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Year 02 of the Undergraduate Training Program in Breast Cancer Research has been completed successfully. In addition to the eight students funded by this grant, two additional students were recruited and funded by the Dean of Pharmacy. Nine different mentors were involved. In the two years of the program thus far, 13 of the 22 participants have been women, who are underrepresented in the biomedical research community. Students carried out research in their mentors' laboratories, attended a weekly journal club, attended a weekly seminar of guest speakers, presented a formal oral report on their work, and wrote a final report. Three grant submissions, one pending and two funded, have resulted in part from work sponsored by this program. Participants have been listed as coauthors thus far on two presentations at meetings. Two program participants thus far are enrolled in graduate school in the life sciences.

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Introduction

This report describes progress in implementing the second year of a three-year summer undergraduate training program in breast cancer research at Purdue University in the School of Pharmacy & Pharmacal Sciences and the School of Veterinary Medicine.

Body of Report

Task 1: Publicity and Notification. We contacted a number of small colleges in the vicinity of Purdue and solicited applications to the Breast Cancer Program. In addition, professional students in the Purdue Schools of Pharmacy and Veterinary Medicine were notified both by a web site and by direct class visitations. We felt that the best possibility of recruiting minority students was to coordinate our efforts with those of the MARC-AIM program at Purdue.

Task 2: Schedule Seminar Speakers. Speakers were scheduled for a weekly seminar in Breast Cancer Research. For these seminars, faculty participating in the program gave talks directed to the undergraduate researchers about their research relevant to breast cancer research. In addition, a special seminar was presented by Professor Martin Tenniswood, an expert in Breast Cancer from Notre Dame University.

Task 3: Mail Publicity and Mount Web Site. (See also Task 1.) We also received contact information from the MARC-AIM program at Purdue and wrote to *all* qualified students who we thought might have an interest in the Breast Cancer program.

Task 4: Accept and Review Applications. Applications were accepted through February 21, 2003. As was the case in Year 01 of the program, our office was operating a more general undergraduate research program with about the same application deadlines. This program was funded by the Office of the Dean of the School of Pharmacy. This year the Dean's Program funded two additional positions. In other words, the Army grant funded eight positions, and the School funded two. *Over the past two years, the Army grant has leveraged support from other sources for a total of six additional undergraduate research opportunities* beyond the 16 supported on this grant.

Task 5: Make Offers to Students. Prof. Riese (the co-PI) and the PI, with consultation by other participating faculty, made offers to students in mid-March. We had no indication of interest by students from the MARC-AIM program. Despite out disappointing results with minority students, it should be pointed out that 13 of the 22 participants to date have been women, which are underrepresented in the research community. The high percentage of qualified women in the Purdue Pharmacy Program (almost 70%) provide fertile ground for recruiting women researchers.

Task 6a: Assign Students to Laboratories of Program Staff. This assignment was made at the time of offer; that is, students were informed with whom they would work.

Task 6b: Coordinate Assignment of Students with MARC/AIM Program. Because we had no indications in interest from MARC-AIM students, this task was not applicable.

Task 7: Complete Program Setup Tasks. Program setup tasks were accomplished prior to the start of the program on June 2.

Task 8: Preside over First Week of Program. An official meeting of students was held on June 2, 2003. Students who had not already done so were briefed on expectations for safety, seminar attendance, and reports. A barbecue was held at the home of the PI later in the summer.

Task 9: Conduct Program. The program was conducted in the manner described in the proposal. Students took part in a weekly journal club, a weekly seminar by a guest speaker, and in weekly laboratory meetings.

Tasks 10 & 11: Final Reports. Students were scheduled about one month in advance for their final reports, and a letter was sent to each describing expectations for their final written report. Each student presented a 15-minute PowerPoint seminar describing his/her work in a public seminar. Each student also provided the PI and Prof. Riese with a short report summarizing their research and evaluating the program in answer to specific questions. Summaries of the research conducted by each student are provided at the end of this report.

Task 12: Review Students' and Members Final Reports. Student final reports were due, and were received, on or before September 1, 2003. Faculty mentors were asked for, and provided, a short summary report and evaluation in April 2004.

Task 13: Survey Students about Attitudes and Career Choices. As part of the summary report, students were asked about their attitudes toward research, and whether they were contemplating a career path in research.

Task 14: Make Yearly Report to Breast Cancer Program. This report fulfills this task.

