

**CRADA Final Report**  
**CRADA No. BG98-053**

**1. Parties:**

Chiron Corporation and UC Regents/LBNL

**2. Title of the Project:**

“Identification of New Therapies with Potential for Treatment of Breast Cancer”

**3. Summary of the specific research and project accomplishments:**

A. The project team combined their knowledge and resources to analyze the changes in gene expression involved in both the development and treatment of breast cancer. This information was used to identify new tumor suppressor genes with therapeutic potential that will be tested in the culture model system. Because the cell lines used in the study were all derived from a single individual, the differences between the experimental samples reflect primarily the changes that occur upon the development of breast cancer and the changes that occur upon the suppression of malignancy. These changes have been difficult to investigate by existing methods, so relatively little was previously known about them. Nonmalignant, malignant, and reverted malignant breast cell lines were cultured in Mina Bissell’s laboratory. The cell lines were harvested and analyzed at Chiron Corporation, and the datasets of gene expression patterns were analyzed in Saira Mian’s laboratory. These results have indicated separate and distinct groups of genes associated only with the nonmalignant cells, with the malignant cells, and the reverted cells. Of these, an invention disclosure has been filed on 367 prospective tumor suppressor genes.

**Analysis of HMT-3522 Experimental Results**

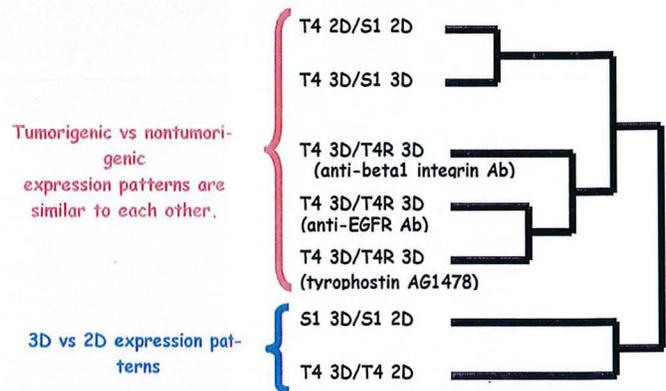
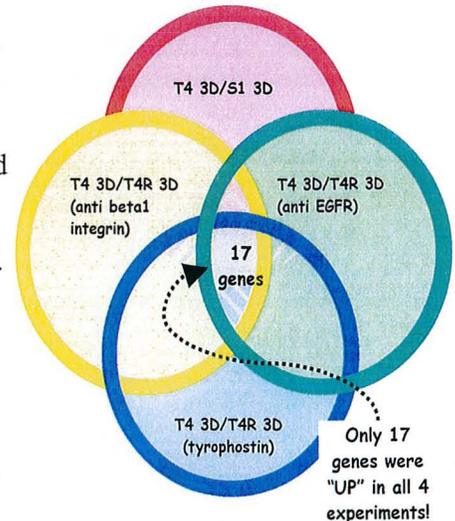


Figure 1. mRNA was prepared from S1, T4, and T4 reverted (T4R) cells, on 2D and 3D, and used for pairwise array analyses with 20,000 target genes. Upper figure indicates clustering results, lower figure shows Venn diagram indicating the relative changes observed with four of the experiments.



B. The 3-dimensional assay will be developed further for a high throughput analysis of anticancer drugs using Chiron’s combinatorial library. The assay has been successfully modified at LBNL to yield a robust end point for high throughput analysis. With the help of engineers at LBNL, an imaging time-lapse system was built and installed inside a modified tissue culture incubator. The modifi-

cation of the assay allows the colonies to be visualized on *top* of a laminin-rich gel. Our prototype "Cell Imager" is an optical-mechanical system integrated into a standard incubator. The prototype accommodates standard culture plates, and any section of the plate can be accurately and reproducibly positioned under a close-up digital camera (a Navitar lens system with a CCD camera). Each image "tile" represents an area of about 1.3 x 1 mm, giving a resolution of about 1 micron per pixel to view cell colonies growing on gel substrates in the plate. The control software allows us to record time-lapse images of any section of the plate, and to assemble the images into "stop-motion movies", in which 40 to 50 images of the same tile, taken at four- to six-hour intervals, are combined into a sequence that allows us to see how the colonies evolve over a period of several days. The stage is thus set for the use of the combinatorial library on the assay to see which compounds can revert the malignant phenotype.

