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TITLE: Mapping genetic modifiers of mammary tumor susceptibility in BALB/c-Trp53+/-mice

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

The *TP53* tumor suppressor gene is defective in the majority of sporadic breast cancers. Breast cancer is also the most frequent tumor type in women with Li-Fraumeni syndrome who inherit germline mutations in *TP53*. This suggests that p53 is fundamental to the growth regulation and prevention of tumors in the mammary epithelium. However, the incidence of mammary tumors in p53-deficient mice varies greatly among strains. We have undertaken the genetic mapping of modifiers of susceptibility by performing genetic crosses between mammary tumor-susceptible BALB/c-*Trp53*+/- mice and -resistant C57BL/6-*Trp53*+/- mice. Mammary tumor susceptibility was shown to segregate as a partially dominant trait with recessive-acting modifiers also detected. BALB/c alleles of the *Cdkn2a* and *Prkdc* genes had been shown to confer tumor susceptibility previously, and therefore examined for association with the mammary tumor susceptibility phenotype. Neither gene segregated as a major modifier, however, *Cdkn2a* was shown to alter latency of lymphomas. Therefore, a genome pan was undertaken. The results indicate the presence of a major modifier gene on mouse chromosome 7. The interval is being narrowed with additional markers and candidate genes will be analyzed for polymorphisms.

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Table of Contents

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* (Kleihues et al., 1997). 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. These modifier alleles may carry a very small risk of breast cancer when considered alone (*i.e.* low-penetrance alleles), but can have dramatic effects when combined with other susceptibility alleles. Low-penetrance alleles would have little selective disadvantage by themselves, and therefore, may represent relatively common alleles in the general population. Therefore, genetic susceptibility may belie a much larger proportion of breast cancer due to the presence of low-penetrance (Ponder, 2001). Though low-penetrance modifier loci may represent important contributors of overall risk as well as targets of therapy, their subtle effects would be indistinguishable from "sporadic" breast cancer due to the complex patterns of inheritance in human populations.

While mammary tumors are observed infrequently in C57BL/6-*Trp53*$^{+/-}$ mice {DONEHOWER1995A}(Donehower et al., 1995), we demonstrated a high incidence of spontaneous mammary tumors in BALB/c-*Trp53*$^{+/-}$ females (Kuperwasser et al., 2000). In this project we exploited this difference in mammary tumor susceptibility among strains to determine the pattern of inheritance of mammary tumor susceptibility 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 (Appendix 1A). A total of 220 females have been monitored for tumor incidence for >15 months. Homozygosity for the candidate genes *Cdkn2a* and *Prdkc*, for which it was known the BALB/c strain harbors variants that reduce protein function, has been analyzed for segregation with the mammary tumor phenotype. A genome-wide pan for linkage of susceptibility loci to markers has been completed (Appendix 2).

Key Research Accomplishments:

- The mapping panel of mice has been generated providing a tool to examine a wide spectrum of candidate genes.
- Interactions between BALB/c and C57BL/6J alleles of *Prkdc* and *Cdkn2a* were detected and shown to differentially affect the probability of developing lymphoma and osteosarcomas.
- Mammary tumor susceptibility was not linked to either *Prkdc* or *Cdkn2a*.
- The genome scan identified linkage of a single major modifier locus on the distal

end of mouse chromosome 7.
- Transcriptional profiling has identified 4 genes within the interval that are differentially expressed.

Reportable outcomes:
- Data presented at the Gordon Research Conference in Mammary Gland Biology (June 2001).
- Data presented at the Era of Hope meeting (Sept. 2002)
- A respository of >80 matched normal and tumor mammary tissue and DNA has been established. Tissues are available for genetic and histologic analyses.
- Two manuscripts are in preparation based on these results. The first describes the patterns of inheritance and interactions between the alleles of the *Cdkn2a* and *Prkdc* loci in different tumor types (lymphoma, osteosarcoma and mammary carcinoma). The second manuscript describes the mechanism by which the wild type allele of *Trp53* is lost in mammary tumors.
- One M.S. graduate student (Christine McLary), 3 undergraduates (Tamar Soffer, Jennifer Brown and Jordyn Boesch) and one post-doctoral fellow (Dr. Anneke Blackburn) were trained during the course of this project. Christy McLary has taken a technical position at the Dana-Farber Cancer Center, Boston, MA. Tamar Soffer is working in a forensics lab in New York City. Jennifer Brown accepted a technical position at U. Mass. Medical School in Worcester. Dr. Blackburn has gained valuable experience with mammary gland and tumor biology in preparation for an independent career. She was awarded a post-doctoral fellowship for her work and has returned to the John Curtin Medical Research Center at the Australian National University in Canberra.

Conclusions:
- Susceptibility to mammary tumors resulted from a combination of dominant-acting modifiers as well as recessive-acting modifiers.
- Loss of heterozygosity of p53 is an obligate part of the process and proceeds via a homologous recombination mechanism.
- The the BALB/c and C57BL/6 alleles of the candidate genes tested (*Cdkn2a, Prkdc*) had significant effects on the probability of lymphomas and osteosarcomas, but did not affect mammary tumors.
- Preliminary efforts suggest linkage of the recessive-acting modifiers to mouse chromosome 7.

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4. Ponder,B.A. (2001). Cancer genetics. Nature *411*, 336-341.