Summary Reports of Key Research Accomplishments

Names of Students, Home Institutions, and Mentors

Marygrace Foster (Purdue University School of Pharmacy), Prof. Robert Geahlen
Jonathan Hoggatt (Purdue University School of Pharmacy), Prof. Richard Gibbs
Megan Koritnik (Purdue University School of Pharmacy), Prof. Ross Weatherman
Jason Lewis (Purdue Biology), Prof. Sophie LeLievre
Joshua McAfee (Indiana University/Purdue University School of Pharmacy), Prof. Robert Geahlen
Grace O'Connor (Purdue University School of Pharmacy), Prof. Suresh Mittal
Cyle Ralston (Ball State/Purdue University School of Pharmacy), Prof. Daitoku Sakamuro
Michael Sawvel (Indiana Wesleyan), Prof. Mark Cushman
Dustin Spencer (Purdue University School of Pharmacy), Prof. Mark Green
Elizabeth Williams (Ball State University), Prof. David Riese

Reports of Students' Accomplishments
(These reports were provided by the students themselves.)

Marygrace Foster. My research focused on Syk nuclear localization in B cells. It was noticed previously that Syk localizes in nucleus and this summer, I tried to find out why. I began by reproducing experiments that had been completed prior to my joining the lab by a graduate student. We used the Syk-GFP and linker-less Syk (fluorescently tagged Syk) and loaded them into Syk negative cells, which allowed us to view the localization of Syk under fluorescence microscopy. As shown previously, the linker-less Syk showed no nuclear localization. From this, our main focus was now on deciphering the area of the linker responsible for nuclear localization. We designed cuts of the linker region into halves and also thirds, sent them to be synthesized and eventually loaded them into the cells. Our results were exciting. We found that

the final section (332-359) of the linker region was responsible for the localization. This was confirmed by both the third portion and the half portion of the linker region exhibiting nuclear exclusion.

Jonathan Hoggatt. My project involved developing a mass spectrometric technique for the detection of post-translational farnesylation of *ras* proteins. Mutated *ras* proteins have been found in approximately 30% of all human cancers and thus present themselves as a possible target for anti-cancer agent development. Mutated *ras* lacks normal GTPase activity, and thus is in a constantly *ras* bound active state. This constant activation causes continuous cell proliferation and tumor growth. *Ras* must be localized on the cellular membrane to be active and localization is achieved through the post-translational addition of a farnesyl group to the carboxy-terminal cysteine residue. Inhibition of farnesylation or alternate farnesylation through farnesyl analogs may prevent membrane localization and thus function of mutated *ras*. A mass spectrometric technique is needed to detect what farnesylation reactions have occurred in various cellular models to work in conjunction with studies currently underway to develop competing farnesyl analogs.

Megan Koritnik. Progesterones play a role in the development of the breasts as well as in the changes during menstruation, pregnancy and lactation in the female body. Progesterones, however, have a less clearly defined effect when it comes to breast cancer. There has been evidence to suggest that different progesterone metabolites have different effects on breast tissue. The predominant metabolite formed by normal, non-tumorous breast tissue is 3α-hydroxy-4-pregnen-20-one (3αHP), but when breast tissue becomes cancerous 5α-pregnane-3,20-dione (5αP) is produced in significantly greater amounts. The 5α-pregnanes, including 5αP, seem to cause increased cellular proliferation and decreased cell attachment, which are favorable to a cancerous result. The 4α-pregnenes, which include 3αHP, have been shown to have an opposite, anti-cancerous effect. The location of the receptors for these metabolites is not entirely clear. The presence of a membrane-associated receptor has proven difficult to verify, and its roles even more elusive. An approach to exploring this hypothetical receptor would be to develop membrane impermeable compounds (by adding a polyacrylamide chain) that would still be able to bind receptors solely on the outer surface of the membrane. The membrane receptors are believed to be responsible for initiating the effects associated with the progesterone metabolites. While our library of polymer/hormone compounds was being synthesized, my project was to seek out just what effects we would be looking for when we used these compounds in an experiment. My assays were meant to show that 5αP would yield greater cell growth and detachment, while 3αHP would show decreased proliferation and increased attachment when compared to controls. The proliferation assays were done by plating known numbers of cells and subjecting them to no treatment, treatment with 5αP and treatment with 3αHP; later counts of the cells yielded the proliferation data. The attachment/detachment assays were ultimately done by Western blot of the soluble and polymerized portions of actin found within cells that had undergone the three treatments. A higher ratio of non-soluble actin is found in non-cancerous cells, lending support to the cytoskeleton and the ability to stay attached. Most of my work this summer was to optimize these assays to hopefully produce conclusive and reproducible data. I also did work with plasmids, their growth, cultivation and transfection into human breast cancer cells. My work with these methods and expansion to new methods will continue into the next semester and is far from complete.

