AD_____

Award Number: DAMD17-02-1-0627

TITLE: Identification of Genes Controlling Mammary Tumor Development and Progression

PRINCIPAL INVESTIGATOR: Amy R. Moser, Ph.D.

CONTRACTING ORGANIZATION: University of Wisconsin-Madison
Madison, Wisconsin 53706-1409

REPORT DATE: April 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2003	Annual (15 Mar 02 - 14 Mar 03)

4. TITLE AND SUBTITLE
Identification of Genes Controlling Mammary Tumor Development and Progression

5. FUNDING NUMBERS
DAMD17-02-1-0627

6. AUTHOR(S):
Amy R. Moser, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Wisconsin-Madison
Madison, Wisconsin 53706-1409

E-Mail: moser@mail.humonc.wisc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)* The genes that predispose to breast cancer development in most women with a family history of breast cancer have yet to be identified. We propose a means to identify genes that play a role in cancer development or progression. A mutant allele of the *Apc* gene results in an increased risk of mammary tumor development in mice. Exposure of mice carrying the mutant allele on one genetic background to a carcinogen results in over 90% of the mice developing carcinomas within 60 days. Another strain of mice carrying the mutant allele are resistant to developing mammary cancer. When these mice are treated with the carcinogen, few develop cancers. However, these mice are still susceptible to the development of hyperplastic, or benign, lesions of the mammary gland. By performing crosses of mutant mice from the susceptible strain with those from the resistant strain, we will determine the chromosomal location of the genes conferring resistance to development of cancer. We will also characterize the changes that occur during the progression from hyperplasia to neoplasia in the mammary lesions that develop in these mice. These studies will be a first step toward the identification of novel genes involved in tumor development and progression.

14. SUBJECT TERMS:
genetic susceptibility, modifier genes, hyperplasia, carcinoma

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

INTRODUCTION

Background: The genes that predispose to breast cancer development in most women with a family history of breast cancer have yet to be identified. One hypothesis is that in some families the predisposing alleles will confer only a slight increase in risk and that cancer development will be the result of the interaction of several genes. The difficulty will be in identifying genes that either moderately increase risk of tumor development or modify the course of tumor progression. Identification of such loci will aid in our understanding of the process of tumor development and progression and may lead to the development of new treatment or prevention strategies. We propose to use genetically predisposed mice to identify genes that modify the course of tumor development or progression. Mice carrying a mutant allele (Apc^{Min}) of the *Apc* gene are predisposed to the development of mammary carcinomas. Treatment with ethylnitrosourea (ENU), a direct-acting alkylating agent, increases the incidence and decreases the latency of tumor development. Genetic background affects the number, latency, and type of lesions that develop after ENU treatment. More than 90% of C57BL/6J (B6) $Apc^{Min}/+$ female mice develop an average of three mammary squamous cell carcinomas by 60 days after ENU treatment, but few hyperplastic lesions. In contrast, ENU-treated FVBxB6 $Apc^{Min}/+$ female mice develop an average of four alveolar hyperplasias but squamous cell carcinomas or adenocarcinomas only rarely and with increased latency.

Hypothesis/Objective: We propose that the loci that control the resistance to tumor development and progression in the FVBxB6 $Apc^{Min}/+$ mice can be identified by backcross analysis. The ultimate goals of this research are to identify novel loci that control the resistance to carcinoma development and determine the molecular mechanism of resistance.

Specific Aims: 1) To use standard backcross analysis to map genes controlling the progression from hyperplastic to neoplastic lesions in FVBxB6 F1 mice. 2) To generate and characterize congenic lines of mice carrying the modifiers as a first step in the molecular identification of the modifier loci. 3) To investigate the time course of molecular and histological development and progression of the hyperplasias and tumors.

Study Design: We will use standard mapping techniques to identify the chromosomal locations of loci that control tumor development and progression in B6x(FVBxB6) $Apc^{Min}/+$ female mice. We will then generate and characterize congenic lines of mice carrying the resistance loci from the FVB strain on the sensitive B6 background. This will allow us to confirm the effect of the loci on tumor development. These congenic lines will provide valuable tools for the investigation of the identity of the modifier loci and the molecular mechanism of resistance. To investigate the time course of tumor development, we will collect mammary tumors and hyperplasias from B6 and FVBxB6 $Apc^{Min}/+$ mice at various time points after ENU treatment. We will also determine the expression of APC and β-catenin in the tumors and hyperplasia as a preliminary characterization of the molecular development of tumors.

Relevance: These studies will provide identify novel loci that affect the process of tumor development and progression. The FVBxB6 $Apc^{Min}/+$ mouse model provides and excellent opportunity to identify genes that control tumor progression. The congenic lines that will be produced will be a unique resource with which to study tumor development and progression. Ultimately, understanding of tumor progression will aid in the development of strategies for prevention and treatment.

