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TITLE: Mapping Genetic Modifiers of Mammary Tumor Susceptibility in BALB/c-p53+/- Mice

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13. ABSTRACT ***(Maximum 200 Words)***

The *TP53* tumor suppressor gene is critical for inhibiting tumors in many tissues. Heterozygosity at *TP53* has been associated with the Li-Fraumeni tumor susceptibility syndrome where breast cancer is the most common tumor in women with this syndrome. We found that the incidence of mammary tumors in mice that were heterozygous for *Trp53* varied among strains. Therefore, the mammary tumor-susceptible strain (BALB/cMed) was crossed with a resistant strain (C57BL/6J) to create a panel of 220 backcross mice to allow genetic mapping of chromosomal intervals containing modifiers of mammary tumor susceptibility that varied between these strains. We have monitored tumor type and latency in these mice for >15 months with 50 mice remaining. The incidence of mammary tumors in the *Trp53+/-* backcross mice was considerably reduced compared to the BALB/c (~60% *vs* 30%) confirming the presence of modifier loci in the BALB/c strain that confer susceptibility. We are evaluating linkage of the tumor phenotype to BALB/c polymorphisms in the *Ink4a* and *Prdkc* genes that result in proteins that have reduced activity in cell cycle checkpoint and DNA repair activities. We have also established the mechanism by which the wild type allele of *Trp53* is lost during mammary tumor formation in these mice.

14. SUBJECT TERMS
Genetic mapping, p53, Susceptibility, Breast cancer

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Introduction:

The *TP53* tumor suppressor gene is critical for inhibiting tumor development in many tissues. In the breast epithelium, this is evident from the high incidence of breast cancers in Li-Fraumeni syndrome (LFS) patients who inherit germline mutations in *TP53* [1]. However, the incidence and age of onset of breast cancer in LFS patients bearing identical mutations varies dramatically. Genes that segregate independently of *TP53* may interact with p53 status to modify tumor susceptibility in LFS patients and in the general population. Modifier loci with subtle effects (*i.e.* low-penetrance alleles) would have a complex pattern of inheritance in human populations that would be indistinguishable from "sporadic" breast cancer and may prove to be important contributors to the overall risk of breast cancer.

While mammary tumors are observed infrequently in C57BL/6-*Trp53*+/- mice [2], we have recently demonstrated a high incidence of spontaneous mammary tumors in BALB/c mice that are heterozygous for *Trp53* (BALB/c-*Trp53*+/-). In female BALB/c-*Trp53*+/- mice, 55% developed mammary carcinomas with a latency of 8-14 months [3]. In this project we have exploited this to determine the pattern of inheritance and genetically map chromosomal intervals harboring genes that alter susceptibility to mammary tumors.

Body:

The initial work of mating mice to prepare female (C57BL/6xBALB/cMed)F1 progeny and backcross to BALB/c-*Trp53*-/- males to prepare the mapping panel of female mice heterozygous for *Trp53* has been completed. A total of 220 females have been monitored for tumor incidence for >15 months. To date a total of 82 mammary tumors have been recovered with latency varying between 21 and 68 weeks (Appendix, figure 1). Normal tissues have been collected from which DNA mas been extracted for analysis of genetic markers. Homozygosity for the candidate genes *Ink4a* and *Prdkc*, for which it is known the BALB/c strain harbors variants that reduce protein function, is being analyzed for segregation with the mammary tumor phenotype.

Key Research Accomplishments:

- The mapping panel of mice has generated the mammary phenotypes anticipated and genomic DNA has been isolated in preparation for genotyping. The number of mice with and without mammary tumors are sufficient to allow a genome-wide scan to provide provisional mapping of loci that alter susceptibility to mammary tumors.

Reportable Outcomes:

- Data presented at the Gordon Research Conference in Mammary Gland Biology (June 2001).
- A respository of >80 matched normal and tumor mammary tissue and DNA has been established. Tissues are available for genetic and histologic analyses.
- These DNA samples have been exploited to determine the mechanism by which the wild type allele of *Trp53* is lost in mammary tumors. One manuscript is in preparation describing this mechanism.
- Dr. Anneke Blackburn was awarded a postdoctoral fellowship (DAMD17-01-1-0315) to continue the phenotypic and genetic analysis of this mapping population.
- Dr. Blackburn has gained valuable experience with mammary gland and tumor biology in preparation for an independent career. During this time she has demonstrated her abilities as an independent scientist.

Conclusions:

Analysis of the inheritance of mammary tumor susceptibility in *Trp53*+/- mice is consistent with recessive-acting alleles from BALB/cMed genetic background. Analysis of the *Ink4a* and *Prdkc* loci is preliminary, but suggests that these loci can be excluded from consideration.

References:

1. P. Kleihues, B. Schauble, H. A. zur, J. Esteve, H. Ohgaki, *Am.J.Pathol.* 150, 1-13 (1997).

2. L. A. Donehower et al., *Mol.Carcinogenesis* 14, 16-22 (1995).

3. C. Kuperwasser et al., *Am.J.Pathol.* 157, 2151-2159 (2000).

Appendices:

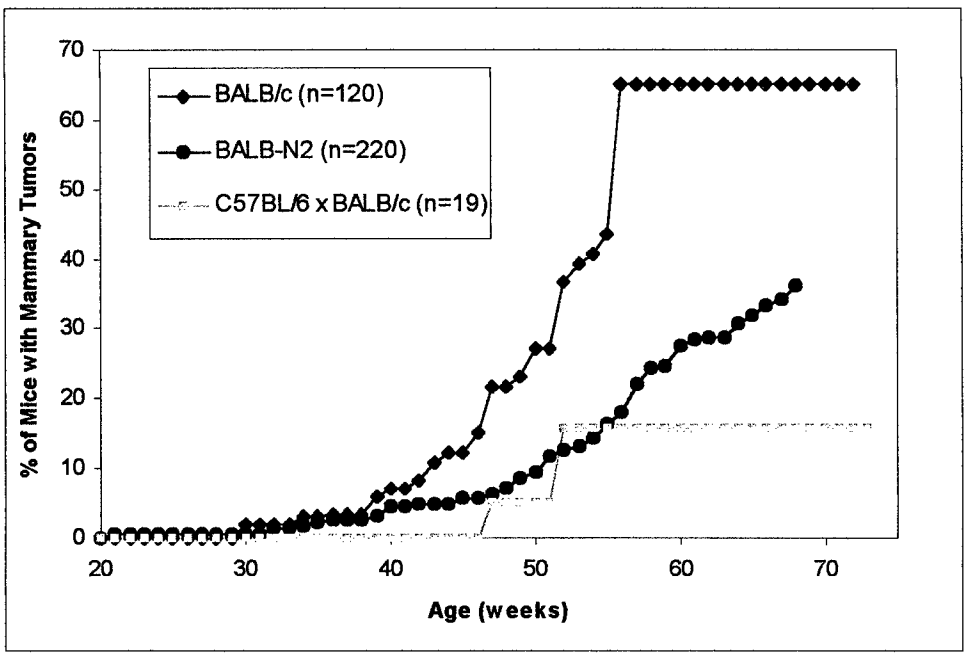

Figure 1: Occurrence of mammary tumors in female Trp53+/- mice of different strain origins. BALB-N2 is the mapping panel created with this grant. BALB-N2 are the offspring of [C57BL/6 x BALB/c] x BALB/c-Trp53-/- matings.

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REPLY TO
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