four cells were seeded per well in the presence of feeder cells ( $2 \times 10^4$  irradiated lymphocytes) to estimate CFE. Positive wells were scored at day 10.

**Molecular and Cytogenetic Studies.** Characterization of the DAP<sup>+</sup> variants is described elsewhere (25). Allele-specific PCR determined whether or not the DAP-resistant phenotype was caused by physical loss of *Aprt*<sup>+</sup>. Clones with physical loss of *Aprt*<sup>+</sup> were designated as class I and were further characterized for LOH of flanking simple sequence repeat (SSR) markers. Some class I clones were further characterized by fluorescence *in situ* hybridization (FISH) with whole chromosome 8 painting probes and an

*Aprt* gene probe (pMAprt11). Clones without physical loss of *Aprt*<sup>+</sup> were designated as class II and were assayed for APRT activity, and their *Aprt* genomic DNA was sequenced, as described previously (25).

## Results

**Comparison of CFE.** Both skin fibroblasts and T lymphocytes from *p53*<sup>-/-</sup> mice have a higher CFE than their *p53*<sup>+/+</sup> counterparts. Whereas an average CFE of  $1.6 \pm 0.1\%$  was observed for *p53*<sup>+/+</sup> skin fibroblasts, it was elevated 1.6-fold, to  $2.6 \pm 0.2\%$ , in *p53* null mice ( $P < 0.001$ ). To rule out the possibility that the increased CFE was caused by the re-seeding of migrating cells from early colonies, CFE was also estimated with 96-well flat-bottom plates. With 20 skin fibroblasts and  $1 \times 10^4$  feeder cells per well, the *p53*-deficient cells still exhibited an increased CFE, 1.5-fold greater than that of the *p53*<sup>+/+</sup> cells (data not shown). The CFE of fibroblasts from *p53*<sup>+/-</sup> mice,  $1.8 \pm 0.1\%$ , was not significantly different from that of *p53*<sup>+/+</sup> mice. T cells of *p53*<sup>-/-</sup> mice were observed to have 3-fold higher CFE than their *p53*<sup>+/+</sup> counterparts,  $4.3 \pm 0.4\%$  and  $15.1 \pm 1.3\%$ , respectively ( $P < 0.001$ ). The increased CFE we observed for skin fibroblasts and T cells was consistent with the enhanced *in vitro* proliferation reported for fibroblasts and epithelial cells of *p53*<sup>-/-</sup> mice (29).

**Frequency of DAP-Resistant Variants in *p53*<sup>-/-</sup> Mice.** After adjustment for CFE, the frequency of DAP<sup>+</sup> fibroblast variants was estimated for each ear (Fig. 1A). Each group of mice showed a wide range in the frequency of DAP<sup>+</sup> cells in individual ears, but the median frequency was about 3-fold higher in *p53*<sup>-/-</sup> than in

**Table 1. Classification of DAP-resistant variants by allele-specific PCR**

	Fibroblasts			T cells		
	<i>p53</i> <sup>+/+</sup> ( <i>n</i> = 35)	<i>p53</i> <sup>+/-</sup> ( <i>n</i> = 18)	<i>p53</i> <sup>-/-</sup> ( <i>n</i> = 19)	<i>p53</i> <sup>+/+</sup> ( <i>n</i> = 19)	<i>p53</i> <sup>+/-</sup> ( <i>n</i> = 9)	<i>p53</i> <sup>-/-</sup> ( <i>n</i> = 11)
Class I	96	50	112	73	70	79
Class II	22	19	74	23	8	14
Total no. clones	118	69	186	96	78	93
Fraction (%) of class I clones	81	72	60	76	90	85

*n*, Number of mice.

*p53*<sup>+/+</sup> mice,  $33.5 \times 10^{-5}$  and  $11.4 \times 10^{-5}$ , respectively ( $P < 0.002$ , Wilcoxon rank sum test). The frequency of DAP<sup>r</sup> fibroblast variants from *p53*<sup>+/-</sup> mice was not significantly different from that of *p53*<sup>+/+</sup> mice ( $P = 0.56$ ). Experiments using 96-well plates showed similar results (data not shown).

