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TITLE: Ph.D. Post-Doctoral Training Program in Breast Cancer Research

PRINCIPAL INVESTIGATOR: Dean P. Edwards, Ph.D.

CONTRACTING ORGANIZATION:

University of Colorado Health Sciences Center
Denver, Colorado 80045-6508

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13. ABSTRACT *(Maximum 200 Words)*

The training program is designed to graduate well-qualified and highly motivated scientists who will make a career in the breast cancer research field and who will have a strong potential for contributing new research approaches to the breast cancer problem. The students accepted into the program have already entered into different Ph.D. degree granting programs that each have their own guidelines, curriculums, and requirements. The curriculum of the Breast Cancer Training Program extends beyond that of the normal Ph.D. requirements to include didactic classroom teaching, journal clubs, seminars, workshops and mini-symposiums on relevant topics in breast cancer. Additionally, the program provides extensive one-on-one laboratory training in breast cancer research that is committed to the discovery of new fundamentals about the biology of breast cancer and its eventual treatment. The faculty who serve as research mentors have established records of successful training of Ph.D. and M.D./Ph.D. students.

14. SUBJECT TERMS
breast cancer, apoptosis, cell-cycle control, steroid hormone receptors, signal-transduction, oncogenes, transcription factors, developmental genes

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TRAINING AND KEY RESEARCH ACCOMPLISHMENTS

I Student Trainees and Training Faculty

A) Students

Three pre-doctoral students supported in the first year of the grant completed their Ph.D. degrees: Richard Lee (M.D./Ph.D. student) returned to medical school, as did Aaron Spalding (M.D./Ph.D. student), and Christine Wu (Ph.D. student) took a post-doctoral position at the Scripps Research Institute with Dr. John Yates to continue work on proteomics and breast cancer. To replace the graduates, three new students joined the training program: G.J. Cappetta, Katherine Lobel-Rice and Suzanne Wardell. Three students from the first year continued to be supported in 2001-2002: Jason Prescott, Sarah Roemer, and Steven Rosinski. The table lists the six students supported during the 2001-02 academic year on the training grant, along with their faculty thesis advisors and Ph.D. programs. A seventh student funded by a private foundation (Avon Fund) joined the training program: Traci Lyons, under the mentorship of Steven Anderson, Ph.D. Traci participates in all the same training program activities as the six DOD-supported students.

Student	Type of Student	Faculty Mentor	Ph.D. Program
Cappetta, G.J.	Ph.D.	Arthur Gutierrez-Hartmann, M.D./Ph.D.	Molecular Biology
Prescott, Jason	M.D./Ph.D.	Arthur Gutierrez-Hartmann, M.D./Ph.D.	Molecular Biology
Roemer, Sarah	Ph.D.	Dean Edwards, Ph.D.	Molecular Biology
Rosinski, Steven	M.D./Ph.D.	Ian McNiece, Ph.D.	Pharmacology
Lobel-Rice, Katherine	Ph.D.	Gary Johnson, Ph.D.	Pharmacology
Wardell, Suzanne	Ph.D.	Dean Edwards, Ph.D.	Molecular Biology
Lyons, Traci	Ph.D.	Steven Anderson, Ph.D.	Molecular Biology

B) Training faculty.

Two new faculty have joined the training program, Drs. Heide Ford and Valerie Fadok. Their biosketches, other support, and approved animal protocols are included in Appendix materials. Katherine Lobel-Rice successfully defended her thesis and is projected to graduate at the end of August, 2002. She will be replaced on the training grant in September for the 2002-2003 academic year by Kelly Jansky, a Biochemistry second-year Ph.D. student working in Dr. Ford's laboratory. Her research project involves studies to understand why the human Six1 homeobox gene is over-expressed in breast cancer and to understand the functional significance of that over-expression. She is using a series of breast cancer cell lines that represent different stages of disease (normal, primary, metastatic breast cancer) to determine the mechanism of Six1 over-expression.

Unfortunately, G.J. Cappetta has elected to leave graduate school. He will be replaced by Catherine Smith in Dr. Fadok's laboratory. She is a second year Immunology Ph.D. student. Her project involves studies on the role of the phosphatidylserine receptor (PtdSerR) in breast cancer. In addition to recognizing and mediating clearance of apoptotic cells by macrophages, the PtdSerR expressed on mammary epithelial cells triggers cell growth and release of VEGF. Catherine Smith's project will be devoted to understanding the signaling mechanisms leading to these biological phenomena, and how they are regulated in normal mammary development compared to breast cancer.

II Training Program Activities

A) Seminar Series. Students participated in a seminar series that was organized jointly with the UC Cancer Center Program on Hormone Related Malignancies. This is held weekly throughout the academic year and includes seminars on all topics related to endocrine and hormone regulated tumors. Students are encouraged to attend all seminars, but were required to attend the 9 breast cancer or breast cancer related seminars. The seminars are presented by outside invited scientists as well as faculty from our training program. In addition, students each gave a 30 min seminar in May 2002 to update the group on the progress of their research projects. A schedule of the seminars is included in Appendix materials.

B) Minisymposium. Students and faculty attended a mini-symposium also jointly sponsored by the DOD breast cancer training program and the UC Cancer Center Hormone Related Malignancies Program. The mini-symposium was held on March 15th, 2002 and featured four prominent invited speakers : James A. Fagin, M.D., University of Cincinnati; Nancy L. Weigel, Ph.D., Baylor College of Medicine; MylesA. Brown, M.D., Dana-Farber Cancer Institute, Harvard Medical School; and V. Craig Jordan, Ph.D., Northwestern University. The DOD breast cancer training program sponsored and funded the two breast cancer speakers, Myles Brown and V. Craig Jordan.

C) Retreat on Mammary Gland Development, Function and Neoplasia. . Students and faculty participated in an off-campus scientific retreat for all faculty, students and staff at UCHSC working in the area of normal mammary gland biology and breast cancer. The retreat was organized as a mixture of seminars by local faculty, invited outside scientists, and poster presentations by students and fellows. The DOD breast cancer training grant supported two of the invited speakers, Piotr Sicinsky (Harvard University) and Elizabeth Anderson (Manchester, UK). The schedule of the retreat is included in Appended materials.

D) Didactic mini-course: " Mammary gland development, function and neoplasia". A 1-credit hr graduate course (Physiology 7840) was given by the training faculty for the students on the DOD breast cancer training grant and other trainees of a Program Project Grant on mammary gland

development. The class was held weekly for 2hrs during the Fall quarter and included lectures on normal mammary development and involution, endocrine regulation of normal breast and breast cancer, cell biology of secretion and genes involved in mammary neoplasia.

