AD_____

Award Number: DAMD17-00-1-0224

TITLE: Training in Mammary Gland Biology and Breast Cancer

PRINCIPAL INVESTIGATOR: Mina J. Bissell, Ph.D.

CONTRACTING ORGANIZATION: University of California at Berkeley
Berkeley, CA 94720

REPORT DATE: September 2004

TYPE OF REPORT: Annual Summary

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2004	Annual Summary (1 Sep 2000 - 31 Aug 2004)

4. TITLE AND SUBTITLE
Training in Mammary Gland Biology and Breast Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0224

6. AUTHOR(S)

Mina J. Bissell, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California at Berkeley
Berkeley, CA 94720

E-Mail: MJBissell@lbl.gov

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
Original contains color plates: ALL DTIC reproductions will be in black and white

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

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The goal of the present proposal is to provide post-doctoral training opportunities in breast cancer research that focus on the role of microenvironment in mammary gland biology. Trainees will benefit from working in a dynamic interactive program under the guidance of the LBNL mentors to investigate the intersection of hormone action, growth factor activity and extracellular matrix remodeling during mammary gland development and carcinogenesis. In addition, trainees will be exposed to a variety of other topics related to breast cancer, as well as research ranging from molecular medicine to genomics, by their participation in working groups, lectures and scientific meetings with other Berkeley Lab and Bay Area researchers.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
10

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

DOD Training Grant in Mammary Biology
2004 Annual Report

Introduction

The goal of the present proposal is to provide post-doctoral training opportunities in breast cancer research that focus on the role of microenvironment in mammary gland biology. Trainees will benefit from working in a dynamic interactive program under the guidance of the LBNL mentors to investigate the intersection of hormone action, growth factor activity and extracellular matrix remodeling during mammary gland development and carcinogenesis. In addition, trainees will be exposed to a variety of other topics related to breast cancer, as well as research ranging from molecular medicine to genomics, by their participation in working groups, lectures and scientific meetings with other Berkeley Lab and Bay Area researchers.

Postdoctoral trainees supported by this grant in 2004 include Anna Erickson and Joanna E. Mroczkowska-Jasinska through December 2003, Celeste Nelson who replaced Joanna in December 2003 and Mark LaBarge who joined the Bissell laboratory in June of 2004.

Postdoctoral Fellows/Research Accomplishments

- Anna Erickson, Ph.D.

Dr. Erickson joined the Barcellos-Hoff lab in June 2001 to conduct research on a joint project with Dr. Barcellos-Hoff and Bissell. She obtained her degree in Cell Biology from the University of Alabama at Birmingham in 2001 in the laboratory of Dr. John Couchman. She has studied E-cadherin mRNA, protein abundance and localization, and its association with other membrane and cytoskeletal proteins in cells surviving ionizing radiation (IR) exposure in the three-dimensional model of alveolar morphogenesis. Her work has shown that IR exposure promotes epithelial-mesechymal transition (EMT) in nonmalignant HMECs in a manner distinct from, but augmented by, that induced by TGF-β1 signaling. Importantly, the effect of IR is heritable and could contribute to its action as a carcinogen in breast. This work is in the process of being submitted for publication (see Abstract 1). In addition, Dr. Erickson has done some important work to show that TGF-β protects mammary epithelial cells from radiation-induced centrosomes amplification which is also in preparation for publication (see Abstract 2).

- Joanna E. Mroczkowska-Jasinska, Ph.D.

Dr. Mroczkowska-Jasinska joined the lab of Dr. Yaswen in June 2002. Joanna used human mammary epithelial cells (HMEC) to study pathways that influence telomerase expression in human mammary epithelial cells during immortalization. In particular, she examined the effects of perturbations in three transcription factors; p53, ZNF217 and BORIS, on telomerase expression and immortalization. The Yaswen/Stampfer group previously reported that inactivation of p53 function can induce expression of telomerase activity in early passage "conditionally" immortal HMEC with low or undetectable levels of telomerase. Employing promoter reporter assays, Joanna demonstrated higher activity of the hTERT promoter in conditionally immortal HMEC exposed to a dominant negative inhibitor of p53 function. These studies provided evidence that the suppression of telomerase by p53 is transcriptional. In collaboration with Dr. Jean Benhattar at the Institut Universitaire de Pathologie in Switzerland, Joanna also investigated the degree of correlation between hTERT promoter methylation and hTERT transcription level in HMEC at different stages of immortalization. In general, there was a good correlation between methylation and hTERT/telomerase expression. Methylation changes were heterogeneous and progressive in a cell line examined at different passages after immortalization. However, three immortal cell lines expressed hTERT in the presence of little or no hTERT methylation. These three cell lines also underwent the least amount of chromosomal instability and/or heterogeneous growth during immortalization. Thus, increased methylation of the hTERT promoter often accompanies HMEC immortalization, but may not be required for expression. Another potential oncogene, BORIS, has been reported to have a role in reprogramming the methylation pattern of particular tracts of DNA, including sites in the hTERT gene. Joanna used retroviral constructs to determine whether exogenous BORIS expression extends the proliferative potential of, or immortalizes finite life span HMEC. Negative results suggested that up-regulated BORIS expression by itself could not extend the life span of HMEC.

