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The objective of the program is to establish at the University of Texas Health Science Center at San Antonio an in-depth training program in the Molecular Genetics of Breast Cancer. One goal of the program is to train highly qualified doctoral students in the genetic, cellular, and molecular basis of Breast Cancer. The training program, conducted within the Molecular Medicine Ph.D. Program, is administered by a select group of faculty whose research projects are intimately involved in breast cancer. An additional goal of the program is to promote synergistic interactions between the various laboratories engaged in breast cancer research. Breast cancer meetings, Molecular Medicine Minisymposia and a distinguished Seminar series are integral parts of the training program. The strength of the program is the quality of the Program faculty, and the interactive breast cancer research community. The faculty are studying breast cancers and their therapy, as well as fundamental mechanisms of cell growth and differentiation. Students supported by the training program had 22 peer-reviewed publications, a 400% increase over 1996. Based on the excellent progress of the training program, a proposal to the National Institutes of Health for continuation of the Program will be submitted June, 1998.

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INTRODUCTION

1. Brief Description of the Training Program and Its Objectives

The ongoing goal of the program is to establish at the University of Texas Health Science Center in San Antonio an in-depth training program in the Molecular Genetics of Breast Cancer. The most important goal of the program is to train highly qualified Ph.D. students in the genetic, cellular, and molecular basis of Breast Cancer. It is our expectation that the background in Breast Cancer Biology these students obtain will lead to significant future discoveries. The training program is conducted within the Molecular Medicine Ph.D. Program by a select group of faculty whose research projects are relevant to breast cancer. An additional goal of the program is to promote synergistic interactions between the various laboratories engaged in breast cancer research. Toward this end, a Breast Cancer Minisymposium for students in the training program was organized by leaders of the Training Program. The agenda (attached) featured a full day of seminars by the leading breast cancer researchers in San Antonio. Another important event is the Annual Breast Cancer Symposium held in San Antonio. All students supported by the program were required to attend. Finally, an outstanding Molecular Medicine Seminar Series sponsored by the Department of Molecular Medicine was also a requirement for all trainees. The following seminars in this series were pertinent to breast cancer:

G. Steven Martin	"Transformation by Src and Ras"
Winship Herr	"Transcriptional regulatory mechanisms"
Glenn D Preswich	"New affinity probes for cell signalling"
Robert Benezra	"Mitotic checkpoint controls"
Richard Baer	"The functional properties of BRCA1"
Alan M. Weiner	"A viral model for chromosome fragility"
Michael Lieber	"Site-specific recombination"
Larry H. Thompson	"Recombination repair in mammalian cells."

One of the major strengths of the program is the high quality of the Program faculty, and the interactive nature of the Breast Cancer research community in San Antonio. The program faculty are organized into four subprograms, which encompass scientists and physicians studying different aspects of breast cancer and cancer therapy, as well as fundamental mechanisms of cell growth, differentiation and molecular genetics. These faculty groupings are listed here, detailed descriptions of individual research programs were included in the original application.

A Breast Cancer Sub-Program

C. Kent Osborne, M.D.
John Chirgwin, Ph.D.
Suzanne Fuqua, Ph.D.
E. Lee, Ph.D.
W.-H. Lee, Ph.D.
Z. Dave Sharp

B. Growth Factor Sub-Program

Douglas Yee, M.D.
Gregory Mundy, M.D.
Robert J. Klebe, Ph.D.
Betty Sue Masters, Ph.D.

C. Drug Development Sub-Program

Daniel Von Hoff, M.D.

D. Molecular Genetics Sub-Program

Robin Leach, Ph.D.
Peter O'Connell, Ph.D.
Alan E. Tomkinson, Ph.D.
Robert J. Christy, Ph.D.

Each of these faculty members maintains an active research program. A listing of their research support is found below.

In this progress report, the relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program is reviewed, and additional or updated information is provided regarding:

Research Support for Program Faculty
Listing of Supported Trainees
Project Summaries of upper level trainees
Appendix: Reprints of Trainee Publications

2. Relationship between the Breast Cancer Training Program and the Molecular Medicine Graduate Ph.D. Program

The Breast Cancer Training Program was implemented within the context of the Molecular Medicine Graduate Ph.D. Program. The Molecular Medicine Ph.D. Program is a recently established interdisciplinary Ph.D. training program in the Graduate School of Biomedical Sciences at the UTHSCSA. For the academic year 1995-96, there was a total of 26 students enrolled in the Molecular Medicine Ph.D. Program -- 24 Ph.D. and 2 M.S. Of those 25 students, only six are supported by the Training Program in the Molecular Basis of Breast Cancer.

The Breast Cancer Training program takes advantage of the internationally recognized breast cancer research program existent in the institution for many years, and offers a unique opportunity for students interested in starting careers in breast cancer research. The participating scientists in this breast cancer program represent diverse departments including the Divisions of Medical Oncology, Hematology and Endocrinology in the Department of Medicine, and the Departments of Cellular and Structural Biology, Pathology and Biochemistry. In addition, the new University of Texas Institute of Biotechnology and the San Antonio Cancer Institute [SACI], an NIH-designated Cancer Center, represent outstanding resources for training opportunities in clinical and basic science research. The national and international reputation of the participating faculty serve to attract a large number of excellent applicants to the breast cancer research track in the Molecular Medicine program. The continuation of a Breast Cancer Specialized Program of Research Excellence (SPORE) grant to the institution documents the quality of breast cancer research available to trainees.

The rationale for administering the breast cancer training program in the Molecular Medicine Ph.D. program is based on several important criteria: [1] The Molecular Medicine curriculum is specifically designed to provide basic science training while integrating fundamental principles of molecular biology with modern medicine. A Molecular Medicine Core course provides students with the mechanisms underlying human disease and provides intensive review of specific diseases [including breast cancer] that may serve as models for how human diseases can be studied at the molecular genetic level. [2] The Molecular Medicine program requires the participation of both clinical and basic scientists in the training process. The inclusion of MDs on all student advisory committees insures that every graduate has a clear perspective on the clinical relevance of the basic research in their program, that in

most instances, will serve as a guide for the project. [3] The Molecular Medicine program is an interdepartmental, interdisciplinary program that offers flexibility to students in terms of research laboratories, advisors and committee members. This arrangement offers a real potential for synergism in breast cancer research not possible in traditional department-bound programs. In summary, our program offers a near perfect environment for Ph.D. training in breast cancer and has attracted many well-qualified applicants.