E) Journal club. We continued with a journal club organized by one of the new training faculty, Dr. Heide Ford, on topics related to developmental genes in breast cancer. This was held on the 2nd Friday of most months. Each journal club was 2hrs and was spent on student discussions and interpretation of two original publications, one on developmental genes involved in mammary neoplasias and another on a different developmental system to parallel the mammary gland.

F) Workshop on Gene Arrays to Analyze Steroid Regulated Genes in Breast Cancer. This was held on October 19th and was attended by trainees. Training faculty engaged in microarray experiments spent a full day presenting details of their experimental design, data analysis and results.

III Outcomes resulting from training grant award.

The accomplishments of student trainees in terms of publications, presentations of abstracts at national/international meetings and degrees awarded are listed below for each student. The meetings listed were supported in part by the DOD training grant award. Descriptions of student research over the last year, or abstracts of each student's research presented at National/International meetings, are included in Appendix Materials.

Student: Aaron Spalding. At the time of the progress report last year, Aaron had submitted two manuscripts for publication that are now in print.

Publications:

Spalding AC, Jotte RM, Scheinman RI, Geraci MW, Clarke P, Tyler KL, and **Johnson GL**. 2002. Trail and inhibitors of apoptosis are opposing determinants for NF-KAPPAB-dependent, genotoxin-induced apoptosis of cancer cells. Oncogene 21(2):260-271.

Clarke P, Meintzer SM, **Spalding AC**, **Johnson GL**, and tyler KL. 2001. Caspase 8-dependent sensitization of cancer cells to Trail-induced apoptosis following retrovirus-infection. Oncogene 20(47):6910-6919.

Student: Sarah Roemer

Publications:

Melvin VS, **Roemer SC**, Churchill ME, and **Edwards DP**. 2002. The C-terminal extenstion (CTE) of the nuclear hormone receptor DNA binding domain determines

interactions and functional response to the HMGB-1/-2 co-regulatory proteins. J. Biol. Chem. 277(28):25115-25124.

Presentations at Meetings: None.

Participation in Meetings:

Keystone Symposia: Nuclear Receptor Superfamily. April 13-19, 2002, Snowbird, Utah.

Student: Steven Rosinski

Publications: None

Presentations at Meetings:

Rosinski S, McNiece I, Sphall E, Clough N, Russel P, McDermott J, Nieto Y. Peripheral T cell levels correlate with outcome in patients undergoing autologous stem cell transplant. Experimental Hematology (ISEH2002), July 5-9, Montreal, Canada

Student: Jason Prescott

Publications: None

Presentations at Meetings:

Prescott JD[1], Eckel K[2], Singh M[1], and **Gutierrez-Hartmann A**[1]. The ESX transcription factor in human breast cancer: Localization of the ESX protein in human breast epithelial cells and identification of ESX target genes. [1]University of Colorado Health Sciences Center, Department of Medicine, Denver, Colorado. [2]University of Washington, Seattle, Washington. The 84th Annual Endocrine Society Meeting, June 18-22, 2002, San Francisco, California, Abstract #P1-625, p. 300.

Student : Suzanne Wardell

Publications:

Wardell SE, Boonyaratanakornkit V, Adelman J, Aronheim A, and **Edwards DP**. 2002. Jun dimerization protein-2 (JDP-2) functions as an N-terminal coactivator of progesterone receptor. Molecular and Cellular Biology 22:5451-5466.

Presentations at Meetings:

Wardell SE and **Edwards DP**. Jun Dimerization protein functions as a progesterone receptor N-terminal domain coactivator. 2002 Nuclear Receptor Superfamily Keystone Symposium in Snowbird, Utah, April 13-19, 2002. The published abstract is P. 108, #268.

Student: Kathrine Lobel-Rice

Publications:

Lobel-Rice K, Kesevan K, Sun W, Webb S, Doan A, Gelfand EG, Henson PM, **Johnson GL**, and Garrington TP. MEKK2 integrates ERK5-JNK signaling for growth factor regulation of c-Jun and AP-1 activation. (2002, submitted).

Sun W, Wei X, KEsevan K, **Lobel-Rice K**, Garrington TP, and Gelfand EG. Growth factor and stress activation of ERK5 requires LAD/RIBP and MEKK2, and is dependent on src kinases. (2002, submitted).

Presentations at Meetings: None

Degree Awarded: Ph.D. in Pharmacology, August 30, 2002. Thesis: Distinct roles for MEKK2 and MEKK3 in cell signaling, gene regulation, and development as defined by targeted gene disruption. (successfully defended, June 20, 2002)

APPENDICES

Biographical Sketches

Provide the following information for the key personnel listed on the budget page for the initial budget period.

NAME	POSITION TITLE
Ford, Heide L.	Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
State University of New York at Geneseo, NY	B.S.	1989	Biology
University of Rochester, Rochester, NY	M.S.	1992	Biochemistry
University of Rochester, Rochester, NY	Ph.D.	1995	Biochemistry
Dana-Farber Cancer Institute/Harvard Med. Sch.	Postdoc	1995-2001	Cancer Res.

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

Employment/Experience

2001-present Assistant Professor, University of Colorado Health Sciences Center, Dept. of Obstetrics and Gynecology
2001-present Adjunct Assistant Professor, Univ. of Colorado Health Sci. Ctr., Dept. of Biochemistry/Molec. Genetics

Professional Affiliations

1994-present American Association for the Advancement of Science
1993-2001 American Association for Cancer Research, Associate Member
2001-present American Association for Cancer Research, Active Member
1994-1996 Metastasis Research Society

Selected Publications

Tulchinsky, E., **Ford, H.L.**, Kramerov, D., Reshetnyak, E., Grigorian, M., Zain, S., Lukanidin, E. (1992) Transcriptional Analysis of the Mts1 Gene with Specific Reference to 5' Flanking Sequences. *Proc. Natl. Acad. Sci. USA* **89**: 9146-9150.

Tulchinsky, E., Kramerov, D., **Ford, H.L.**, Reshetnyak, E., Lukanidin, E., Zain, S. (1993) Characterization of a Positive Regulatory Element in the Mts1 Gene. *Oncogene* **8**: 79-86.

Ford, H.L. and Zain, S. (1995) Interaction of Metastasis Associated Mts1 Protein with Nonmuscle Myosin. *Oncogene* **10**: 1597-1605.

Ford, H.L., Salim, M., Chakravarty, R., Aluiddin, V., and Zain, S. (1995) Expression of Mts1, a Metastasis Associated Gene, Increases Motility but not Invasion of a Nonmetastatic Mouse Mammary Adenocarcinoma Cell Line. *Oncogene* **11**: 2067-2975.