- Celeste M. Nelson Ph.D.

Dr. Nelson joined the Bissell Laboratory in November 2003. She obtained a Ph.D. in biomedical engineering from John Hopkins University in 2003 under Dr. Chris Chen where she studied the effects of cell shape and cytoskeletal tension on cellular proliferation and differentiation. Her current project focuses on branching morphogenesis, the formation of branched epithelial tubes from a preexisting unbranched structure. This process, common throughout development, is responsible for generating the complex fractal structures building the lung airways, kidney collecting ducts, and the salivary and mammary glands. Although it is clear from studies of several tissues that branching morphogenesis is regulated by interactions between the epithelium and its underlying mesenchyme, the exact molecular and physical mechanisms responsible for the initiation of branching, especially in the mammary gland, remain to be determined. Dr. Nelson's project aims to understand the biochemical and physical cues regulating the site of branch-point initiation during branching morphogenesis of the mammary gland. Dr. Bissell's lab has previously determined that clusters of mammary epithelial cells embedded in collagen gels will be induced to branch when provided with three cues: one of several growth factors, matrix metalloproteinase activity, and the mesenchymal morphogen, epimorphin (Simian *et al*, 2001). However, it remains unclear why some of the cells in the clusters branch out while others do not. One of the main difficulties in studying the spatial regulation of branching in culture is the inability to compare branching structures with each other and with the branching gland of the animal. In order to circumvent this obstacle, we have developed a three-dimensional micropatterning approach to specify *a priori* the architectures of

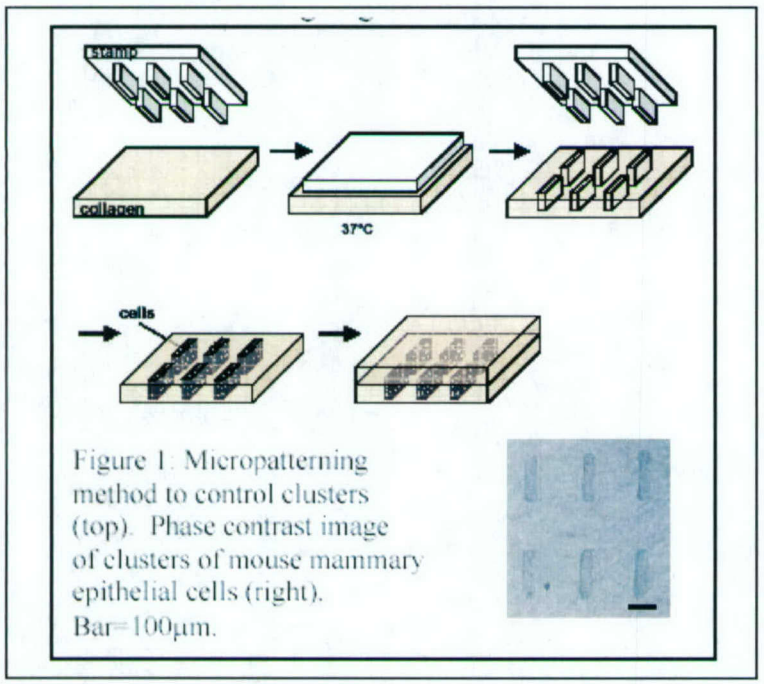

Figure 1: Micropatterning method to control clusters (top). Phase contrast image of clusters of mouse mammary epithelial cells (right). Bar=100μm.

the cellular clusters (Figure 1). When stimulated with the appropriate cues, cells branch out from characteristic locations in the cellular clusters (Figure 2).