3. Research Support for Program Faculty

An essential component of maintaining a successful and aggressive training program in Breast Cancer Research is the continued research funding of the individual Program Faculty laboratories. Current funding for each member of the Program faculty is detailed in the attached table. As can be readily seen from the table, the faculty have been extremely successful in obtaining research funding, including over $10,725,133 in direct costs.

4. Listing of Supported Trainees

Trainees receiving support from the Training Program in the Molecular Basis of Breast Cancer Research are selected from among entering first year students in the Molecular Medicine Ph.D. Graduate Program. In subsequent years of their training, they may be maintained on the Training Program, or transferred to other funding sources, depending on the nature of their research interests, and the availability of grant support. The following trainees were supported on the Breast Cancer Training Program

Reporting Period 09/23/96 to 09/22/97

09/23/96 to 07/01/97
Upper Level Students

*Jerry Alan Bates
*Jill Gilroy
Shang Li
Zachary Mackey
*Jonathen Mlocek
*Hongyi Pan

07/01/97 to O9/22/97
Upper Level Students

*Jackie Lin
*Frank Yuan, M.D.
*Qing Zhong
Hongyi Pan
Shang Li
*Ashby Morrison

* New to the program this year, see report below.

Record of Previous Year's Trainees:

Jim Fitzgerald	Graduated from the program with an M.S. degree.
Christa Hargraves	Left the program for academic reasons.
Zachary Mackey	Continues in the program as an upper level student [see report below].
Harold Pestana	Left the program for academic reasons.

Yuewei Qian	Graduated from the from the program with a Ph.D. Postdoc in James Maller's laboratory at the Howard Hughes Medical Institute at The University of Colorado School of Medicine. Dr. Qian's research involves understanding the cell cycle and cell proliferation. This is a problem that is relevant to all types of cancer, including those of the breast.
James Wang	Continues in the Molecular Medicine Ph.D. Program, currently funded by advisor's grant. Although no longer in the Training program, his work on the mechanism of viral latency is important in some cancers.
Linda deGraffenried	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
Jennifer Gooch	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
David Levin	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below].
Ernesto Salcedo	Continues in the Molecular Medicine Ph.D. Program as an upper level student [see report below]. Ernesto was removed from the training grant since he elected to pursue work in a non-program facutly's laboratory [Dr. Steve Britt].
Jerry Alan Bates	Continues in the Molecular Medicine Ph.D. Program as Masters Student in the laboratory of Dr. Robert Clark, Professor and Chair of the Department of Medicine whose work in on signal transduction. See below for Mr. Bates work on breast cancer in Dr. Yee's laboratory where he worked before changing to Dr. Clark's laboratory.
Jill Gilroy	Continues in the Molecular Medicine Ph.D. Program as Ph.D. Student in the laboratory of Dr. Hanna Abboud, Professor and Chief of the Nephrology Division in Department of Medicine. Ms. Gilroys work centers on signal transduction in kidney development.
Jonathen Mlocek	Resigned from the Molecular Medicine Ph.D. Program for personal reasons.

The 1996-1997 academic year marks the fourth full year of operation for the Molecular Medicine Ph.D. Program, and the third for the Training Program in the Molecular Basis of Breast Cancer Research. The availability of highly qualified applicants to the Molecular Medicine Program has proven to be excellent. Over 150 applications were received for admission to the Fall 1996 entering class. Eight students began classes in August of 1995. The total number of students at the start of the Fall semester, 1995 the Molecular Medicine Ph.D. Program at all levels was 26, which includes 12 women, and 3 minorities (1 black, 2 Hispanic students). All three minority students were supported by the Training Program in the Molecular Basis of Breast Cancer Research.

5. Project Summaries of Upper Level Trainees

Linda DeGraffenried ***Mentor -- Dr. Suzanne Fuqua***

Tamoxifen is an effective therapy for estrogen receptor (ER)-positive breast cancer patients, however almost all women will eventually become resistant and fail this hormonal therapy. Clinical data suggests that in some patients, tamoxifen might actually stimulate tumor proliferation. To understand one potential mechanism for the stimulatory effects of tamoxifen, we have studied regulation of the rat prolactin promoter because tamoxifen is known to increase prolactin levels in rat pituitary cell lines. The rat prolactin promoter contains four pit-1 transcription factor binding sites which are important in its regulation. In addition, there is a

nonconsensus estrogen response element in the proximal region of the promoter which may play a role in the hormonal regulation of this gene. We have analyzed the hormonal regulation of the rat prolactin promoter using transient transactivation assays in human breast cancer cells. In the absence of pit-1 expression, the rat prolactin promoter was induced by tamoxifen ten-fold in ER-positive MCF-7 cells, but not ER-negative MDA-MB-231 cells. Estrogen did not induce this promoter in the absence of Pit-1. In the presence of Pit-1, the rat prolactin promoter was induced by estrogen, but not tamoxifen in MCF-7 cells. We are currently examining whether tamoxifen stimulation of the rat prolactin is working through a classical estrogen response pathway, or a novel mechanism such as a putative AP-1 site buried within the four pit-1 binding sites. We hypothesize that the rat prolactin promoter may serve as a model for cell and type-specific tamoxifen agonist effects in human breast cancer cells.

Ms. De Graffenried's current project is to determine the cis-acting sequences responsible for the regulation of the human estrogen receptor gene. Deletion and site-directed mutagenesis of the ER promoter combined with transient transfection assays have revealed elements located both proximal and distal to the transcription start site are responsible. A detailed elucidation of these elements as well as the DNA-binding proteins that mediate transcriptional response will be characterized.

This project is directly relevant to breast cancer. Elucidating the basis for tamoxifen resistant and regulation of the ER are both important issues in breast cancer research.