Ford, H.L., Silver, D.L., Kachar, B., Sellers, J.R., Zain, S. (1997) Effect of Mts1 on the Structure and Activity of Nonmuscle Myosin. *Biochemistry* **36**: 16321-16327.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY.
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Ford, H.L., Silver, D.L., Kachar, B., Sellers, J.R., Zain, S. (1997) Effect of Mts1 on the Structure and Activity of Nonmuscle Myosin. *Biochemistry* **36**: 16321-16327.

Ford, H.L. and Pardee, A.B. (1998) The S-phase: Beginning, Middle, and End: A Perspective. *J. of Cellular Biochem.* Supplements **30/31**, 1-7.

Ford, H.L., Kabingu, E.N., Mutter, G.L., Bump, E., and Pardee, A.B. (1998) Abrogation of the G2 Cell Cycle Checkpoint Associated with Overexpression of HSIX1: A Possible Mechanism of Breast Carcinogenesis. *Proc. Natl. Acad. Sci. USA* **95:** 12608-12613.

Ford, H.L. (1998) Homeobox genes: A Link Between Development, Cell Cycle, and Cancer? *Cell Biol. Int.* **22**, 397-400.

Ford, H.L. and Pardee, A.B. (1999) Cancer and the Cell Cycle. *J. of Cellular Biochem.* **75** (S32), 166-172.

Guan, R.L., **Ford, H.L.**, Fu, Y., Li, Y., Shaw, L.M., Pardee, A.B. (2000) Drg-1 as a Differentiation-Related, Putative Metastatic Suppressor Gene in Human Colon Cancer. *Cancer Research.* **60**, 749-755.

Pardee, A.B., **Ford, H.L.,** and Sager, R. (2000) *Expression Genetics and Early Cancer Detection.* In: Current Options for the Human Genome Project (ed. Grisolia, S.), Edita Fundacion BBV Documenta, Bilbao, pp. 151-162.

Ford, H.L., Landesman-Bollag, E., Dacwag, C.S., Stukenberg, P.T., Pardee, A.B., Seldin, D. (2000) Cell Cycle Regulated Phosphorylation of the Human SIX1 Homeodomain Protein. *J. Biol. Chem.* **275**, 22245-22254.

Pardee, A.B., **Ford, H.L.**, Biswas, D.K., Martin, K.J., and Sager, R. (2001) Expression Genetics of Hormone Dependent Human Tumors. In: Hormonal Carcinogenesis III (eds. Li, J.J., Daling, J.R., and Li, S.A.), Springer-Verlag, New York, pp. 37-43.

Geng, Y., Yu, Q., Whoriskey, W., Dick, F., Tsai, K., **Ford, H.L.**, Biswas, D.K., Amati, B., Jacks, T., Richardson, A., Dyson, N., and Sicinski, P. (2001) Expression of cyclins E1 and E2 during mouse development and in oncogenesis. *Proc. Natl. Acad. Sci. USA* **98:** 13138-13143.

Ford, H.L. and Pardee, A.B. (2002) *Cell Cycle Checkpoints* In: Encyclopedia for Molecular Medicine (ed. Biderman, A.), John Wiley & Sons, Inc., New York, pp. 720-722.

Ford, H.L., Biswas, D.K., Martin, K.J., and Pardee, A.B. (2002*) Discovery of Expressed Genes by Differential Display and Their Applications.* In: Perspectives in Gene Expression. Eaton Publishing /Biotechniques Press, One Research Drive, Suite 400A, Westboro, MA 01581-6-070. In press.

OTHER RESEARCH SUPPORT:

ACTIVE

ACS IRG/UCCC 10/1/01-9/30/02
CANCER RESEARCH SEED GRANTS $20,000 direct
"Examining the Role of the HSIX1 homeobox gene in normal mammary gland development and tumorigenesis using inducible, mammary gland specific HSIX1 transgenic mice"

AVON FOUNDATION SEED GRANT 10/1/01-9/30/02
"Identification of HSIX1 Targets Important in Cell Cycle Control and Breast Cancer"
$50,000 direct

1 RO1 CA95277-01 4/1/02-3/31/07 60%
National Cancer Institute $178,000 direct/year
HSIX1 in Cell Cycle Control and Tumorigenesis

START-UP FUNDS
Cancer Center: $125,000
Dean's Fund: $125,000
Dean's Fund salary support: $75,000
Obstetrics/Gynecology Fund: $50,000

F. VERTEBRATE ANIMALS

(1) 8 week old, female, nude mice (NCR:nu) will be used in all three specific aims to test the role of HSIX1 phosphorylation, HSIX1 degradation, and downstream target genes in tumorigenesis (see #2 for more detailed explanation as well as Preliminary Data and Research Design and Methods for methodology). For each experiment, 60-day release 17 β-estradiol pellets will be placed into the mice one day before injection of the cells. The following day, 5-6 mice will be injected in the left flank with 10 x 10^6 cells from each cell line to be tested (therefore 5-6 mice/group). Mice will be monitered twice a week for the next 6 weeks, and tumor size will be measured over this time. Total number of nude mice to be used will range from 110-165 (justification below).

(2) An animal model must be used if we want to assess the effect of HSIX1 and the various mutants and targets in tumorigenesis, as the complexity of this process cannot be duplicated in simpler systems. As we are injecting human cell lines into the mice, we need immunocompromised animals (nude mice) to avoid rejection of the cells. The numbers to be used are justified as follows:
Specific aim 1- At least two independent clones and one pooled population of MCF7 cells stably transfected with the control plasmid, HSIX1, and the HSIX1 phosphomutant (S204A) will be injected into the mice. With 5 mice per group, the minimum number of mice for this experiment is 45.
Specific aim 2- The experimental design is as above, except rather than comparing the phosphomutant, we will be comparing MCF7 cells stably transfected with non-degradable HSIX1. Therefore will need a minumum of 45 mice for this experiment. However, it is possible that these experiments are done in parallel (aims 1 and 2), and therefore less mice can be utilized as the same controls are necessary for both experiments.
Specific aim 3- In this aim, we will need to inject the same control lines as above (MCF7 cells stably transfected with control plasmid as well the HSIX1 overexpressing MCF7 cells). In addition, we will test at least two independent clones and one pooled population of MCF7-HSIX1 cells that have been altered in an identified downstream target. We anticipate testing at least 3 downstream targets in this fashion. Therefore we will need approximately 75 nude mice for this experiment.