Jason Lewis. This summer my studies focused on the protein, NuMA. NuMA stands for nuclear mitotic apparatus and its functions include stabilization of the mitotic spindle during interphase as well as being involved in RNA metabolism and cell differentiation. Blocking of the carboxy-

terminus of the protein in normal, differentiated breast epithelial cells leads to a loss of differentiation. My studies this summer focused upon showing an association between NuMA and chromatin. Previously my laboratory had show that upon DNAse I digestion of normal, differentiated breast epithelial cells, there was a loss of staining for the protein, NuMA. This summer I developed a chromatin isolation technique in which I was able to isolate chromatin and the nuclear matrix from a nuclear pellet. I then performed Western Blots using the chromatin isolation samples and, using an antibody against NuMA, I was able to determine the presence of the protein in the different wells. My results showed that NuMA was present both in the chromatin fraction and the nuclear matrix fraction (the protein had been shown to be present in the nuclear matrix by numerous other studies) of malignant and non-malignant breast epithelial cells. These results indicate that NuMA associates with chromatin.

Joshua McAfee. My research focused on Syk, a non-receptor protein tyrosine kinase, found in breast epithelial cells. Syk expression in highly malignant breast cancer cell lines is absent or highly reduced. This suggests that Syk is functioning as a tumor suppressor gene or anti-metastatic gene in human breast epithelial cells. Despite this connection, little is known about the function of Syk in human breast epithelial cells. Therefore my research goal was to identify proteins that interact with Syk in human breast epithelial cells. To detect protein interactions *in vivo*, graduate student Qing Zhou and I used a yeast two-hybrid system. The yeast two-hybrid system is a transcriptional assay, in which one can screen a library for novel proteins that interact with a bait protein. In our case we used the cDNA from human mammary gland for the library protein and wild type Syk for the bait protein. After we isolated the interacting proteins, the DNA was purified and sent to the Purdue Genomics Center for identification of the proteins involved in interacting with Syk in human mammary gland cells. Some of the proteins that Qing Zhou will further explore are TRIP and Cbl-3. She plans to do coimmunoprecipitation reactions and mammalian two-hybrid systems to further confirm these interactions. Ultimately, she would like to elucidate the physiological function of Syk in relation to the proteins of interest.

Grace O'Connor. My project involved work with EphA2, a gene overexpressed in over half of aggressive breast cancer cell lines. Normal breast cells have low levels of EphA2, all of which binds to its ligand, EphrinA1, and is then phosphorylated and degraded. A previous study showed that overexpression of EphA2 alone is enough to cause malignant transformation. There are cell morphology differences and EphA2 is not phosphorylated or degraded. I used a replication deficient adenovirus with EphA2 gene added (HAdEphA2) to determine if various cell lines infected with HAdEphA2 virus caused EphA2 expression and EphA2 phosphorylation levels to increase. I used Western blots and collected the cell lysates 12, 24, 36, and 48 hours post-infection. All cell lines I used (NIH-3T3, MCF-10A, MTIA2, and MDA-231) showed increased EphA2 expression with the HAdEphA2 compared with the control (HAd□E1,E3) and mock (PBS) infected groups. More importantly, EphA2 phosphorylation levels dramatically increased with the HAdEphA2 infected groups, most noticeable in the cancer cell lines.

Cyle Ralston. My project was to observe the differences between the interacting proteins p53 wild-type (wt) and p53ΔPP (wild type p53 with a deletion in the poly-proline region) in breast cancer cells (MCF 7). The interaction of proteins are critical for p53 (in either form) to serve its function. Wild type p53 has two functions in the body: apoptosis and tumor suppression. As was already determined, deletion in the poly-proline region disenables p53's function of apoptosis. However, p53 ΔPP retains its tumor suppression ability. My role in the lab was to first transfect MCF 7 cells with pcDNA3 (vector), CMV-p53, and CMV-p53ΔPP. I was attempting to observe the difference in the number of colonies by using the selecting medium

G418. With the utilization of mass spectrometry, Sakamuro lab will determine if there is a difference in relation to the interacting proteins.

Michael Sawvel. My project involved the synthesis of several 2-methoxyestradiol (2ME2) analogs with substituents at the 2- and 3-postions. 2ME2 has shown both antitumor and antiangiogenic properties *in vivo.* Both breast cancer and prostate cancer have demonstrated susceptibility to the drug. However, some issues with drug stability occur *in vivo.* In order to address these issues, I worked on synthesizing an analog with a propynyl group at the 3-position and a sulfamate group at the 2-position. This combination would allow both greater absorption and stability. Secondly, synthesis of an amine at the 2-position was done to better explore the role of hydrogen bond donating in the active site. Both compounds were almost synthesized completely.