David Levin ***Mentor -- Dr. Alan Tomkinson***

DNA joining events are required to maintain the integrity of the genome. Three human genes encoding DNA ligases have been identified. David is identifying the cellular functions involving the product of the *LIG1* gene. Previous studies have implicated DNA ligase I in DNA replication and some pathways of DNA repair. During DNA replication, DNA ligase I presumably functions to join Okazaki fragments. However, under physiological salt conditions, DNA ligase I does not interact with DNA. It is Mr. Levin's working hypothesis that DNA ligase I involvement in different DNA metabolic pathways is mediated by specific protein-protein interactions which serve to recruit DNA ligase I to the DNA substrate. To detect proteins that bind to DNA ligase I, David has fractionated a HeLa nuclear extract by DNA ligase I affinity chromatography. PCNA was specifically retained by the DNA ligase I matrix. To confirm that DNA ligase I and PCNA interact directly, Mr. Levin found that *in vitro* translated and purified recombinant PCNA bind to the DNA ligase I matrix. In similar experiments, he has shown that DNA ligase I interacts with a GST (glutathione S transferase)-PCNA fusion protein but not with GST. Using *in vitro* translated deleted versions of DNA ligase I, Mr. Levin determined that the amino terminal 120 residues of this polypeptide are required for the interaction with PCNA. During DNA replication PCNA acts as a homotrimer that encircles DNA and tethers the DNA polymerase to its template. He showed that DNA ligase I forms a stable complex with PCNA that is topologically linked to a DNA duplex. Thus, it appears that PCNA can also tether DNA ligase I to its DNA substrate. A manuscript describing these studies has been published in the *Proc. Natl. Acad. Sci. U.S.A.*

In addition to interacting with PCNA, the amino terminal domain of DNA ligase I also mediates the localization of this enzyme to replication foci. To determine whether these are separable functions David fine mapped the region that interacts with PCNA and, in collaboration with Dr. Montecucco's group, the region required for recruitment to replication foci. Since the same 19 amino acids are necessary and sufficient for both functions and the same changes in amino acid sequence inactivate both functions, we conclude that DNA ligase I is recruited to replication foci by its interaction with PCNA. A manuscript describing these studies has been submitted to *Molecular Cell.*

This project is relevant to breast cancer since problems with DNA replication and repair will undoubtedly be involved in the development of all tumors at some stage in their progression.

Publications;

Mackey, Z.B., W Ramos, **DS Levin**, CA Walter, JR McCarrey and AE Tomkinson. 1997 An alternative splicing event, which occurs in mouse pachytene spermatocytes, generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. *Molec. Cell. Biol.* **17**, 989-998.

Tomkinson, A.E. and **DS Levin** Mammalian DNA ligases. *Bioessays.* **18**, 803-901 (1997)

Levin, D.S. W Bai, N Yao. and M O'Donnell and AE Tomkinson. 1997 Interaction between DNA ligase I and Proliferating Cell Nuclear Antigen; implications for Okazaki fragment DNA metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 94, 12863-12868.

Montecucco, A., R Rossi, **DS Levin**, R Gary, MS Park, TA Motycka, G Ciarrocchi, A Villa, G Biamonti and AE Tomkinson. DNA ligase I is recruited to sites of DNA replication by an interaction with proliferating cell nuclear antigen: Identification of a common targeting mechanism for the assembly of replication factories. (Submitted, 1998)

Shang Li ***Mentor -- Dr. Wen-Hwa Lee***

Mutations of the *BRCA1* gene predisposes women to the development of breast cancer. The *BRCA1* gene product [BRCA1] is a nuclear phosphoprotein that is mislocated to the cytoplasm of breast cancer cells. To understand the basis of its cellular partitioning and function, one of Mr. Li's project is to identify BRCA1-interacting proteins, confirm their *in vivo* interactions and to elucidate their relevance to the development of breast cancer. First, site-directed mutagenesis identified the functional nuclear localization sequence of BRCA1. Second, he used the yeast two-hybrid assay to identify BRCA1-interacting proteins. From a human B-lymphocyte cDNA library, he identified four clones [hBRAPs] that encode polypeptides capable of interacting with BRCA1. When compared to the currently available GenBank, he found that one is novel, one has homology to an uncharacterized zinc-finger domain-containing protein, and two bear sequence homology to previously cloned cDNAs. Interestingly, the sequence of hBRAP21 is identical to that of the nuclear localization signal receptor hSRP1α, also known as importin-α or karyopherin-α. He is also analyzing another of the clones which appears to encode a protein located in the cytoplasm which he postulates may be a tethering BRCA1. This would be very significant to the issue of cytoplasmic mislocation of BRCA1 in advanced breast cancer cells. The last of Mr. Li's projects is the identification of proteins that interact with the C-terminal region of BRCA1. This domain is notable for the presence of BRCT repeats, sequences frequently found in proteins involved in DNA repair and cell cycle control.

This project is directly relevant to breast cancer since it involves the study of a protein whose malfunction or mislocation leads to tumor development in the mammary gland.

Publications:

1. **Chen C-F, S. Li, Y. Chen**, P-L Chen, ZD Sharp, and W-H Lee. 1996 The Nuclear Localization Sequences of the *BRCA1* Protein Interact with the Importin-a Subunit of the Nuclear Transport Signal Receptor. **J. Biol. Chem.**, 271: 32863-32868 *Note: The three authors in bold contributed equally to this work.*

2. Liu, C. Y., A. Flesken-Nikitin, **S. Li,** Y. Y. Zeng, and W. H. Lee. 1996. Inactivation of the mouse *Brca1* gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. Genes Dev. **10**:1835-1843.

3. **Li S**, C-Y Ku, A. Farmer, Y-S Cong, C-F Chen, and W-H Lee. Identification of a novel cytoplasmic protein that specifically binds to nuclear localization signal motifs. In Press -- J. Biol. Chem. (1998).