(3) Veterinary care will be provided by The Center for Lab Animal Care (CLAC) at UCHSC, which has three veterinarians on staff. The animals will be monitored over the 6 week period to ensure that their tumor size does not exceed limitations (2 cm). In addition, the veterinarians will be consulted in all cases where the animal appears to be in distress (below).

(4) To ensure that discomfort, distress, pain, and injury will be limited to that which is unavoidable, we will moniter the animals twice a week and check for the following parameters: weight loss, change in behavior, activity, or posture, and decrease in abilitity to move. We will euthanize the mice if the changes observed indicate that the animals are suffering. Decisions regarding when euthanization is appropriate will be done in conjunction with the veterinarians on staff. Furthermore, if any mice obtain tumors greater than 2 cm, they will be immediately sacrificed. No analgesics, anesthetics, tranquilizing drugs, or restraining devices are anticipated for this study.

(5) The method of euthanasia to be employed throughout and at the end of the study is carbon dioxide exposure, which is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. It was selected because it is rapid, humane, and can be performed on multiple mice with relative ease.

57703101(02)2E "The study of HSIX1 in Mammary gland development/tumorigenesis"- approved 2/16/01
57703101(02)1E "Production of transgenic Mice by DNA or stem cell injection"- approved 2/12/01
57703101(07)3E "Tumorigenic potential of mutant Six1"- approved 7/16/01

Biographical Sketches

Provide the following information for the key personnel listed on the budget page for the initial budget period.

NAME	POSITION TITLE
Fadok, Valerie A.	Associate Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include post-doctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Washington State University, Pullman, WA	B.S.	1976	Veterinary Med
Washington State University, Pullman, WA	D.V.M.	1978	Veterinary Med
Univ. of Colorado Health Sci. Ctr., Denver, CO	Ph.D.	1991	Exp. Pathology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

Employment/Experience

1978-1979	Intern, Small Animal Med. & Surgery, West Los Angeles Veterinary Medical Group, Los Angeles, CA.
1979-1981	Resident, Comparative Dermatology, College of Veterinary Medicine, University of Florida, Gainesville, FL
1981-1983	Asst. Prof./Staff Dermatologist, College of Veterinary Medicine, University of Tennessee, Knoxville, TN
1982	Board Certification: American College of Veterinary Dermatology
1983-1986	Asst. Prof./Large Animal Dermatologist, College of Veterinary Medicine, Univ. of Florida, Gainesville, FL
1986-1991	Graduate Student, Dept. of Pathology, Univ. of Colorado Health Sci. Ctr., Denver, CO
1991-1993	Post-doctoral Fellow, National Jewish Ctr. for Immunology & Respiratory Medicine, Denver, CO
1993-1995	Assoc. Prof., Dept. of Small Animal Med. & Surgery, College of Vet Med., Texas A&M Univ., College Station, TX
1995-2001	Asst. Faculty Member, Dept. of Pediatrics, National Jewish Med. & Research Ctr., Denver, CO
2001-present	Assoc. Prof., Dept. Pediatrics, National Jewish Med. & Research Ctr., Denver, CO

Professional Affiliations

1979-present	American Academy of Veterinary Dermatology
1982-present	American College of Veterinary Dermatology
1979-1999	Academy of Veterinary Allergy and Clinical Immunology
1987-present	Society of Leukocyte Biology
1996-present	American Society of Cell Biology

Selected Publications (Total of 78 peer reviewed articles and 42 Chapter and review articles)

Fadok, VA, Voelker Dr, Campbell PA, Cohen JJ, Bratton DL and Henson PM. 1992. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J. Immunol. 148:2207-2216.

RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY.
DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

Bratton DL, Dreyer E, Kailey JM, **Fadok, VA**, Clay KL, and Henson PM. 1992. The mechanism of internalization of platelet-activating factor in activated human neutrophils. J. Immunol. 148:514-523.

Cohen JJ, Duke RC, **Fadok VA** and Sellins KS. 1992. Apoptosis and programmed cell death in immunity. Ann. Rev. Immunol. 10:267-293.

Fadok VA, Savill J, Haslett C., Bratton DL, Doherty D., Campbell PA, and Henson PM. 1992. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognize and remove apoptotic cells. J. Immunol. 149:4029-4035.

Fadok VA, Laszlo DJ, Noble PW, Weinstein L, Riches DWH, and Henson PM. 1993. Particle digestibility is required for induction of the PS recognition mechanism used by murinc emacrophages to phagocytose apoptotic cells. J. Immunol. 151:4274-4285.

Savill J, **Fadok VA**, Henson PM, and Haslett C. 1993. Phagocyte recognition of cells undergoing apoptotis. Immunology Today 14:131-136.

Rose DM, **Fadok VA**, Riches DWH, Clay KL, and Henson PM. 1994. Autocrine/paracrine involvement of platelet-activating factor and transforming growth factor-beta in the induction of phosphataidylserine recognition by murine macrophages. J. Immunol. 155:5819-5825.

Gossett R, Kier AB, Schroeder F, McConkey D, **Fadok V**, and Amoss MS Jr. 1996. Cycloheximide-induced apoptosis in melanoma cells derived from regressing cutaneous tumours of Sinclair Swine. J. Comp. Path. 115:353-372.

Fournier T, **Fadok V**, and Henson PNM. 1997. Tumor necrosis factor-α inversely regulates PGD2 & PGE2 production in murine macrophages. J. Biol. Chem. 272:31065-31072.

Bratton DL, **Fadok VA**, Richter DA, Kailey JM, Guthrie LA, and Henson PM. 1997. Phosphatidylserine expression on apoptotic cells: the relative importance of calcium-mediated nonspecific phospholipids flip-flop and loss of the aminophospholipid translocase. J. Biol. Chem. 272:26159-26165.

Fadok VA, Konowal A, Freed PW, Wetscott JY, Bratton DL, and Henson PM. 1998. Macrophages which have ingested apoptotic cells inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGFβ, PGE2, and PAF. J. Clin. Invest. 101:890-898.

Frasch SC, Nick JA, **Fadok VA**, Bratton DL, Worthen GS, and Henson PM. 1998. p38 MAP kinase-dependent and -independent intracellular signal transduction pathways leading to apoptosis in human neutrophils. J. Biol. Chem. 273:8389-8397.

Fadok VA, Bratton DL, Frasch SC, Warner ML, and Henson PM. 1998. The role of phosphatidylserine in recognition of apoptotic cells by phagocytes. Cell Death Differentiation 5:551-561.

Fadok VA and Henson PM. 1998. Apoptosis: Getting rid of the bodies. Current Biol. 8:R693-R695.