The frequency of DAP<sup>r</sup> T cells also varied among individual *p53*<sup>+/+</sup> mice, ranging from less than  $1 \times 10^{-5}$  to  $73 \times 10^{-5}$  in a group of 17 mice. Although the median frequency of  $4 \times 10^{-5}$  in *p53*<sup>-/-</sup> mice was slightly higher than that of the  $3 \times 10^{-5}$  median frequency observed in *p53*<sup>+/+</sup> mice, the difference was not significant ( $P = 0.64$ , Fig. 1B).

**Mitotic Recombination as the Primary Cause of LOH in *p53*<sup>+/+</sup> and *p53*<sup>-/-</sup> Mice.** As reported earlier, DAP<sup>r</sup> variants can be classified into class I or class II by simultaneous amplification of the targeted (*Aprt*<sup>neo</sup>) and nontargeted (*Aprt*<sup>+</sup>) alleles (25). Clones exhibiting physical loss of *Aprt*<sup>+</sup> (no amplification of *Aprt*<sup>+</sup>) were designated as class I, and those retaining *Aprt*<sup>+</sup> as class II. Class I clones were further genotyped with SSR markers flanking *Aprt* to determine their putative mechanistic origin, e.g., mitotic recombination, chromosome loss or interstitial deletion. As shown in Tables 1 and 2, about 80% of the DAP<sup>r</sup> fibroblast variants isolated from *p53*<sup>+/+</sup> mice were caused by mitotic recombination. Point mutation and epigenetic inactivation accounted for the remaining 20%. Chromosome loss/duplication and interstitial deletion were not observed in 88 clones.

The DAP<sup>r</sup> T cell variants recovered from *p53*<sup>+/+</sup> mice display a similar mutational spectrum as the DAP<sup>r</sup> fibroblasts (Tables 1 and 2). A majority (76%) of the DAP<sup>r</sup> T cell variants were of the class I type, and an overwhelming majority of the class I clones (98%) exhibited LOH at the most distal, but not the most centromeric, locus (Table 2), suggesting mitotic recombination for their origin. Putative interstitial deletion/gene conversion (retention of heterozygosity at both *D8Mit155* and *D8Mit56*) was detected in only one T cell clone. The mutational spectrum of the DAP<sup>r</sup> T cells we observed is consistent to that reported for 8-azaadenine-resistant T cells in *Aprt*<sup>+/-</sup> mice (30).

To determine whether or not deficiency of p53 may modulate the pathways of LOH, we characterized DAP<sup>r</sup> fibroblasts and T cells recovered from *p53*<sup>-/-</sup> mice. For fibroblasts, the majority of clones were of class I (60%). However, the fraction of class II

variants was significantly increased ( $P < 0.005$ ) in comparison to *p53*<sup>+/+</sup> mice. In contrast, for T cells, the fractions of class I and class II clones were similar to those in *p53*<sup>+/+</sup> mice (Table 1).

Genotyping with SSR markers showed that most class I variants from *p53*<sup>-/-</sup> mice exhibited LOH at all loci distal to *Aprt*, but not at loci near the centromere (Table 2), indicative of mitotic recombination. To confirm mitotic recombination as a mechanism of LOH, representative clones were analyzed by FISH with whole chromosome 8 painting probes and an *Aprt* gene probe, pMAprt11. All metaphases examined exhibited two normal copies of chromosome 8 and heteromorphic centromeres characteristic of each parental strain (25). FISH analysis with pMAprt11 DNA showed *Aprt* sequences on both copies of chromosome 8. Taking into consideration the increased overall frequency of the DAP<sup>r</sup> fibroblasts in *p53*<sup>-/-</sup> mice, the frequency of the fibroblast variants derived from mitotic recombination was calculated to be about 2-fold higher in *p53*<sup>-/-</sup> mice ( $2 \times 10^{-4}$  vs.  $1 \times 10^{-4}$ ).