Zachary Mackey ***Mentor -- Alan Tomkinson***

DNA joining events are required to maintain the integrity of the genome. Three human genes encoding DNA ligases have been identified. In this project we are intending to identify the cellular functions involving the product of the LIG3 gene. Mammalian cell lines with reduced DNA ligase III activity exhibit spontaneous genetic instability and increased sensitivity to DNA damaging agents. We have cloned human and mouse cDNAs encoding DNA ligase III. In both mouse and humans, we have identified two forms of DNA ligase III cDNA that differ at their 3' end and encode polypeptides with different C-termini. At the site where the cDNA sequences diverge, the nucleotide sequence resembles consensus splice donor/acceptor sequences. We have confirmed that these cDNAs represent alternatively spliced products from the same gene by cloning and analysis of the 3' end of the mouse LIG3 gene. Analysis of DNA ligase III expression by northern blotting demonstrated that this gene is highly expressed in the testes. Using RT-PCR, we have examined the expression of the two forms of DNA ligase III cDNA in mouse tissues and cells. One form of DNA ligase III mRNA, DNA ligase III-a is ubiquitously expressed. In contrast, expression of DNA ligase III-b mRNA is restricted to the testis. During spermatogensis, DNA ligase III-b mRNA expression occurs during the latter stages of meiotic prophase. This restricted expression pattern suggests that DNA ligase III-b mRNA may have a specific role in the completion of meiotic recombination. In support of this idea we have shown that DNA ligase III-a interacts with the DNA strand break repair protein Xrcc1 whereas DNA ligase III-b does not. We suggest that the DNA ligase III-a/Xrcc1 complex functions in DNA repair in both somatic and germ cells whereas DNA ligase III-b functions in meiotic recombination. A manuscript describing these studies has been published in Molecular and Cellular Biology.

A unique feature of the DNA ligases encoded by the LIG3 gene is an amino terminal zinc finger that binds to DNA single-strand breaks. Deletion of this motif does not inactivate the ability of DNA ligase III to complement the conditional lethal phenotype of an E. coli DNA ligase mutant nor does it effect in vitro DNA joining. Using site-directed mutagenesis, we have identified amino acid residues within the catalytic C-terminal domain that are required for interaction with nicked DNA. Our current working model is that the zinc finger functions in vivo to displace another enzyme, poly (ADP-ribose) polymerase (PARP) from the nicks. To provide support this model, we will reconstitute reactions in vitro with nicked DNA, PARP, Xrcc1 and DNA ligase III.

This project is relevant to breast cancer since genomic instability is likely to be involved at many of the several stages of breast cancer progression leading to malignancy. Methods to intervene and stabilize the genome could prevent progression and spread of the disease. In addition, information about DNA repair processes in normal and cancer cells may lead to the development of treatment regimes that more effectively kill cancer cells and minimize damage to normal tissues and cells.

Publications:

Wang, Y.-C.J., WA Burkhart, **ZB Mackey**, MB Moyer, W Ramos, I Husain, J Chen, JM Besterman and AE Tomkinson. 1994 Mammalian DNA ligase II is highly homologous with Vaccinia DNA ligase. Journal of Biological Chemistry 269, 31923-31928.

Husain, I., AE Tomkinson, WA Burkhart, MB Moyer, W Ramos, **ZB Mackey**, JM Besterman and J Chen. 1995 Purification and characterization of DNA ligase III from bovine testes. Journal of Biological Chemistry 270, 9683-9690.

Chen, J., AE Tomkinson, W Ramos, **ZB Mackey**, S Danehower, RA Schultz, JM Besterman and I Husain. 1995 Mammalian DNA ligase III: Molecular cloning, chromosomal localization and involvement in meiotic recombination during spermatogenesis. Molec. Cell. Biol. 15, 5412-5422.

Mackey, Z.B., W Ramos, DS Levin, CA Walter, JR McCarrey and AE Tomkinson. 1997 An alternative splicing event, which occurs in mouse pachytene spermatocytes, generates a form of

DNA ligase III with distinct biochemical properties that may function in meiotic recombination. Molec. Cell. Biol. 17, 989-998.

Tomkinson, AE and **ZB Mackey**. Structure and Function of Mammalian DNA ligases. Mutation Research. (In press, 1998).

Hongyi Pan ***Mentor -- Dr. Wen-Hwa Lee***

Mutations in the breast cancer susceptibility gene, *BRCA1*, is involved in the development of hereditary breast cancer. The *BRCA1* gene product [BRCA1] is a nuclear phosphoprotein that is mislocated to the cytoplasm of breast cancer cells. To understand the basis of its cellular partitioning and function, one of Mr. Pan's project is to identify BRCA1-interacting proteins. One protein identified in this screen, named AP12, is a zinc-finger-containing protein. Since AP12 has the hallmarks of a Krab-domain repressor protein, Mr. Pan first identified the recognition sequence necessary for DNA-binding. Next, he inserted this sequence into mammalian reporter constructs and demonstrated that AP-12 can, indeed, repress transcription. He is currently, performing experiments to determine if BRCA1 can influence AT-12-mediated repression. The hypothesis under test is that BRCA1 can influence positively or negatively the expression of a repertoire of AP12-regulated genes. This control function may be important in BRCA1-mediated suppression of breast cancer.

This project is relevant to breast cancer since BRCA1 function is hypothesized to be involved in suppressing the formation of breast cancer.

Qing Zhong ***Mentor -- Dr. Wen-Hwa Lee***

Mr. Zhong's project in Dr. Lee's laboratory is a study of the tumor suppressor protein, TSG101. *tsg101* was identified as a tumor susceptibility gene by homozygous function inactivation of allelic loci in mouse 3T3 fibroblasts. To confirm its relevance to breast cancer that was originally reported, antibodies specific for the putative gene product were prepared and used to identify cellular 46 kDa TSG101 cytoplasmic protein. A full size 46 kDa TSG101 protein was detected in a panel of 10 breast cancer cell lines and 2 normal breast epithelial cell lines with the same antibodies. A full-length *TSG101* mRNA was also detected using rtPCR. These results indicate that homozygous intragenic deletion of *TSG101* is rare in breast cancer cells.

This work is important for breast cancer research since it indicated that TSG101 may not be an important component for suppressing tumorigenesis, at least directly, since it is present in all the breast cancer lines.

Qing Zhong, CF Chen, Y Chen, PL Chen, and WH Lee 1997 Identification of Cellular TSG101 protein in multiple human breast cancer cell lines. Cancer Res. 57, 4225-4228.

Jerry A. Bates ***Mentor -- Dr. Doug Yee***

The IGFs stimulate breast cancer cell proliferation by interacting with specific cell surface receptors and initiating an intracellular signaling cascade. Recently, a new member of the IGF signaling pathway, grb10, has been cloned. Unlike other IGF signaling molecules, it appears that grb10 inhibits IGF action. To study the function of grb10, Alan created breast cancer cells that would allow the inducible expression of this protein after exposure to doxycycline. He first transfected MDA-435 and MCF-7 cells with a fusion transactivator (RTA), then selected stable clones proved to be inducible by transient transfection with a luciferase expression vector. He created a grb10 expression vector that allowed dual expression of grb10 and β-galactosidase. He then transfected individual RTA clones with the grb10/βgal vector. While he was able to show excellent induction of β-galactosidase, grb10 was not detected by immunoblot. It is possible that these cells are sensitive to the inhibitory effects of grb10, and would not tolerate even a very low level of basal expression. We are continuing to use the cells created by Alan to examine grb10 expression using the single reporter construct.