Fadok VA, Warner ML, Bratton DL, and Henson. 1998. CD36 is required for phagocytosis of apoptotic cells by human macrophages which utilize either a phosphatidylserine receptor or the vitronectin receptor ($\alpha_v\beta_3$). J. Immunol. 161:6250-6257.

Bratton DL, **Fadok VA**, Richter DA, Kailey JM, Frasch SC, Nakamura T, Henson PM. 1999. Polyamine regulation of plasma membrane phospholipid flip-flop during apoptosis. J. Biol. Chem. 274:28113-28120.

Fadok VA. 1999. Clearance: The last and oft forgotten stage of apoptosis. J. Mammary Gland Biology and Neoplasia, 4:203-211.

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RESEARCH AND PROFESSIONAL EXPERIENCE (CONTINUED). PAGE LIMITATIONS APPLY.
DO NOT EXCEED 3 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

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C. Research Support

Ongoing Research Support

RO1 GM60449 Fadok (PI) 1/1/00 – 12/31/04
NIH/NIGMS
Phosphatidylserine-specific Receptor for Apoptotic Cells
This grant specifically addresses the nature of the phosphatidyl serine receptor. It will involve determination of its distribution within cells and in different tissues, orientation in the membrane, mechanism of PS recognition, relationship *to C. elegans* and *Drosophila* homologs, synthetic regulation, etc.
Role: PI

PO1 HD 38129 Fadok (PI) 8/1/00 – 7/31/05
NIH/NICHD
Functional Development of the Mammary Gland
Dr. Margaret Neville, Program Director
This project in the PPG entitled "Functional Development of the Mammary Gland" will address the recognition mechanisms for removal of apoptotic epithelial cells during involution of the mammary gland. It does not overlap with the current proposal. Valerie Fadok is the prinicipal investigator for this project.
Role: PI

SCOR: 1 P01 HL67671 Fadok (Co-PI, Project 1) 9/1/01 – 7/31/06
NIH/NHLBI
Program Director: Robert Mason, MD, Pathology of Fibrotic Lung Disease
Title of Project 1: Role of Cell Debris Removal in Fibrosis, Peter Henson, Principal Investigator
Role: Co-PI

Completed Research Support:

R01 HL60980 Fadok (Co-PI) 8/1/98 – 8/31/01
NIH/NHLBI
Neutrophil Apoptosis in Resolution of Inflammation
(Relinquished to SCOR mentioned above)
Peter Henson, Principal Investigator
Role: Co-PI

RO1 HL50319 Fadok (Co-PI) 9/1/93-8/31/98
NIH
Control of Neutrophil Function by Volume
Peter Henson, Principal Investigator
Role: Co-PI

R01 GM 48211 Fadok (Co-PI) 5/1/97 - 4/30/01
NIH
Phosphatidylserine-specific Removal of Apoptotic Cells
Peter Henson, Principal Investigator
Role: (Co-PI)

Development Support for Cancer Center, 1995-1997

Phosphatidylserine-specific receptor for apoptotic cells

F. Vertebrate Animals:

Mice, rats, and rabbits will be used for production of monoclonal antibodies and polyclonal antisera, for production of bone marrow derived macrophages and thioglycollate-elicited macrophages, and for production of embryos.

a. Female balb/c mice will be used for monoclonal antibody production. 12 mice will be used for each injection of protein or peptide. We estimate producing 8 new antibodies; therefore we will need 96 mice. When possible, we will produce the resulting monoclonals in the bioreactor; however, not all hybridomas grow well under those conditions. We therefore may need mice for ascites production. I am estimating 50 male balb/c's for that purpose.
TOTAL: 146 MICE

b. C3H/Hej LPS-resistant mice are used to make bone-marrow derived macrophages; these will be required for the studies in Aim 2. I am estimating that 5 mice will be used for each preparation, and that we will need at least 10 different preparations. Therefore 50 male C3H/Hej mice will be used. In addition, we will need an additional 50 males for production of thioglycollate-elicited macrophages.
TOTAL: 100 MICE

c. Breeding mice will be needed for production of embryos. We will use balb/c breeding triplets and will require 10 embryos for each time point. We will initially assess 4 time points in embryonic life: day 11.5, 12.5, 13.5, 14.5; therefore we will need 40 embryos. I am estimating that we will need 5 breeding triplets to ensure this amount. We will therefore use 5 males, 10 females, and 40 embryos .
TOTAL: 55 MICE

d. Approximately 10 female balb/c mice will be needed to make thymocytes.
TOTAL: 10 MICE

e. Sprague-Dawley rats will be used to make rat anti-mouse PSR hybridomas if the mouse
anti-human antibodies do not cross react with mouse antigen. Under those conditions, we
would require 24 females.
TOTAL: 24 RATS

f. New Zealand white rabbits will be used for production of polyclonal antisera against
whole protein and peptides. I am estimating 3 rabbits per each injection with a minimum
of 4 proteins and 4 peptides.

Mice, rats, and rabbits are required because there are no other ways to make the antibodies we need for these studies. Also, we have found no cell line that can substitute for primary macrophages in terms of the phagocytic function we study.

The animals are housed in an AALAC-approved facility, and are under the care of Dr. Ron Banks, a board-certified laboratory animal veterinarian at University of Colorado Health Sciences Center.

Every effort is made in our facility to ensure that the animals do not suffer distress or pain. Animals used for bone-marrow macrophages or for embryo production will be humanely euthanatized prior to harvesting any tissue. Those animals receiving intraperitoneal injections are momentarily restrained and the needle size is 25 gauge or smaller. For thioglycollate-elicited macrophages, the animals are injected once then humanely euthanatized prior to harvesting cells. For antibody production, the animals do receive multiple injections but they are very rapid. In the case of mice and rats, they are given intraperitoneally. In the case of rabbits they are given subcutaneously. No tranquilizing drugs are required, as the injection of the drug would be more noxious and has the potential for more adverse side effects than the injection of the cells or proteins. Ascites production will be minimized, and will not be used at all if the hybridomas can adapt to the bioreactors. If ascites is required, the animals are monitored daily and they are euthanatized prior to harvesting ascitic fluid. No animal receives multiple peritoneal taps for ascites.

The method of euthanasia for mice and rats is CO2 narcosis; for rabbits, an overdose of sodium pentobarbital. These methods are in keeping with the recommendations of the Panel on Euthanasia of the AVMA.

Mechanisms of Apoptotic Cell Clearance in Mammary Involution
Valerie A. Fadok, P. I.