We reported previously that there were more than the expected number of mitotic crossovers in the interval between 59 and 67 centimorgans (cM) in  $129 \times$  C3H hybrids of *p53*<sup>+/+</sup> mice (25). To test whether this is also true for the *p53*<sup>-/-</sup> mice, 61 class I clones isolated from *p53*<sup>-/-</sup> mice were characterized for mitotic crossover breakpoints. Although meiotic map predicts that seven clones would have a crossover in the 59- to 67-cM interval, 13 clones with a crossover in this interval were observed ( $P < 0.02$ ). This observation indicates that the distribution of mitotic crossover breakpoints was similar in *p53*<sup>+/+</sup> and *p53*<sup>-/-</sup> mice.

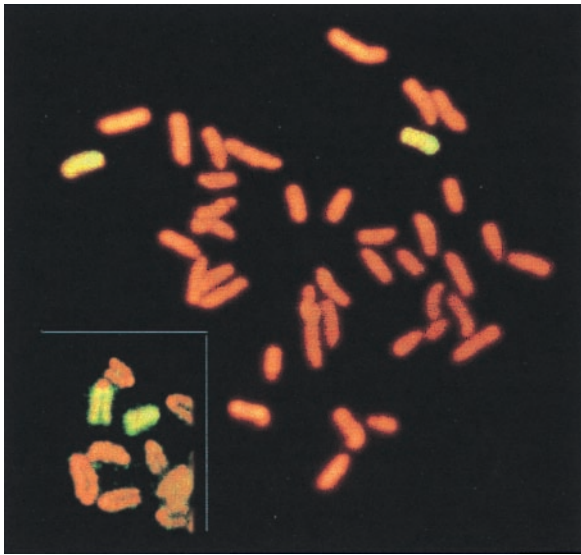
Taken together, mitotic recombination still predominates as the pathway of LOH in both fibroblasts and T cells of *p53*<sup>-/-</sup> mice. Thus, lack of p53 has minimal effect, if any, on mitotic recombination in T cells, although it may cause a modest increase in fibroblasts.

**Prevalence of Somatic Variants with Chromosomal Abnormalities in *p53*<sup>-/-</sup> Mice.** Molecular characterization of class I DAP<sup>r</sup> clones showed that two categories of clones were over-represented in *p53*<sup>-/-</sup> mice as compared with *p53*<sup>+/+</sup> controls (Table 2). First, seven fibroblast clones and two T cell clones exhibited LOH at all loci tested, including *D8Mit155*, which is 1 cM from the centromere. This particular pattern of LOH could have arisen

**Table 2. Classification of class I clones by SSR genotyping**

	Fibroblasts			T cells		
	<i>p53</i> <sup>+/+</sup> ( <i>n</i> = 35)	<i>p53</i> <sup>+/-</sup> ( <i>n</i> = 18)	<i>p53</i> <sup>-/-</sup> ( <i>n</i> = 19)	<i>p53</i> <sup>+/+</sup> ( <i>n</i> = 19)	<i>p53</i> <sup>+/-</sup> ( <i>n</i> = 8)	<i>p53</i> <sup>-/-</sup> ( <i>n</i> = 11)
A. Mitotic recombination	88	42	93	57	65	63
B. Chromosome loss with or without reduplication	0	0	7	0	0	2
C. Deletion/gene conversion	0	5	6	1	1	11
Total no. clones	88	47	106	58	66	76
Fraction (%) of B and C	0	11	12	2	2	17

*n*, Number of mice.



**Fig. 2.** Cytogenetic evidence that LOH was caused by chromosome loss followed by duplication in a class I clone. Genotyping showed loss of all C3H alleles of chromosome 8. Whole chromosome painting revealed two copies of chromosome 8 that are characteristic of the strain 129. Typical chromosome 8 of strains 129 and C3H are shown in inset, with strain C3H exhibiting a large centromeric spot.

either by chromosome loss/duplication or by a mitotic crossover between the centromere and *D8Mit155*. Whole chromosome 8 painting of one such clone, 758R2A, showed two apparently normal copies of chromosome 8 in diploid metaphase spreads. However, the characteristic heteromorphic centromeric marker of the C3H strain was absent from each chromosome 8 (Fig. 2), indicating that C3H/HeJ chromosome 8, which contributes the *Aprt*<sup>+</sup> allele, was lost and the 129/Sv parental chromosome 8 was duplicated, presumably by nondisjunction. Unless p53 deficiency enhances crossover between the centromere and *D8Mit155*, it is most likely that all these clones were derived from chromosome loss/duplication.