This project was relevant to breast cancer since IGF signalling may be important for stimulating the growth of breast cancer cells.

Ashby Morrison ***Mentor -- Dr. Kent Osborne***

Ms. Morrison worked in three labs, breast cancer research being the primary area of research in each lab. My first lab rotation, which was in the lab of Peter O'Connell, Ph.D., She was involved in the preliminary work of locating a gene that when mutated may be involved in process of metastasis. The second lab rotation was done in the lab of Jolene Windle, Ph.D. During the months I spent in this lab I was exposed to the technique of using mouse models to study breast cancer. Specifically, my project involved transgenic and knockout mice to research the effects of oncogenes and tumor suppressors on breast cancer development. During her third lab rotation, in the lab of Kent Osborne, MD., Ms. Morrison was involved in a more clinical area of breast cancer research. Her project was to study the effects of varying levels of estrogen receptor coactivators and corepressors during tamoxifen treatment. Ms. Morrison was accepted into Dr. Osborne's laboratory where she continues to make good progress on the identification of estrogen receptor-associated proteins that are hypothesized to be co-activator/repressor proteins.

Jennifer Gooch ***Mentor -- Dr. Doug Yee***

Dr. Yee's laboratory is interested in the growth regulation of breast cancer cells by insulin-like growth factors (IGFs). Data from several laboratories had suggested that interleukin-4 (IL-4) and IGFs share common signaling pathways. Since it was known that IL-4 could directly inhibit breast cancer cell proliferation, Jennifer began examining the potential overlap of growth stimulatory and growth inhibitory signaling pathways in breast cancer cells.

Ms. Gooch first confirmed that IL-4 was inhibitory for breast cancer cells. This inhibition was dependent on expression of the IL-4 receptor and blocking antibodies to the receptor neutralized the effects of IL-4. She discovered that IL-4's growth inhibitory effects were dependent on cell proliferation. Quiescent cells were not affected by IL-4. Moreover, IL-4 induced apoptosis in estradiol-stimulated cells. She documented apoptosis by morphologic change, TUNEL assay, PARP cleavage, DNA laddering and generation of a sub-G1 peak by flow cytometry. Thus, she has shown that IL-4 inhibits breast cancer cell growth by inducing apoptosis to some, but not all, growth stimuli.

Because IL-4 and IGF-I share a common signaling pathway through insulin receptor substrate protein-1 (IRS-1), it is possible that this molecule coordinates both growth promoting and cell death signals. It is also possible that additional signals generated by IL-4 are responsible for its growth inhibitory effects. To date, she has documented Stat-6 activation by IL-4. She has shown that IL-4 treatment induces Stat-6 binding to a synthetic oligonucleotide in gel mobility shift assays. She has also shown that IRS-1 is activated by IL-4 in responsive cell lines. However, IL-4 differs from IGF-I in its kinetics of IRS-1 activation. While IGF-I rapidly phosphorylates IRS-1 to high levels followed by rapid dephosphorylation, IL-4 causes tonic levels of IRS-1 to appear in the cell. Furthermore, it appears that IRS-1 is rapidly degraded after IGF-I treatment, while such degradation does not occur after IL-4. Preliminary evidence suggests that IRS-1 may be ubiquitinated after IGF-I treatment, but not IL-4. Her future projects involve the detailed characterization of these pathways and determination of their contribution to IL-4's growth inhibitory effects.

Finally, she has shown that interferon-gamm (IFNg) stimulates Jak/Stat activation in human breast cancer cells. As in other epithelial tumors, activation of Stat-1 and Stat-3 appear to be growth inhibitory compared to their function in lymphocytes.

This project is relevant to breast cancer since intracellular signaling pathways are almost certainly involved in the growth stimulation at some stage of mammary cell tumor development

or progression. Since growth inhibitory (IL-4) and growth stimulatory (IGF-I) pathways may be coordinated through a single molecule, the precise definition of the mechanism of IL-4 action, as compared to IGF-I action, could define molecular targets to inhibit breast cancer cell growth.

Lee AV, Jackson JG, **Gooch JG**, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Synergistic enhancement of insulin-like growth factor signaling in human breast cancer: Estrogen regulation of insulin receptor substrate-1 *in vitro* and *in vivo.* Submitted.

Gooch JL, Lee AV, Yee D. Interleukin-4 induces growth inhibition and apoptosis in human breast cancer cells. Submitted.

Yee D, **Gooch JL**, Jackson JG. IGF-I, insulin, and IL4 activate IRS1 in human breast cancer cells: Differential IRS1 tyrosine phosphorylation by IGF-I is associated with increased MAPK and P13K activation. Proc AACR 38: A2910, 1997.

Jackson JG, **Gooch J**, Yenushe L, White MF, Lee AV, Yee D. Expression and activation of insulin receptor substrate-1 and -2 (IRS-1 and -2) in human breast cancer cells. 78th Annual Meeting of the Endocrine Society, 1996.

Gooch JL, Yee D, Lee AV. Ligand dependent degradation of insulin receptor substrate-1 in human breast cancer. Submitted to 1998 Endocrine Society Annual Meeting.

Lee AV, Jackson JG, **Gooch JL**, Hilsenbeck SG, Coronado-Heinsohn E, Osborne CK, Yee D. Enhancement of the insulin-like growth factor signaling pathway by estrogen in human breast cancer. Submitted to 1998 Endocrine Society Annual Meeting.

Gooch JL, Jackson JG, Yee D. Interluekiin-4 induced apoptosis is associated with STAT6 activation, IRS-1 phosphorylation, and activation of the SAPK pathway in human breast cancer cells. Accepted at the 1998 Keystone Symposium on Signal Transduction.