G. EXPERIMENTAL ANIMALS.

1. The animals to be used are balb/c mice, C57Bl/6 mice, C1q-deficient mice, and hamsters or rats. Female mice are bred at approximately 8 weeks and will be used during their first pregnancy. I am estimating that 200 mice/year will be required and that at any one time, we will maintain 100. Thirty Sprague-Dawley female rats and or 30 Armenian hamsters will be used for production of monoclonal antibodies against mouse CD36, mouse apoptotic primary epithelial cells, mouse apoptotic HC-11 cells, mouse viable primary cells, viable HC-11 cells; each will be housed for 12 weeks at a cost of 1.00/day.

2. In all experiments, discomfort and injury to the animals is limited to that which is unavoidable in conducting the research. Anesthetics will be used to minimize discomfort and pain.

3. Animals will be euthanatized with CO2, a method consistent with the recommendations of the Panel of Euthanasia for the American Veterinary Medical Association.

4. Mice will be housed in a licensed animal care facility at University of Colorado Health Sciences Center, which is under the supervision of veterinarians certified in the specialty of laboratory animal medicine.

University of Colorado Cancer Center

Program in Hormone-Related Malignancies

SEMINAR SERIES: FALL 2001

Tuesdays, 4:00 p.m: BRB Room 623

September 11	**Donghai Dai, Ph.D.** Postdoctoral Fellow Obstetrics and Gynecology	**"Mechanisms of Progesterone Control of Endometrial Cancer Cell Growth"**
October 2	**Peter Jones, Ph.D.** Assistant Professor Pediatrics, UCHSC	**"Regulation of Paired-related Homeobox Genes by the Extracellular Matrix"**
October 9	**Melissa Allen, Ph.D** Fellow Endocrinology, VAMC	**"A Novel Mechanism for GnRH Neuron Migration Involving Gas6/Ark Signaling to p38 MAPK"**
October 23	**John Pawlowski, Ph.D.** Instructor Endocrinology, VAMC	**"Mechanisms of Transcriptional Repression by Liganded Steroid Receptors"**
October 30	**Phil Zeitler, M.D.** Assistant Professor Pediatrics, UCHSC	**"Growth Hormone Releasing Hormone (GHRH) and Breast Cancer"**
November 13	**Doug Wolf, Ph.D.** Assistant Professor Obstetrics and Gynecology	**"Progesterone Regulated Genes in Endometrial Cancer: Effectors of Growth, Migration and Apoptosis"**
December 11	**Robert Sclafani, Ph.D.** Professor Biochemistry and Molecular Genetics UCHSC	**"Cell Cycle Regulation in Breast Cancer Cells"**
December 18	**Jay Wimalasena, Ph.D.** Professor & Director of Research Department of Obstetrics and Gynecology University of Tennessee Medical Center Knoxville, TN	**"Regulation of Growth and Apoptosis in Breast Cancer Cells by Estrogen"**

University of Colorado Cancer Center

Program in Hormone-Related Malignancies

SEMINAR SERIES: Winter/Spring 2002

Tuesdays, 4:00 p.m: BRB Room 623

January 29	**Carol Sartorius, Ph.D.** Instructor, Division Endocrinology UCHSC	**"Role of Progesterone Receptors A and B in Hormone-Dependent Human Breast Tumor Growth"**
February 5	**Lori Sussel, Ph.D.** Assistant Professor Barbara Davis Center	**"Transcriptional Regulation of the Mouse Embryonic Pancreas"**
February 26	**Trevor Williams, Ph.D.** Professor Timpte/Brownlie Endowed Chair in Craniofacial/ Molecular Biology, UCHSC	**"AP-2 Genes and the Control of Development and Disease"**
Friday **March 15** **8:00am -12:00pm** **Denison Auditorium**	**Myles Brown, M.D.** **James A. Fagin, M.D.** **V. Craig Jordan, Ph.D., D.Sc.** **Nancy Weigel, Ph.D.**	**"Program in Hormone Related Malignancies Mini Symposium"**
March 26	**Angie Ribera, Ph.D.** Associate Professor Physiology & Biophysics	**"Using Zebrafish Mutants to Study Neuronal Development"**
April 2	**William Schieman, Ph.D.** Assistant Professor National Jewish Medical and Research Center	**"TGF-beta Signaling: Looking for Some Positive Reinforcement"**
April 9	**Russell V. Anthony, Ph.D.** Hill Professor Department of Physiology Colorado State University	**"Transcriptional Regulation of Placental Hormones"**
April 23	**Anthony Elias, M.D.** Associate Professor Director, Clinical Breast Cancer Program UCHSC	**"Newer Therapies in Breast Cancer"**
May 7	**DOD Breast Cancer training grant Student seminars**	

May 14	**DOD Breast Cancer training grant Student seminars**	
May 28	**Shlomo Melmed, M.D.** Professor and Director Department of Endocrinology & Metabolism Cedars-Sinai Medical Center for Human Nutrition Los Angeles, CA	**"Molecular pathogenesis of Pituitary tumors"**

University of Colorado Cancer Center

Program in Hormone Related Malignancies

Traci Lyons — Graduate Student, Breast Cancer Training Program

"Proteomic Analysis of Akt Substrates in Breast Cancer cells"

Steve Rosinski — Graduate Student, Breast Cancer Training Program

"Peripheral T Cell Levels Correlate with Outcome for Patients with Breast Cancer Undergoing Autologous Stem Cell Transplantation"

Tuesday, May 7th, 2002
4:00 p.m.

6th Floor Conference Room
623 Biomedical Research Building

University of Colorado Cancer Center

Program in Hormone Related Malignancies

Katherine Lobel — Graduate Student, Breast Cancer Training Program

"MEKK2 Integrates ERK5-JNK Signaling for Growth Factor Regulation of c-Jun Expression and AP-1 Activation"

Suzanne Wardell — Graduate Student, Breast Cancer Training Program

"Novel Progesterone Receptor Coactivators"

Tuesday, May 14th, 2002
4:00 p.m.