Second, 6 fibroblast and 11 T cell class I clones remained heterozygous at the most centromeric locus, *D8Mit155*, and at the most telomeric locus, *D8Mit56*, but exhibited LOH at a variable number of loci flanking *Aprt* (Table 3). These DAP<sup>r</sup> variants were presumably caused by interstitial deletion or gene conversion. Clones with LOH extending to at least one flanking SSR marker were probably caused by interstitial deletion. Thus, the majority of class I clones in both *p53*<sup>+/+</sup> and *p53*<sup>-/-</sup> mice are caused by mitotic recombination, whereas chromosome loss/

duplication and interstitial deletion, which are rare in *p53*<sup>+/+</sup> mice, account for more than 10% of the class I clones in *p53*<sup>-/-</sup> mice.

**Characterization of Class II Variants.** The CFE corrected frequency of DAP<sup>r</sup> T cells is not significantly higher in *p53*<sup>-/-</sup> mice than in *p53*<sup>+/+</sup> mice. However, the frequency of DAP<sup>r</sup> fibroblasts, especially class II variants, was much higher in *p53*<sup>-/-</sup> mice. To determine whether the increase was because of increased *Aprt* point mutation, all five exons, introns 1, 3, and 4, and part of the promoter region of *Aprt* of 16 clones were sequenced. Only one clone revealed a point mutation, suggesting that most of the class II variants arose by other mechanisms.

We next determined the resistance of the class II fibroblast variants to 2-fluoroadenine (FA), an adenine analog that is more toxic than DAP to cells with residual APRT activity. Although all of the class I variants can grow in FA (2 μg/ml), only 1 of 18 class II variants survived the FA, suggesting that most of the class II variants were not completely APRT-deficient.

Representative fibroblast clones of class II were assayed for their residual APRT activity. Clones recovered from *p53*<sup>-/-</sup> mice were observed to retain higher residual APRT activity than those from *p53*<sup>+/+</sup> mice. Whereas only 4 of 18 clones from *p53*<sup>+/+</sup> mice have more than 40% of the APRT activity of the parental *Aprt*<sup>+/+</sup> cells, all 9 clones from *p53*<sup>-/-</sup> mice exhibited as much APRT activity (*P* < 0.01), indicating that *p53*<sup>-/-</sup> cells with APRT activity are more resistant to the toxicity of DAP.

**Characterization of DAP-Resistant Variants from p53 Heterozygous Mice.** Mice of *p53*<sup>+/-</sup> genotype did not exhibit an increased frequency of DAP<sup>r</sup> cells compared with *p53*<sup>+/+</sup> mice. Sixty-nine DAP<sup>r</sup> fibroblast variants isolated from *p53*<sup>+/-</sup> mice were characterized by allele-specific PCR. Fifty (72%) were designated as class I, and 19 (28%) as class II. Interestingly, 5 of the 47 class I clones examined exhibited interstitial LOH. These five clones were independently derived and recovered from three different mice (Tables 2 and 3). Because none of the 88 class I fibroblast clones recovered from *p53*<sup>+/+</sup> mice exhibited interstitial LOH, the *p53*<sup>+/-</sup> mice may differ from *p53*<sup>+/+</sup> mice in the generation or surveillance of this category of variants. The fact that fibroblast clones with interstitial deletion in *p53*<sup>-/-</sup> mice are not more common than in *p53*<sup>+/-</sup> mice suggests that complete deficiency of p53 protein is not a prerequisite for the generation of interstitial deletion. Consistent with that notion is that none of the five clones exhibited LOH at the *p53* locus (data not shown). Probably a mere reduction of p53 dosage is enough for the generation of fibroblast variants with interstitial deletion. This notion is consistent with the finding that tumors from *p53* heterozygous mice frequently retain a functional, wild-type *p53* allele (31). Also, the transcriptional activity of p53 is less