Dr. Nelson's working hypothesis is that the location of cells affects the regulation of branching-specific pathways. In order to dissect the cues that regulate the site of branching, she will compare the patterns of gene expression of cells in the locations that branch (ie, the corners of the rectangular clusters in Figure 2) with those in the locations that do not branch (ie, the long edges of the rectangular clusters in Figure 2). We will use laser capture microdissection to isolate these cells from each other prior to the addition of the branching cues. We willthen 1) use RT-PCR to examine differences in expression of candidate genes with suspected involvement in branching in other systems (including fibronectin, fibroblast growth factor receptor, etc.), and 2) use cDNA arrays to identify other possible genes involved in the decision to branch. All candidates will be verified confocal analysis of immunofluorescence staining of the clusters before and after branching. We anticipate that our approach will allow the identification of factors involved in branching that can then be verified in the mouse mammary gland.

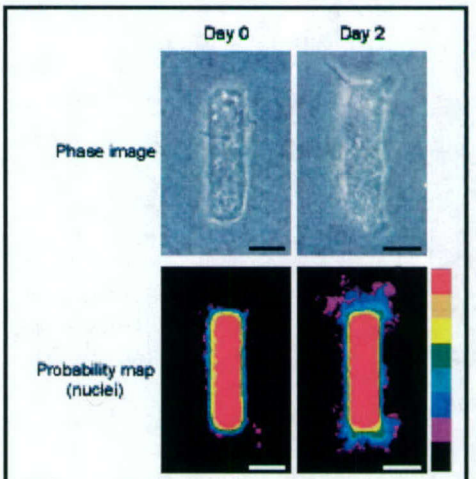

Figure 2: Branching is affected by the location.

- Mark LaBarge, Ph.D.

Dr. Mark LaBarge joined the laboratory of Dr. Bissell in June of 2004. He received his Ph.D. from Stanford University under the mentorship of Dr. Helen Blau where he studied the mechanisms of bone marrow cell-derived myogenesis. His proposed project will focus on how cells integrate information from their environment to control the regulatory networks that modulate polarity, proliferation, and differentiation. The Bissell Laboratory cuture model was among the first to show that mammary luminal epithelial, myoepithelial, and stem cells could be cultured in 3-dimensional (3D) environments that effectively simulated the mammary gland and that organ genesis, morphology, and cell signaling can be effectively studied using this approach. Combining his experience in adult stem cell biology with the lab's 3D culture models and cell

signaling expertise he proposes to dissect molecular mechanisms that allow adult mammary epithelial and stem cells to integrate signaling information from pro-proliertive (EGF/MAPK), differentiation (Notch), and adhesion (integrins) signaling pathways during organ genesis and tumor genesis.

To that end, in the short while that he has been a recipient of funds from this grant, June-Sept, he has been able to demonstrate that Notch pathway antagonists do mediate reversion of malignant luminal epithelial cells in 3D culture. Conversely, Notch pathway ligands stimulate growth of the malignant cells in 3D culture. Although preliminary, these data provide the initial evidence that the Notch pathway is important in mammary acinus development and is a pathway worthy of more attention during the remainder of the granting period.

Training Activities

The trainees are exposed to a wide range of research approaches, tools, and methods that are encompassed in the mentor's laboratories. In addition to weekly **laboratory meetings** with the preceptor, a monthly **Cell and Molecular Biology department meeting** is held to bring together the investigators and the trainees to discuss research and literature relevant to the program. The department will hosts an annual a Postdoctoral Research Day which features poster presentations and a speaker chosen by postdoctoral fellows (http://www.lbl.gov/lifesciences/postdoc/index.htm contains the details of the LSD postdoc society). **Division seminars** are held weekly (see http://www.lbl.gov/lifesciences/resources/seminars.html for a roster of speakers for 2004).

Of particular relevance is the monthly **Mammary Gland Affinity Group**, which is a long standing tradition. LBNL mammary biology and breast cancer groups meet for informal research presentations. Additional participants from UC San Francisco Medial Center and UC Berkeley campus attend regularly. Approximately 30-40 participate. The format consists of two short talks by postdoctoral fellows.

The Life Sciences Division currently hosts approximately 50 research grants in breast cancer and mammary biology, totaling over $16 million in funds. Dr. Mina Bissell, the Principal Investigator of the Training Grant, the director of the Life Sciences Division is Dr. Joe Gray. Dr.