Appendices:

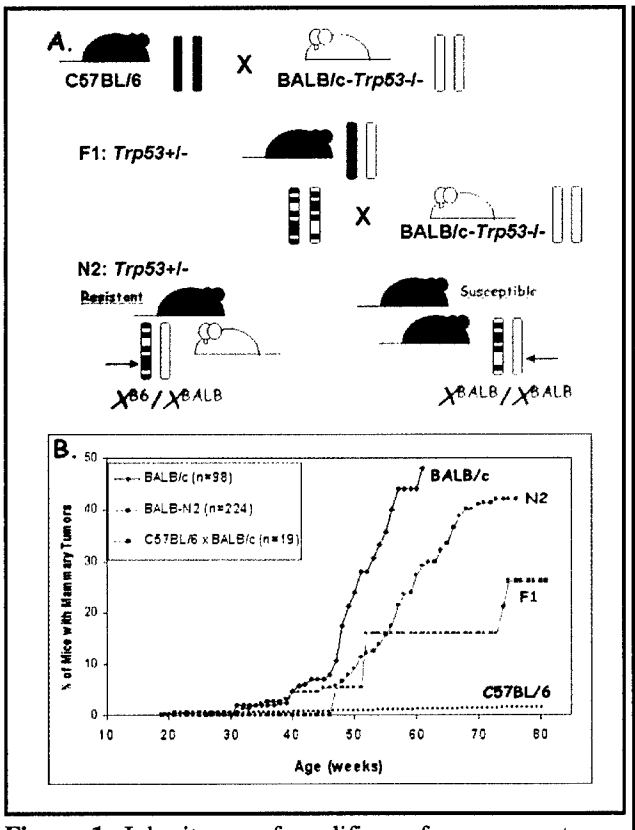

Figure 1: Inheritance of modifiers of mammary tumor susceptibility in C57BL/6 (resistant) and BALB/c (susceptible) mice. (A) F1 intercross mice and N2 backcross mice were prepared to establish the dominance and recessive characteristics of the mammary tumor phenotype. (B) The frequency of mammary tumors in each population of *Trp53*+/- mice is shown.

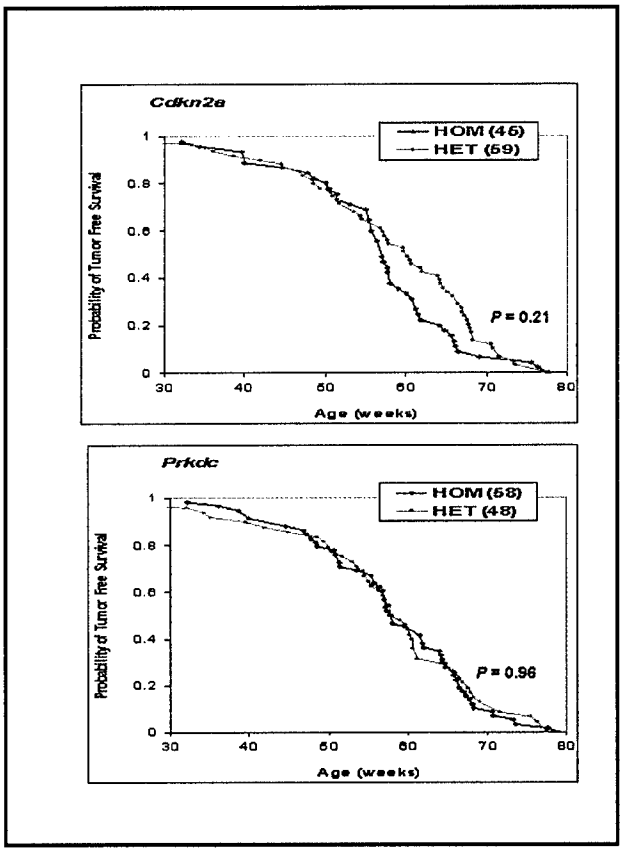

Figure 2: Analysis of relationship between candidate genes and probability of mammary tumors. No statistically significant difference was detected between genotype for either *Cdkn2a* (upper panel) or *Prkdc* (lower panel).

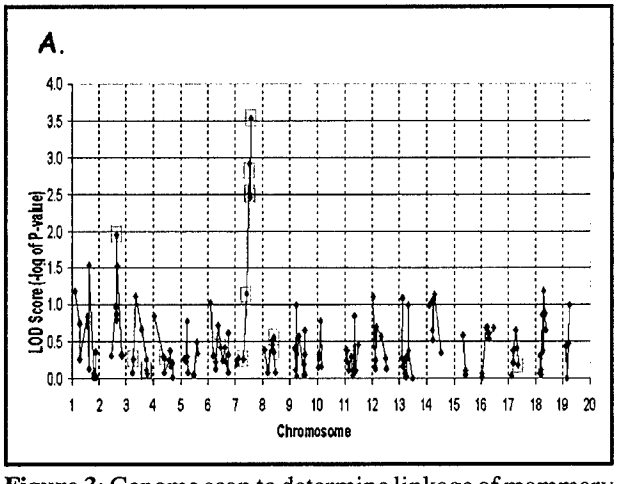

Figure 3: Genome scan to determine linkage of mammary tumor susceptibility in N2 backcross mice. A single point analysis was conducted. The P-values were calculated to determine the probability of linkage. The P-values were converted to approximate a LOD score using the (-Log) transformation.

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