

AD _____

Award Number: DAMD17-00-1-0349

TITLE: Prevention of Breast Cancer in IGFBP

PRINCIPAL INVESTIGATOR: Douglas Yee, M.D.

CONTRACTING ORGANIZATION: University of Minnesota
Minneapolis, MN 55455

REPORT DATE: June 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.

20040413 025

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE June 2003	3. REPORT TYPE AND DATES COVERED Annual (1 Jun 2002 - 31 May 2003)	
4. TITLE AND SUBTITLE Prevention of Breast Cancer in IGFBP			5. FUNDING NUMBERS DAMD17-00-1-0349	
6. AUTHOR(S) Douglas Yee, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Minnesota Minneapolis, MN 55455 E-Mail: yeexx006@tc.umn.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) In this proposal, we hypothesize that inhibition of IGF action by IGFBP-1 will prevent breast cancer in a SV40 Tag transgenic model of breast cancer. We will test this hypothesis with two specific aims: 1) to inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter and 2) to test the ability of IGFBP-1 to suppress tumorigenesis by mating these animals with C3/Tag transgenic mice. To date, we have generated two founder lines containing the IGFBP-1 transgene and several F1 and F2 animals were analyzed. Unfortunately, while the transgene was clearly integrated into these animals, we were unable to detect expression of IGFBP-1 protein. To correct this problem we have generated more founders with a modified construct involving insulator sequences. Oocytes have been injected and we are awaiting the offspring.				
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 4	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

COVER.....	1
SF 298.....	2
Introduction.....	4
BODY.....	4
Key Research Accomplishments.....	4
Reportable Outcomes.....	4
Conclusions.....	4
References.....	none
Appendices.....	none

Introduction

The purpose of this project was to create transgenic mice expressing IGFBP-1 in the mammary gland. We hope overexpression of this binding protein can neutralize IGF action and inhibit breast cancer development.

Body

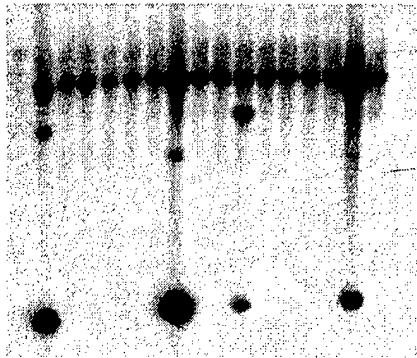
Specific Aim (Task) #1 - To inhibit IGF action at the mammary epithelial cell by creating transgenic mice that express IGFBP-1 under the control of the whey acidic protein (WAP) promoter

- a. Months 0-3 - Create WAP-IGFBP-1 transgene vector
- b. Months 3-9 - Create and identify IGFBP-1 F1 progeny
- c. Months 9-16 - Characterize level of IGFBP-1 expression in mammary gland, determine influence of IGFBP-1 expression on lactation, examine activation of IGFR1

As noted in last year's progress report, we showed that we were able to identify integration of the transgene, but were not able to identify F1 generation animals with mRNA or protein expression of IGFBP-1. In last year's report, we felt that we selected too few animals.

In order to address this concern, we decided to re-inject animals with the original WAP-IGFBP-1 vector. Our collaborator (Dr. David Largaespada) reviewed our progress and suggested that addition of an insulator sequence may potentially enhance the ability of the transgene to be expressed. With Dr. Largaespada's assistance, we created a new transgene construct bearing the insulator sequence.

While we generating the new construct in preparation for oocyte injection, our specific pathogen free (SPF) animal facility suffered an unfortunate outbreak of pinworms and mouse hepatitis virus. Our Animal Use Committee decided to completely close the facility, transfer the animals to the animal facility in a newly constructed building, sterilize the old facility, then re-open the transgenic core facility for investigator use.



These outbreaks in the SPF facility essentially delayed this work for 12 months as the transgenic facility was closed for that period of time. Obviously, this contamination of the mouse facilities has significantly delayed our progress on this project.

Despite this setback, we were able to derive new founder animals. Figure 1 shows Southern blots of several of the separate founder lines we are now analyzing. We currently have 17 separate founder lines, a substantially increased number of animals compared to last year's progress report. We are in the progress of generating F1 animals and analyzing them for protein and mRNA expression.

Figure 1 – Southern Blot of Founders

Key Research Accomplishments

- Generated insulator constructs and injected into oocytes
- Generated 17 founder animals

Reportable outcomes

Given the necessity to generate new founder animals, we do not yet have reportable outcomes.

Conclusions

The progress in this proposal has been unfortunately delayed. Our initial oocyte injections yielded only a small number of animals who had integrated the transgene, but did not express IGFBP-1 mRNA or protein.

Unfortunately, we were unable to re-inject oocytes for nearly a year because of an infectious outbreak in our animal SPF facility. This has now been rectified, and we are back on track regarding generation of founders. We have been granted a no-cost extension and hope to complete the original aims of the proposal within the upcoming year.

References – None

Appendices - None