**4. Deliverables:**

Deliverable Achieved	Party (LBNL, Participant, Both)	Delivered to Other Party?
Developed technology for culturing human mammary epithelial cells on top of 3D ECM	LBNL	yes
Produced high-quality RNA for use in cDNA microarray experiments	LBNL	yes
Acquired cDNA microarray data	Chiron	yes
Statistical analyses of cDNA microarray data	LBNL	yes

**5. Identify publications or presentations at conferences directly related to the CRADA?**

Some of this work was published in (Chen HM, Schmeichel KL, Mian IS, Lelievre S, Petersen OW, Bissell MJ. AZU-1: a candidate breast tumor suppressor and biomarker for tumor progression. *Mol Biol Cell*. 2000, 11, 1357-67), and results from these studies have been discussed in numerous keynote talks and featured presentations by Mina Bissell.

**6. List of Subject Inventions and software developed under the CRADA:**

An invention disclosure was filed on 367 of the prospective tumor suppressor genes as potential therapeutic agents for treatment of breast cancer.

**7. A final abstract suitable for public release:**

This project represents a collaboration between two research groups at Lawrence Berkeley National Laboratory (LBNL) and the research facility at the Chiron corporation, in which each group supplied unique and essential contributions. Mina Bissell, at LBNL, provided the resources and expertise of her research group in a physiologically relevant culture system with particular utility for investigating the development of breast cancer. Chiron Corporation, of Emeryville, California, generated high-quality cDNA microarrays, hybridized cDNA prepared from cultures and cell lines developed in the Bissell laboratory, and performed preliminary analysis of the resultant dataset. Saira Mian, at LBNL, used sophisticated statistical and Bayesian techniques for analysis of the enormously complex dataset to reveal key genes involved in signaling pathways responsible for development of breast cancer. These results have indicated separate and distinct groups of genes associ-

ated only with the nonmalignant cells, with the malignant cells, and the reverted cells. Currently, the project team is involved in data verification, to be followed by testing selected genes for utility as potential tumor suppressors. However, the results already obtained were so striking that a disclosure has been filed on 367 of the selected genes for potential use in therapy.

**8. Benefits to DOE, LBNL, Participant and/or the U.S. economy.**

One of the most significant taxes on the U.S. economy is the loss of productivity due to an ailing workforce. Cancer affects at least 4 in 1000 people in the United States. In breast cancer (the area of initial investigation) there will be:

- ~180,200 new cases among women in US in 1997.
- ~1,400 new cases among men in 1997.
- ~44,190 deaths (43,900 women, 290 men) in US in 1997.

Eighty percent of all breast cancers are in women over 50, but because many women get afflicted between 40-65 much productivity is lost above and beyond the health care expenses. Five-year survival rate >as increased from 72% in the 1940s to 97% in 1997. To be able to provide early detection and treatment of this diseases would save the nation billions in health care costs and offset substantial loss in productivity.

Over the last fifteen years Mina Bissell, with the support of DOE's Office of Biological and Environmental Research has established that the microenvironment surrounding cells plays a crucial role in maintenance of functional differentiation, tissue-specific gene expression, branching morphogenesis and growth. For these studies she has developed a unique model system to study the regulation of normal function in the breast in a culture dish. The overall aim of this work has been to understand the molecular mechanisms involved in regulating tissue-specific gene expression in normal breast and how these are altered as cells become cancerous.

Dr. Bissell's work in rodents has demonstrated the feasibility of this approach to understanding breast cancer induction and progression in humans. The availability of a unique human breast cell line provides an exciting opportunity to extend this model to human cells. When grown on basement membrane, these cells gradually change from morphologically normal to pre-malignant to malignant cells, and the biochemical changes associated with these morphological changes can be followed. The program has demonstrated that critical biochemical changes can be related not only to the loss of response to the extracellular matrix (ECM)—the mass of fibrous and globular proteins that surround the cell—but also to the loss of microenvironmental control. Furthermore, it is now becoming clear that correct tissue structure fundamentally regulates homeostasis. The expertise at LBNL provided a unique opportunity for Chiron, a company with a well-established interest in cancer and its major contributing risk factors, to develop and identify novel compounds and potential therapeutics which likely impact on this human disease condition. In addition to health care considerations, the technology developed within the scope of this collaboration will help maintain the nation's preeminence in biotechnology, and serve to enhance the level of domestic employment in areas related to molecular biology, and gene and cell therapy activities.

**9. Financial Contributions to the CRADA:**

DOE Funding to LBNL	\$	700,000
Participant Funding to LBNL	\$	200,000
Participant In-Kind Contribution Value	\$	500,000
Depreciation and DOE-Added Factor	\$	37,458
Total of all Contributions	\$	1,437,458

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