Jill Gilroy ***Mentor -- Dr. Doug Yee***

STATs, signal transducers and activators of transcription, are activated in the cytosol by tyrosine phosphorylation, and are translocated to the nucleus where they bind specific DNA sequences. STATs are downstream signalling molecules of Interferon α and γ, as well as many other growth factors. Tyrosine phosphorylation and dimerization of the STATs are necessary for DNA binding. In my rotation I was trying to identify if binding of STAT α to its DNA binding element reflect transcriptional activation. This was done by transfecting cells with a plasmid containing the DNA binding element, 4X SIE, as well as the luciferase gene. A Beta-galactosidase plasmid was cotransfected for an internal control. The inducible effects of transcription by growth factors, Interferon gamma and platlet derived growth factor, were also done in this system.

Also, we wanted to determine if the interaction between PDGF receptor and STAT 1α is mediated through the SH2 domain of the STAT 1α. This was done by amplifying the SH2 domain and cloning it into a GST fusion vector. Tyrosine kinase binding assays were performed and confirmed this event.

This project is relevant to breast cancer since signal transduction pathways are a vital part of tumorigenesis.

Frank Yuan, M.D. ***Mentor -- Dr. Eva Lee***

The response of mammalian cells to DNA damage is complex, involving cell cycle arrest, DNA repair and, under certain conditions, apoptosis. Cells from individuals with the recessive disorder ataxia telangiectasia (AT) are hypersensitive to ionizing radiation. ATM (mutated in AT) protein contains a PI-3 kinase domain and is predominantly localized in the nucleus. c-Abl, a non-receptor tyrosine kinase, interacts with ATM and is a substrate of ATM kinase. Dr. Yuan

demonstrated that ATM, c-Abl, and Rad51, a homologue of bacterial RecA protein required for DNA recombination and repair, can be co-immunoprecipitated from cell extracts. c-Abl interacts with and phosphorylates Rad51 *in vitro*. This phosphorylation enhances complex formation between Rad51 and Rad52, which functions with Rad51 in recombination and repair. After γ-irradiation, an increase in both tyrosine phosphorylation of Rad51 and association between Rad51 and Rad52 occurs in wild-type cells but not in $ATM^{-/-}$ or $c\text{-}Abl^{-/-}$ cells. These findings implicate the ATM/c-Abl signaling pathway in promoting the assembly of the recombinational repair machinary. This work is submitted to Nature.

This study is relevant to breast cancer since defects in ATM are likely to predispose individuals to the development of breast cancer.

Suh-Chin(Jackie) Lin ***Mentor -- Dr. Eva Lee***

The tumor suppressor gene, p53, is frequently mutated in human tumors, including breast carcinoma. P53 null mice develop multiple spontaneous tumors, predominantly lymphoma and sarcoma, within the first 6 months of age. To establish a mouse model of p53-mediated mammary tumor development, a bigenetic approach employing the cre-loxp system was initiated by Ms. Lin. Through gene-targeting in embryonic stem (ES) cells, mice carrying floxed p53 genes in which exons 5 and 6 are flanked by the loxp sequence were generated. A second mouse line carrying a cre transgene under the control of mouse mammary tumor virus LTR (MMTV-cre) has also been generated. Floxed p53 mice are being mated with MMTV-cre transgenic mice to produce mice with p53 inactivation in the mammary tissue. This bigenetic approach should provide a mouse mammary tumor model for studies of mammary tumor propagation resulting from p53 mutation and potential therapeutic interventions.

This project is relevant to breast cancer since p53 is frequently mutated in sporadic cases of breast cancer.

Lin, S-C. J., S. X. Skapek, and E. Y.-H. P.Lee Genes in the RB pathway and their knock in mice. Seminars in Cancer Biology 7:279-289, 1996

6. Changes to the Program Faculty: None this year

7. Course Changes: None this year.

8. Appendix: Reprints of Trainee Publications.

CONCLUSIONS: The Breast Cancer Training Program continued to make excellent progress toward attracting and retaining excellently qualified students in breast cancer research. The students are receiving a high level of training in the modern research methods and theory. A total of 22 publications on breast cancer was achieved by students supported by the program. Combined with the basic instruction they receive in the Molecular Medicine Ph.D. Program, they will graduate as highly skilled researchers who will be very competitive for post doctoral positions in the premiere breast cancer laboratories in the world. Based on the excellent progress of the training program, a proposal to the National Institutes of Health for continuation of the Program will be submitted June, 1998.

OTHER SUPPORT

Funding Agency	Title & Grant Number	Project Period	Current Direct Costs
Chirgwin, J.M.			
VA	Associate Research Career Scientist	04/01/94-03/31/98	41,351
DOD	Role of Autocrine Motility Factor in Osteolytic Metastasis	01/01/98-12/31/00	63,391
VA (pending)	PTHrP and Prostate Cancer Metastasis to Bone	03/01/98-02/29/01	96,100
NIH (pending)	Coagulation Factor XIII a Chain: Mechanism of Secretion 1 R01 HL58588	07/01/96-06/30/01	164,004
NIH (pending)	Regulation of Renin in Preeclamptic Hypertension 1 R01	07/01/98-06/30/01	54,533
NIH (pending)	Endosomal Proteolytic Processing of Prorenin in Decidna 1 R01	04/01/98-11/30/01	15,750
Christy, R.J.			
Juvenile Diabetes Foundation International	Dietary and Hormonal Regulation of the Stearoyl-COA Desaturase 1 (SCDI) Gene	09/01/96-08/31/98	45,454
NIH	Insulin and Adipose Cell Committment	05/01/93-04/30/98	71,798
Fuqua, S.A.W.			
NIH/NCI	SPORE in Breast Cancer, Project 1 Clinical Tamoxifen Resistance: Mechanisms and New Agents 5 P50 CA58183-05	09/30/95-07/31/00	184,867
NIH/NCI	SPORE in Breast Cancer, Project 2 Heat Shock Proteins and Drug Resistance 5 P50 CA58183-05	09/30/95-07/31/00	141,695
NIH/NCI	Hypersensitive Estrogen Receptor in Premalignant Breast Disease R01 CA72038-01	09/01/96-05/31/01	149,893
Department of Defense	New Mechanisms of Tamoxifen Resistance in Breast Cancer Patients DAMD17-94-J-4112	10/15/94-09/30/98	62,830
Department of Defense	Involvement of Heat Shock Proteins in Drug Resistance in Human Breast Cancer DAMD17-95-1-5025	01/05/95-09/30/98	68,080