**Table 3. Intervals of interstitial LOH in representative clones**

cM*	<i>D8Mit</i>	No. of clones													
		<i>p53</i> <sup>-/-</sup>										<i>p53</i> <sup>+/-</sup>			
		Fibroblasts					T cells					Fibroblasts		T cells	
1	1	1	3	1	1	3	2	4	1	2	1	1	1		
1	155	●	●	●	●	●	●	●	●	●	●	●	●	●	
59	321	○	●	●	●	●	○	●	ND	●	●	○	○	●	
67	<i>Aprt</i>	○	○	○	○	○	○	○	○	○	○	○	○	○	
67	14	○	○	○	●	○	●	○	●	●	○	●	○	ND	
72	280	●	ND	●	●	○	●	●	●	●	●	●	●	ND	
73	56	●	●	●	●	●	●	●	●	●	●	●	●	●	

●, Retention of heterozygosity; ○, LOH; ND, not determined.  
\*Distance from centromere.



efficient in *p53* heterozygous mice in response to radiation (32). In contrast to the overrepresentation of variants with interstitial deletion in fibroblasts of *p53*<sup>+/-</sup> mice, the spectrum of mutational pathways of the DAP<sup>r</sup> T cells recovered from *p53*<sup>+/-</sup> mice appeared to be similar to that in *p53*<sup>+/+</sup> mice (Table 2).

## Discussion

**In Vivo Chromosome Instability and p53 Deficiency.** We compared the spontaneous frequency of DAP<sup>r</sup> fibroblasts and T cell variants in *Aprt*<sup>+/-</sup> mice with different *p53* genotypes and characterized the spectrum of mutational pathways of these DAP<sup>r</sup> variants. The DAP<sup>r</sup> variants from *p53*-deficient mice exhibited more complex and diverse mutational pathways compared with those from *p53*<sup>+/+</sup> mice. First, the frequency of fibroblast variants, but not T cell variants, was increased in *p53*<sup>-/-</sup> mice. The increase was, in part, a consequence of an enhanced level of mitotic recombination, but not of point mutation. Second, in both fibroblasts and T cells, chromosome loss/duplication and interstitial deletion/gene conversion emerged as prominent mechanisms of LOH in *p53*<sup>-/-</sup> mice. Third, interstitial deletion appears to be more common in fibroblast cells of *p53*<sup>+/-</sup> mice compared with wild-type mice. We conclude that chromosomal abnormalities, which are extremely rare in *p53*<sup>+/+</sup> mice, account for a significant fraction of LOH in *p53*<sup>-/-</sup> and *p53*<sup>+/-</sup> mice.

Increased interstitial deletion in fibroblasts of *p53*<sup>+/-</sup> mice supports the notion of haploinsufficiency of p53 in heterozygotes (31, 32). It appears that, at least in fibroblasts, a mere reduction of p53 dosage is enough for the generation and propagation of somatic variants with chromosomal deletion. Thus, two functional *p53* alleles may be required for the proper maintenance of chromosomal integrity in some tissues.

The increase of chromosomal abnormalities was not reflected in the overall frequency of DAP<sup>r</sup> variants due to the fluctuation in the frequency of variants derived from mitotic recombination. Mitotic recombination still predominated in *p53*<sup>-/-</sup> mice, accounting for more than half of the total variants, thus making a moderate increase (10–20%) in frequency indistinguishable in a small sample.

**Regulation of Cell Cycle Checkpoint, Apoptosis, and DNA Repair by p53.** Characterization of the mutational pathways exhibited by DAP<sup>r</sup> variants in *p53*<sup>-/-</sup> mice serves to test hypotheses about possible roles for p53 *in vivo*. First, the increase of chromosomal abnormalities in *p53*<sup>-/-</sup> mice supports the notion of p53 playing a key role in regulation of cell cycle checkpoints and/or apoptosis. For example, failure of a spindle checkpoint would result in increased aneuploidy. Failure of apoptosis might result in an accumulation of interstitial deletions or other chromosomal abnormalities. It is possible that *p53*<sup>+/+</sup> cells with two double strand breaks (DSBs) in one chromosome most likely undergo apoptosis. But when apoptosis is compromised by the absence or reduction of p53, they might be more likely to survive and allow joining of fragment ends. Misjoining may produce deletions or other chromosomal abnormalities.