6th Floor Conference Room
623 Biomedical Research Building

University of Colorado Cancer Center

Program in Hormone-Related Malignancies

MINI SYMPOSIUM

"Molecular Mechanisms of SERMs for the Treatment and Prevention of Breast Cancer"

V. Craig Jordan, PhD, DSc.
Diana, Princess of Wales Professor of Cancer Research
Director, Lynn Sage Breast Cancer Research program
Robert H. Lurie Comprehensive Cancer Center
Northwestern University

"Nuclear Receptors: A Therapeutic target in Androgen Dependent and Androgen Independent Prostate Cancer"

Nancy L. Weigel, PhD.
Professor
Molecular and Cellular Biology
Baylor College of Medicine

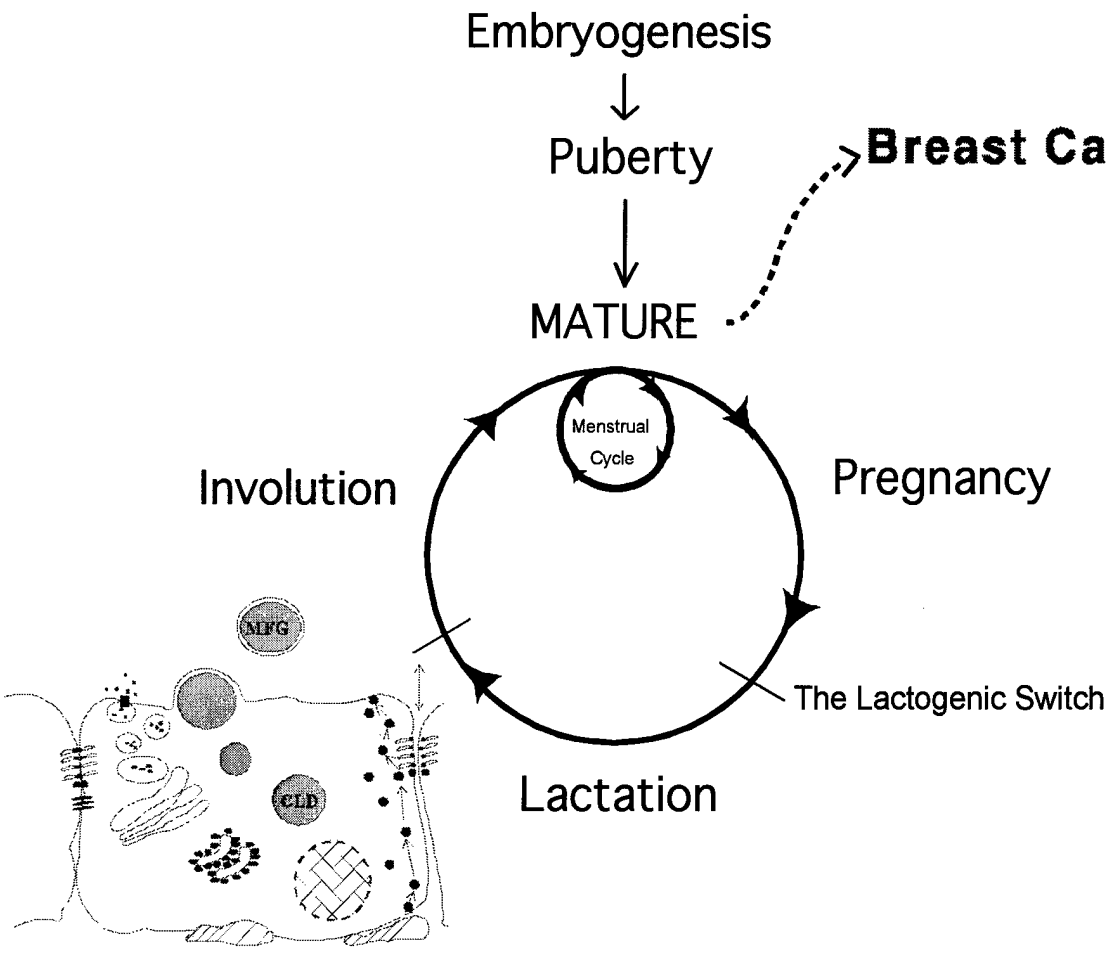
"Pathogenesis of Thyroid Cancer: Insights from Chernobyl and New Targets for Therapy"

James A. Fagin, MD.
Heady Professor of Medicine and Cell Biology
University of Cincinnati College of Medicine

"Role of Co-regulators in Hormone Action"

Myles A. Brown, MD.
Associate Professor
Dana-Faber Cancer Institute
Harvard Medical School

Friday, March 15th, 2002
8:00 a.m.–12:30 p.m.
Denison Auditorium, UCHSC



Mammary Development, Function, and Neoplasia

January 31-February 1
Doubletree Hotel
3203 Quebec St.
Denver, CO

Retreat: Mammary Development, Function and Neoplasia

5:30 PM Thursday, January 31

Reception and buffet –(posters may be put up at this time)

7:00 PM Welcome and Introduction, Peggy Neville

Session 1 Hormones and Development, Steve Anderson, Chair

7:10 Jeff Rosen, Baylor College of Medicine
Systemic hormones and local growth factor regulation of mammary gland development

8:00 Elizabeth Anderson, Manchester, UK
Steroid receptors, proliferation and differentiation in the normal and the "at risk" human breast

8:50 Steve Anderson, *UCHSC*
Prolactin signaling

9:10 Poster Discussion: Peggy Neville,
Studies of Mammary Differentiation

Friday, February 1

7:30 Continental Breakfast—Put up posters

8:30 Announcements

8:35 AM Session 2 The extracellular matrix, Pepper Schedin, Chair

8:35 Introduction

8:40 Mary Helen Barcellos Hoff, Lawrence Berkeley Laboratory
Spheres within spheres: TGF_, ovarian hormones and proliferation

9:20 Pepper Schedin, AMC
Endocrine-induced changes in mammary gland stroma modulate metastatic phenotype of breast cancer cells

9:40 Viroj Boon, *UCHSC*
Molecular mechanisms of progesterone receptor non-genomic actions

10:00 Jerry Schaack, Poster discussion
New adenoviral tools

10:15 AM Coffee Break and posters

10:45 AM Session 3 Milk Secretion, Kathryn Howell, Chair

10:45 Introduction

10:50 Ian Mather, University of Maryland
Function of Butyrophilin in Lactation

11:40 Jim McManaman and Mike Lewis, UCHSC and Baylor College of Medicine
Hedgehog regulation of alveolar differentiation

12:00 Kathryn Howell
Getting out of the Golgi

12:20 PM Buffet Lunch and posters

2:00 PM Session 4 Immune System, Valerie Fadok, Chair

2:00 Introduction

2:10 Jeffrey Pollard, Albert Einstein College of Medicine, N.Y.
The changing stroma and mammary gland development and tumorigenesis.