NIH/NCI	Markers of Breast Cancer Evolution and Progression Program Project 2 Molecular Variants and Overexpression of ER in Clinical Breast Cancer Development 5 P01 CA30195-17	07/01/97-06/30/02	166,162
NIH/NCI	Markers of Breast Cancer Evolution and Progression Program Project 3 Development and Prognostic Factors in Premalignant Breast Disease	07/01/97-06/30/02	146,012
NIH/NCI	Training Program for Translational Breast Cancer T32 CA70091-01	09/01/96-06/30/01	82,008
Department of Defense (pending)	The Role of Estrogen Receptor B in Breast Tumor Progression	09/30/98-09/29/01	69,927
Klebe, R.J.			
NIH	Initial Events in Bone and Tooth Morphogenesis 2 R01 DE08144-08	07/01/88-06/30/98	189,129
Leach, R.J.			
Genetech	Chromosome 8 Radiation Hybrid Mapping Panel No Grant Number	03/26/92-01/01/99	4,505
NIH	Saturation Mapping of Human Chromosome 3, Project 2 5 P01 HG00470-05	06/01/92-05/31/98	228,790
MacDonald Microsoft	Brian MacDonald 18Q Research No Grant Number	10/20/93-01/01/99	165,193
Aging Research and Education Center	Identification of a Gene for Paget Disease on Human Chromosome 18	01/01/97-08/31/97	10,000
NIH	The Role of Aging in Autoimmune Myasthenia Gravis	03/01/97-02/28/98	72,500
Nathan Shock Aging Center	Subgrant 2 5 P30 AG13319-03	07/01/97-06/30/98	3,750

Lee, E.Y.-H.P.			
NIH/NHLBI	Tumor Suppressor Function of RB and P53 in the Mammary Gland 2 R01 CA49649-07	07/01/94-04/30/99	149,450
A-T Children's Project	ATM in Neurodegeneration D1047	03/15/97-03/14/99	50,000
The Council for Tobacco Research	Biological Function of the Retinoblastoma Gene in Small Cell Lung Carcinoma	08/08/97-12/31/98	30,435
Texas Higher Education Coordinating Board	ATM Protein in DNA Repair and in Breast Cancer Predisposition	01/01/98-12/31/99	52,798
NIH (pending)	ATM Signaling and Neurodegenration	04/01/98-03/31/03	172,081
NIH (pending)	Antigen and Antibody Production Shared Resource (Cancer Center Grant with SACI)	08/01/98-07/31/99	97,906
Lee, W.-H.			
NIH/NEI	Molecular Basis of Retinoblastoma Formation 5 R01 EY05758-14	03/01/93-02/28/98	236,286
NIH/NCI	Cancer Suppression by the Retinoblastoma Gene 5 R01 CA58318-05	05/01/97-04/30/98	170,958
NIH/NCI	SPORE in Breast Cancer Project 5 Tumor Suppressor Genes in Breast Cancer Development	08/01/97-07/31/98	144,559
NIH	Markers of Breast Cancer Evolution and Progression Project 5 BRCA-1 Malfunction in Breast Cancer	08/01/97-07/31/98	151,001
NIH/NEI (pending)	Molecular Basis of Retinoblastoma Formation Renewal of 5 R01 EY05758	03/01/98-02/28/99	255,947
American Institute for Cancer Research (pending)	The efficacy of Diet and Chemopreventatives on Cancer Progression in a Novel Mouse Model Mimicking Human Tumorigenesis	07/31/98-07/30/99	75,000

Masters, B.S.S.			
NIH	Prostaglandin 19- and 20-Hydorxylation by Cytochrome P450 GM31296	06/01/97-05/31/01	170,000
NIH	Structural & Functional Modularity in Nitric Oxide Synthase GM52419	04/01/96-03/31/00	134,000
Welch Foundation	Structure-Function Relationships in the FAD- and FMN-Containing Enzymes, NADPH-Cytochrome P450 Reductase and Nitric Oxide Synthase AQ1192	06/01/96-05/31/99	52,000
NIH(Pending)	Structural Determinants of FAD- and FMN- Requiring Enzymes HL30050	04/01/98-03/31/03	171,640
Mundy, G.R.			
NIH	General Clinical Research Center M01 RR01346	12/01/93-11/30/98	1,196,527
NIH	Training Program in Bone and Mineral Metabolism T32 AR07464	07/01/93-06/30/98	70,472
NIH	The Cytokines and Bone Cell Function R01 AR28149	04/01/97-03/31/98	105,703
NIH	Effects of Tumors on the Skeleton Project 3 Mechanisms of Bone Resorption and Hypercalcemia in Hematologic Malignancies 2 P01 CA40035	06/01/95-05/31/99	129,342
NIH	Effects of Tumors on the Skeleton Administrative Core	06/01/95-05/31/99	52,536
NIH (pending)	General Clinical Research Center M01 RR01346	12/01/98-11/30/03	8,201,846
NIH (pending)	Training Program in Bone and Mineral Metabolism T32 AR07464	05/01/98-04/30/03	494,525

O'Connell, P.			
NIH/NCI	Training Program in Academic Medical Oncology/Hemeatology 2T32 CA09434-10A1	05/01/93-04/30/98	57,700
NIH/NCI	San Antonio Cancer Institute 2 P30 CA541174	08/01/94-07/31/98	61,644
NIH	NIDDM Suspectibility Genes in Mexican Americans R01 DK47482	09/30/93-09/29/98	216,481
NIH/NCI	Translational Research in Breast Cancer 2 P50 CA58183	09/01/95-08/31/99	160,857
NIH	Genetic Epidemiology of NIDDM in Mexican Americans 2 R01 DK42273	04/01/96-03/31/01	418,134
NIH/NCI	Molecular and Genetic Epidemiology of Gliomas 2 P01 CA55261	01/01/96-12/31/01	81,433
NIH/NCI	Markers for Breast Cancer Evolution and Progression 4 P01 CA30195	07/01/97-06/30/02	101,955
NIH/NCI (pending)	San Antonio Cancer Institute 3 P30 CA541174 (renewal)	08/01/98-07/31/03	188,767
NIH (pending)	CCR5 Regulation and Promoter Variants in HIV-1 Infection	04/01/98-03/31/03	178,837
DOD Prostate Cancer Idea Development Award (pending)	Genetic Markers of Prostate Cancer Progression	10/01/98-03/31/01	381,478
Osborne, C.K.			
NIH/NCI	SPORE in Breast Cancer 5 P50 CA58183-04	09/30/95-07/31/00	1,749,018
NIH/NCI	Markers of Breast Cancer Evolution and Progression 5 PO1 CA30195-17	08/01/97-07/30/02	1,245,400
NIH/NCI	San Antonio Cancer Institute 5 P30 CA54174-04	08/01/94-07/31/98	56,414
NIH/NCI	Training Program in Academic Medical Oncology/Hematology 5 T32 CA9434-09	05/01/93-04/30/98	64,300
Susan G. Komen	Mechanisms of Tamoxifen Resistance No Grant Number	10/01/96-09/30/99	35,000