It has been reported that p53 may suppress homologous recombination via the inhibition of RecA/Rad51 (5). Increased mitotic recombination observed in fibroblasts in *p53*<sup>-/-</sup> mice is consistent with that notion, so that repair of DSBs is shifted, to some extent, from end joining to homologous recombination repair in fibroblasts in the absence of p53. But it is also possible that cells without p53 may be more likely to survive DSBs because of the failure of apoptosis, thus allowing more recombinants to form. The dramatic

increase of interstitial deletion in both fibroblasts and T cells of *p53*<sup>-/-</sup> mice suggest that direct end-joining still plays an important role in the repair of DSBs in the absence of p53.

Although the frequency of class II variants was elevated about 5-fold, from  $2.4 \times 10^{-5}$  in *p53*<sup>+/+</sup> mice to  $1.3 \times 10^{-4}$  in *p53*<sup>-/-</sup> mice, most were not caused by point mutation. The lack of increased point mutation is consistent with the studies using *lacI* transgene as a reporter (19, 20). If *lacI* reflects only the efficiency of global nucleotide excision repair, *Aprt* as a reporter should be capable of detecting changes in transcription-coupled repair as well. Our results suggest that the effect of p53 on transcription-coupled repair of spontaneous DNA damage is minimal, if any, in the mouse, and is in agreement with the report that p53 mutant human fibroblasts exhibit normal transcription-coupled repair (33).

**Cytotoxicity of DAP and p53.** The relative scarcity of variants with demonstrable *Aprt* point mutations and the retention of high levels of residual APRT activity in class II variants suggest that mechanisms other than *Aprt* point mutation may be responsible for the increased frequency of class II variants of *p53*<sup>-/-</sup> mice. The apparent increased frequency of class II variants in *p53*<sup>-/-</sup> mice may have been due to differential recovery *in vitro*. *p53*<sup>-/-</sup> cells with residual APRT activity appeared to have higher tolerance to the toxic affect of DAP, thus allowing more DAP<sup>r</sup> colonies. DAP is known to exert its toxic effects by blocking RNA synthesis, and it can also lead to abnormal nucleotide pools (34). The latter are known to be inducers of p53 (35, 36). Thus, the cytotoxic affect of DAP may be mediated by p53 to some extent, so that some *p53*<sup>-/-</sup> cells with residual APRT can divide to form colonies in the presence of DAP.

**Chromosome Instability and Tumorigenesis.** The increased chromosome instability in T lymphocytes and fibroblasts of *p53*<sup>-/-</sup> mice may be pertinent to the high incidence of lymphomas, including T cell lymphoma and sarcomas in these mice. It is likely that increased chromosome instability is one of the major contributing factors to the increased predisposition to malignancy. Interestingly, chromosome instability seems to correlate to the observed tumor spectrum in *p53*<sup>+/-</sup> mice, as it is known that sarcomas replace lymphomas as the predominant tumor type in *p53*<sup>+/-</sup> mice (27). Correspondingly, we observed an increased interstitial deletion in fibroblasts, but not in T cells, of *p53*<sup>+/-</sup> mice. Consistent with this correlation is the report that *p53*<sup>-/-</sup> mice did not display an increased mutation frequency at the *Dlb-1* locus within the cells of intestinal epithelium (37), whereas *p53*<sup>-/-</sup> mice are known not to be particularly predisposed to intestinal carcinoma (38).

In summary, we observed an increased level of chromosome abnormalities in normal tissues of *p53* null and heterozygous mice. It should be noted that some types of chromosomal abnormalities like gene amplification and translocation (unless it disrupts *Aprt*) would not be detected by our assay. Assuming more cells with DSBs survived and the DSBs were not properly repaired in the absence or reduction of p53, an increase in the prevalence of translocation should also be expected. Chromosomal translocations are known to be a major cause of activation of protooncogenes. A population of cells with diverse chromosomal abnormalities should be favorably disposed to the initiation and progression of tumorigenesis.

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