Dustin Spencer. My project investigated the KB cell line. I studied both the drug sensitive KB-3-1 and the drug resistant KB-8-5 lines. The resistant KB-8-5 cells are maintained in media containing the drug, colchicine. The 3-1 cells are maintained in the same media minus the colchicine. Dr. Green's lab is working on making novel radiopharmaceuticals for diagnostic use in detecting breast cancer tumors. The lab will use KB cells to form tumors in mice in order to develop and study these radiopharmaceuticals. The mice will be free of drug when the cells are introduced in their systems. It was necessary to know the rate at which the resistant KB-8-5 cells lost resistance once they are introduced into the "drug-free" environment of the mice. I started with the resistant 8-5 line and removed these cells from the colchicine media and studied the rate at which the cells lost resistance. Their loss of P-glycoprotein expression during the experiment showed that they were losing multi-drug resistance.

Elizabeth Williams. My project identified what amino acid residues on ErbB4 are necessary and sufficient for activation of ErbB4 by NRG2β. It also identified residues that are necessary and sufficient for high affinity binding of NRG2β to ErbB4. This project involved three members of the ErbB family of tyrosine kinases: EGFR, ErbB2, and ErbB4. When EGFR and ErbB2 are over expresseed in breast cancer tissue, there is increased cell proliferation and a poor patient prognosis. Conversely, over expression of ErbB4 in breast cancer tissue does not lead to increased cell proliferation and leads to a good patient prognosis. NRG2β is a ligand that binds to ErbB4 and activates ErbB4 signaling but does not bind to nor activate EGFR or ErbB2. Previous experiments in this lab have shown interactions between the NRG2β Phe45 and the ErbB4 Leu437 side chains, as well as between the NRG2β Phe45 and the ErbB4 Lys438 side chains. This experiment investigated whether the ErbB4 Leu437 and Lys438 are necessary and sufficient for ErbB4 activation and binding of NRG2β. To do this, I created or helped create a series of mutants. First, twelve ErbB4 loss of function mutants were generated by replacing the ErbB4 Leu437 or Lys438 with a corresponding residue on EGFR or ErbB2. These mutants should show decreased NRG2β binding and decreased tyrosine phosphorylation compared to wild-type ErbB4. I also created seven EGFR and ErbB2 gain of function mutants by substituting ErbB4 Leu437 and Lys438 for corresponding residues in EGFR and ErbB2. These mutants should display greater levels of tyrosine phosphorylation as well as higher affinity binding of NRG2β than wild-type EGFR and ErbB2. By the end of the summer, all twelve ErbB4 loss of function mutants plus one EGFR gain of function mutant were being generated in cells. The other loss of function mutants were in the process of mutation.

Reportable Outcomes

- Ten undergraduate students, each working with one of nine mentors, carried out breast cancer research in the Summer of 2003, bringing the total number of students sponsored by the program over two years to 22.
- Two of the students were supported by the Office of the Dean of Pharmacy.
- Ms. Grace O'Connor applied for a national-level Undergraduate Research Fellowship from the American Foundation for Pharmaceutical Education, but did not receive the award. However, an additional summer of research will position her well to compete for national awards.
- Two of the students from Year 01 of this program have entered Graduate School. Ms. Emily Rickert entered graduate school in the Department of Medicinal Chemistry and Molecular Pharmacology at Purdue and is carrying out breast cancer research with Prof. Ross Weatherman. Mr. Barry Steinkamp has entered graduate school in the Biochemistry and Molecular Biology program at the Indiana University Medical Center. A third student from the 2002 group, Ms. Yurika Ito, entered the interdisciplinary Biochemistry and Molecular Biology program at Purdue, but withdrew for personal reasons and returned to Japan in the Fall of 2003.
- All students provided favorable reviews of the program.
- Of the 22 participants for the first two years, 13 have been women. Women are underrepresented in the life science academic and research community.
- One major grant application, an NIH R01 grant, has been submitted by Prof. Sophie LeLievre: "NuMA function in non-malignant and malignant breast tissues." This application is pending. Two *funded* grant applications resulting in part from participants' work have been submitted by Prof. Ross Weatherman:

  U.S. Army Medicinal Research and Materiel Command Idea Award BC030507
  1/1/04–12/31/06 Direct Costs first year: $125,000

  American Association of Colleges of Pharmacy New Investigator Program
  12/1/03-11/31/04 Direct Costs: $10,000

- There are two manuscripts in preparation on which participants will be coauthors. These will be reported next year.
- Two papers coauthored by participants were presented at regional meetings:

  New insights into global demethylating drugs for breast cancer therapy. C. Plachot, CM Turner, and SA Lelievre. Walther Cancer Institute retreat, WestLafayette, IN August 2002

  Implications of NuMA carboxi-terminus in breast acinar differentiation and cancer. PC Abad, J Lewis, S Mian, and SA Lelievre. Amelia Research Day, Indianapolis, IN February 2004.

- Unused travel funds have been rebudgeted for student support and a one-year no-cost extension of this grant has been requested and approved. This will allow us to offer this program for an additional year.