NIH/NCI	Physician Scientist Training Grant in Oncology K 12	09/01/97-08/31/02	349,364
Zeneca, Ltd.	A Double-blind Randomized Multicenter Trial Comparing the Efficacy and Tolerability of 125 and 250 MG of Faslodex in Post-Menopausal Women with Advanced Breast Cancer No Grant Number	11/01/96-04/31/99	67,000
NIH/NCI	Training Program in Academic Medical Oncology/Hematology 5 T32 CA9434	12/01/98-11/30/03	66,000
Sharp, Z.D			
NIH/NCI	SPORE Developmental Grant The role of the ATM protein in Human Breast Cancer 5 P50 CA58183-06	08/01/96-07/31/98	35,000
NIH	Transgneic Models Testing Role of DNA Damage in Aging	04/03/97-02/28/02	141,414
USAMRMC (pending)	The role of the BRCA1 C-terminal domain in breast cancer	10/01/98-09/30/99	70,000
NIH (pending)	Gene Expression Profiles in Breast Cancer	01/01/98-12/31/99	144,000
American Institute for Cancer Research (pending)	The Efficacy of Diet and Chemopreventatives on Cancer Progression in a Novel Mouse Model Mimicking Human Tumorigenesis	07/31/98-07/30/00	75,000
NIH (pending)	Nutritional Probe of the Aging Process	06/01/98-05/31/03	77,108
Tomkinson, A.E.			
NIH	Cellular Functions of Eukaryotic DNA Ligases R29 GM47251-03	08/01/97-07/31/98	51,392
The Council for Tobacco Research	DNA Nucleotide Excision Repair in Eukaryotes 3786A	01/01/97-12/31/98	43,478
NIH (pending)	Cellular Functions of Eukaryotic DNA Ligases Competing Continuation	08/01/98-07/31/03	121,064

Von Hoff, D.D.			
NCI	Phase I Clinical Trials of Anticancer Agents 1-U01 CA69853-02-S2	07/01/95-02/28/98	115,331
NCI/NIH	Telomere and Telomerase Interactive Agents 1 U19 CA67760-01	09/30/95-06/30/00	96,136
US Army MRMC	DNA Topoisomerase I-Targeted Therapy for Breast Cancer DAMD 17-96-6008	05/06/96-06/06/99	47,962
National Foundation for Cancer Research	Intermediates in Gene Amplification No Grant Number	10/01/92-09/30/98	10,000
Workshop on Methods in Clinical Cancer Research	1-R-25-CA68647-01A1	07/22/96-06/30/99	48,319
Yee, D.			
NIH/NCI	The IGF System as a Treatment Target in Breast Cancer 5 P01 CA30195-16	07/28/92-07/30/98	130,411
NIH/NCI	SPORE in Breast Cancer, Sub-project Career Development Awards in Translational Breast Cancer Research	09/01/95-07/31/00	154,022
San Antonio Cancer Institute	Inhibition of IGFBP-3 Expression in Human Cancer Cells No Grant Number	01/01/97-12/31/97	20,000
NIH/NCI	SPORE in Breast Cancer, Pilot Project to Develop a Model of Gene Therapy for the Prevention of Breast Cancer 5 P50 CA58183	09/01/96-07/31/98	31,548
NIH/NCI	SPORE in Breast Cancer, Supplemental funds for project enetiled Prevention of Breast Cancer by Adenovira Gene Transfer 5 P50 CA58183	08/01/97-07/31/98	103,448
NIH/NCI	Targeting the IGF System in Breast Cancer R01 CA74285-01	01/01/98-12/31/03	172,497

Department of Defense (pending)	IDEA Award Prevention of Breast Cancer by Adenoviral Gene Transfer	09/30/98-09/29/01	69,914
NIH/NCI (pending)	Enhancement of HSV-tk Gene Therapy in Breast Cancer R01 CA72621	04/01/98-03/31/99	136,682

U.S. Army Breast Cancer Training Grant Mini-Symposium
April 21, 1997

Location:	Room 3.046, Institute of Biotechnology
10:20 - 11:00 am	C. Kent Osborne, M.D. Professor, Department of Medicine *Introduction and Overview*
11:00 - 11:40 am	Peter O'Connell, Ph.D. Professor, Department of Pathology *Genetics of Breast Cancer Evolution*
11:40 - 12:20 pm	Richard M. Elledge, M.D. Assistant Professor, Department of Medicine *Hereditary Breast Cancer*
12:20 - 1:00 pm	Lunch will be provided
1:00 - 1:40 pm	Powel H. Brown, M.D., Ph.D. Assistant Professor, Department of Medicine *Screening and Prevention*
1:40 - 2:20 pm	Sami G. Diab, M.D. Assistant Professor, Department of Medicine *Surgery and Radiation Therapy*
2:20 - 3:00 pm	Gary M. Clark, Ph.D. Professor, Department of Medicine *Prognostic Factors*
3:00 - 3:20 pm	Break
3:20 - 4:00 pm	Suzanne A. Fuqua, Ph.D. Associate Professor, Department of Medicine *Estrogen Receptors*
4:00 - 4:40 pm	Peter M. Ravdin, M.D., Ph.D. Associate Professor, Department of Medicine *Adjunct Chemotherapy and Hormone Therapy*
4:40 pm	Wen-Hwa Lee, Ph.D. Professor and Director, Department of Molecular Medicine *Closing Remarks*