Gray maintains a joint appointment with the UCSF Cancer Center where he is the program leader of the Breast Oncology Program. This program contains s the NCI-funded Bay Area Breast Cancer Specialized Program of Research Excellence (SPORE). http://cc.ucsf.edu/breast_spore/index.html The Breast Oncology Program has a weekly seminar series which we now video conference to LBNL. These seminars provide the postdoctoral fellows with a good mixture of basic research and clinical research. It also provides a good opportunity for the postdoctoral fellows to hear and understand the concerns of breast cancer advocates.

Reportable Outcomes

Publications: Two manuscripts will be submitted shortly, the abstracts are below.

ABSTRACT 1

Ionizing Radiation Promotes TGF-β Mediated EMT in Human Mammary Epithelial Cells

Anna C. Erickson, William S. Chou, Mina J. Bissell and Mary Helen Barcellos-Hoff
Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

Abstract
We have previously shown that ionizing radiation (IR) acts through a heritable, nonmutational mechanism to compromise pathways affecting cell adhesion, extracellular matrix interactions, epithelial cell polarity and cell-cell communication in human mammary epithelial cells (HMEC), grown in a 3D rBM assay (Park et al. 2003). This irradiated phenotype was augmented by the addition of TGF-β; these colonies displayed some of the hallmarks of epithelial-mesenchymal transition (EMT). Since colonies arising from irradiated HMECs grown in the presence of TGF-β exhibited traits similar to EMT we postulated that IR predisposes HMECs to TGF-β induced EMT. To test this hypothesis we characterized the irradiated phenotype in nonmalignant HMEC, S1 HMT-3522 and MCF-10A, monolayer cultures while focusing on the basis for the disruption of cell-cell communication in the progeny of irradiated HMECs. Irradiated HMECs exhibit increased nuclear SMAD, suggesting an increase in TGF-β signaling; consistent with our previous finding that TGF-β1 is rapidly activated in response to IR in mouse mammary gland (Ehrhart et al, 1997). IR resulted in reduced levels of the adherens junction components, E-cadherin and β-catenin, which could be restored with TGF-β neutralizing antibody treatment. E-cadherin and β-catenin protein abundance was decreased by TGF-β treated cultures in both unirradiated and irradiated HMECs. E-cadherin immunoreactivity was significantly decreased in TGF-β, irradiated cells compared to TGF-β controls. To determine the nature of this discrepancy we used differential detergent extraction of soluble proteins followed by immunoprecipitation or immunofluorescence. E-cadherin and β-catenin cytoskeletal association was significantly reduced in irradiated, TGF-β1 treated cells compared to either irradiated or

TGF-β1 treated cells. Irradiated, TGF-β1 treated cells also exhibited and altered actin cytoskeleton and increased vimentin immunofluorescence and protein abundance. These features are consistent with epithelial-mesenchymal transition (EMT), and suggest that IR exposure promotes EMT in nonmalignant HMECs in a manner distinct from, but augmented by, that induced by TGF-β1 signaling. Importantly, this effect of IR is heritable and could contribute to its action as a carcinogen in breast.

ABSTRACT 2

TGF-β Protects Human Mammary Epithelial Cells from Radiation-Induced Centrosomes Amplification

Anna C. Erickson[1], Rishi J. Gupta[1], Jeffrey L. Salisbury[2] and Mary Helen Barcellos-Hoff[1]

[1]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA; [2]Tumor Biology Program, Mayo Clinic Foundation, Rochester, MN 55905, USA

Abstract

TGF-β is rapidly activated in response to ionizing radiation (IR) in mouse mammary gland and plays a critical role in epithelial cell fate decisions. IR is a known human breast carcinogen and has been shown to cause genomic instability. Because previous studies have shown that TGF β1 plays a role in regulating genomic instability in epithelial cells (Glick et al., 1997) we postulated that TGFβ1 could modulate IR induced genomic instability in non-malignant human mammary epithelial cells (HMEC). For this study centrosome abnormalities, assessed by immunofluorescent staining for γ-tubulin, were used as an index of potential genomic instability. We show that TGF-β levels mediate centrosome status in HMECs. IR of single cell HMECs increased the frequency of daughter cells with abnormal centrosomes as a function of dose. The frequency of abnormal centrosomes was decreased in the presence of exogenous TGFβ1 (400 pg/ml). This data indicates that TGF-β acts as a surveyor of genomic instability by protecting HMECs from radiation-induced centrosome amplification.