BODY

I have listed below each of the tasks outlined in the Statement of Work from the original grant proposal. After each task, the progress made is described.

Task 1: **Generate FVBxB6 F1 female mice and B6 *Min/+* male mice for the production of the backcross progeny.**

- Production of 20 FVBxB6 F1 female mice. Months 1-3. ***Ongoing, we continue to generate F1 mice to continue backcross production.***
- Production of 20 B6 *Min/+* male mice. Months 1-8. ***Ongoing: as B6 Min/+ mice only live about 100 days so we continually produce them.***

Task 2. **Backcross production and analysis.**

- Production of backcross mice. Months 3-14. ***Ongoing: to date have produced and genotyped about 140 Min/+ backcross progeny.***
- ENU-treat 300 female *Min/+* backcross mice. Months 4-12. ***Ongoing: we have ENU treated 125 backcross progeny to date.***
- Dissection of 300 backcross mice. Months 7-15. ***Ongoing: we have dissected about 100 mice to date. As we have never tested for tumor development in these mice, we first did a pilot study of about 40 mice to identify the best time to euthanized the mice and count and collect tumors and mammary glands. Through observation, we have found that some of the backcross mice have tumors and must be euthanized by 80 days after ENU-treatment. However, few needed to be euthanized before 70 days after treatment. As many mice developed tumors by that time, we chose to dissect all subsequent mice 70 days after treatment.***
- Collection and processing of mammary glands and tumors. Months 7-16. ***Ongoing: glands have been collected from all of the dissected mice and are being processed (this takes about 3 weeks from collection).***
- Evaluation of stained mammary glands, enumeration, and dissection of hyperplasias and neoplasias. Months 8-24. ***Ongoing: glands are evaluated as they are ready.***
- Processing and sectioning of hyperplasias and neoplasias. Months 8-24. ***Pending: we will collect tumors and hyperplasias from the first 100 mice before we send them for sectioning.***
- Evaluation and interpretation of histological sections of tumors and hyperplasias. Months 9-30. ***Pending***

Task 3. **Mapping of modifier loci.**

- Identification of SSLP markers for analysis. Months 6-12. ***Ongoing: we have identified markers from databases on all chromosomes and are in the process of confirming that they will be useful. We are also in the process of identifying markers to fill in gaps where there are no known markers and testing them for polymorphisms. To date we have identified sufficient markers on 4 chromosomes.***
- Isolation of DNA from spleen samples from the BC mice. Months 12-16. ***Pending: we will start this when the marker identification is complete.***
- Genotyping of BC mice with SSLP markers and data entry. Months 16-20. ***Pending***
- Analysis of BC. Months 20-22. ***Pending***
- Preparation of publication. Months 22-26. ***Pending***

Task 4. Analysis of time course of tumor development in B6*Min/+* and FVBxB6 *Min/+* mice.

- Production of 100 B6 *Min/+* and 100 FVBxB6 *Min/+* mice. Months 1-12. ***Ongoing: We have produced 80 F1 mice and are beginning production of B6 Min/+ mice. This should be completed over the summer.***
- ENU treatment and tumor collection. Months 3-15. ***Ongoing: about 40 F1 mice have been treated and glands and tumors collected.***
- Preparation and evaluation of mammary whole mounts and tumors for histology. Months 6-24. ***Ongoing: this will continue as mice accrue in the study.***
- Histological evaluation of tumors and hyperplasias (in collaboration with Dr, R. Cardiff) and analysis of data. Months 8-30. ***Pending collection of more samples***.
- Preparation of publications. Months 30-36.

Task 5. Molecular characterization of tumors.

- Characterization of antibody reagents for tumor analysis. Months 12-18. ***Pending***.
- Analysis of tumors and hyperplasias. Months 18-32. ***Pending***.
- Preparation of publications. Months 32-36. ***Pending***.

Task 6. Production and characterization of congenic lines. ***Pending***.

- Production of up to 9 congenic lines, 3 for each of three "modifier" loci. Up to 180 mice/ generation. Months 18-36.
- Production and testing of N4 congenic *Min/+* females for preliminary testing. 40 females/congenic line. Months 28-36.
- Analysis of results and preparation of publication. Months 34-36.

KEY RESEARCH ACCOMPLISHMENTS

- We have identified a time after ENU treatment for the dissection of the backcross mice. We are finding that some of the mice develop tumors and need to be euthanized by 80 days after treatment. Therefore, to minimize the number of mice that must be dissected early, we have chosen 70 days after ENU.

REPORTABLE OUTCOMES

Oral Presentation:
None
Manuscripts:
None

CONCLUSIONS

None, all experiments are ongoing.

REFERENCES

none

APPENDICES

none