3:00 Poster Discussion: Valerie Fadok, NJC
Phagocytosis by mammary epithelial cells

3:15 Poster Discussion: Robert Ullrich, CSU
Genetic susceptibility to radiation-induced breast cancer in a mouse model

3:30 Poster Discussion: Pamela Cowin, NYU
Catenin and Gli signaling in the mammary gland

3:45 PM Break and posters

4:00 PM Session 5 Mammary stem cells, Peggy Neville, Chair

4:00 Introduction

4:05 Kay-Uwe Wagner, University of Nebraska Medical Center
An adjunct mammary epithelial cell population in parous females: its role in functional adaptation, tissue renewal, and neoplastic transformation.

4:55 Poster Discussion: Monical Richert
Deletion of downstream effectors of PRLR signaling affect mouse mammary gland development

5:10 Poster Discussion: Jennifer Richer
New transcriptional regulators of progesterone receptors discovered by cDNA microarray methods

5:25 Cocktails (Cash bar) and Posters

7:00 PM Banquet

After dinner speaker: Piotr Sicinsky, Harvard University (introduced by Heide Ford)
Cyclins D1 and E in mammary gland development and neoplasia

Saturday Morning

8:00 AM—10:00 AM Advisory Board meets with PPG members, guests

ABSTRACTS

1. Susan M. Bailey1, Edwin H. Goodwin2, Lila Ramaiah1, Scott Pearson1 and Robert L. Ullrich1
   Impaired Telomere Function, Radiation-Induced Instability and Tumorigenesis

2. Heide Ford,
   Role of the homeobox gene HSIX1 in mammary development and neoplasia

3. D.M.E. Harvell, T.E. Streeker, B. Xie, K.I. Pennington, R.D. McComb, and J.D. Shull
   Dietary Energy Restriction Inhibits Estrogen-Induced Mammary Carcinogenesis in the Female ACI Rat.

4. Sarah Hatsell, Tracey Rowlands, Suzzette Arnal & Pam Cowin
   Catenin and Gli Signaling in the Mammary Gland

5. Metz, R. P., Kaeck, M. Stacewicz-Sapuntzakis, M., Mitrenga, T., McCarty, H. and P. Schedin.
   Effects of vitamin A intake on adolescent mammary gland development and MNU-induced carcinogenesis susceptibility in the Sprague-Dawley rat.

6. Jenifer Monks, F. Jon Geske, Lehman and Valerie A. Fadok
   Phagocytosis by mammary epithelial cells

7. Carol Palmer, Roman Drews, Timothy K. Lee, Henryk Lubon.
   Effect of Recombinant Human Protein C Expression on Mouse Mammary Gland Development

8. Lila Ramaiah, Scott Pearson, Susan M. Bailey, Robert L. Ullrich
   The Search for Breast Cancer Susceptibility Genes Using a Mouse Model

9. Tanya D. Reed, Neal E. Beeman, Emily F. Freed and Margaret C. Neville
   Transduction of the Mammary Epithelium with Adenoviral Vectors

10. Jennifer K. Richer, Nicole G. Manning, and Kathryn B. Horwitz
    New Transcriptional Regulators of Progesterone Receptors Discovered by cDNA Microarray Methods

11. Monica M. Richert and Steven M. Anderson
    Deletion of Downstream Effectors of Prlr Signaling Affect Mouse Mammary Gland Development

12. Michael Rudolph, Valerie Sawicki, Tzu Phang, Sonia Leech, Tanya Reed, and Margaret Neville
    Microarray Analysis of Secretory Activation in the Mouse Mammary Gland

13. Jerome Schaack, Michael Bennett, and John Moorhead
    Construction of Improved Adenovirus Vectors for Delivery *In Vivo*

14. Brigitte Troskie, Valerie Sawicki, Julia Foo, Margaret C. Neville
    Potential Role of Claudins in Tight Junction Closure in the Mouse Mammary Gland

15. Yihong Wan and Steven K. Nordeen
    Differential Gene Regulation by Glucocorticoids and Progestins

16. Suzanne E. Wardell, Dean P. Edwards
    Jun Dimerization Protein-2 Functions as a Progesterone Receptor N-terminal Domain Coactivator

17. Harriet Watkin, Hazel Weir* and Charles Streuli, University of Manchester and *Astra Zeneca
    Using Adenovirus to Explore the Role of Focal Adhesion Kinase in Mammary Epithelial Cell Differentiation

1. Impaired Telomere Function, Radiation-Induced Instability and Tumorigenesis

Susan M. Bailey1, Edwin H. Goodwin2, Lila Ramaiah1, Scott Pearson1 and Robert L. Ullrich1
1Dept. of Radiological Health Sciences, Colorado State University, Fort Collins, CO
2Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM

Telomeres are an often under-appreciated element in preserving genomic stability because, under normal circumstances, they protect chromosome ends extremely well. When telomere dysfunction does occur however, the consequences are severe. They include the induction of cellular senescence and chromosomal rearrangements that may promote carcinogenesis. The molecular basis of telomere function is only now coming to light. Previously we demonstrated that effective end-capping of mouse telomeres has a paradoxical requirement for proteins more commonly associated with DNA double-strand break (DSB) repair. Ku70, Ku80, and DNA-PKcs (the catalytic subunit of DNA-dependent protein kinase), the constituents of DNA-PK, participate in DSB repair by non-homologous end-joining (NHEJ). Mutations in any of these genes cause spontaneous chromosomal end-to-end fusions that contribute significantly to the background level of chromosomal aberrations [Bailey et al., PNAS 96 (1999), 14899]. Additionally, we have demonstrated that telomeres produced via leading-strand DNA synthesis are especially susceptible to end-to-end fusion [Bailey et al., Science 293 (2001), 2462]. We now find that impaired end-capping also allows telomeres to join to ends created by radiation-induced DSB in DNA-PKcs-deficient genetic backgrounds. Three such cell lines were exposed to gamma rays and examined utilizing the specialized cytogenetic technique of CO-FISH in order to distinguish true telomere-to-DSB events from telomere-to-telomere fusions. Telomere-to-DSB fusions were seen in each cell line, including BALB/c, which retains some DNA-PK activity and is susceptible to radiation-induced mammary cancer. These results demonstrate that dysfunctional telomeres in cells with DNA-PKcs deficiency can inappropriately fuse to DSB ends, creating novel chromosome structural rearrangements. Thus, the radiation-sensitive phenotype associated with DNA-PKcs deficiency is not due solely to ineffective DSB repair. Rather, telomere-to-DSB joining provides an additional pathway for misrepair and the initiation of genomic instability not existing in repair-proficient cells.

2. Dietary Energy Restriction Inhibits Estrogen-Induced Mammary Carcinogenesis in the Female ACI Rat.

D.M.E. Harvell[1,2], T.E. Streeker[1,3], B. Xie[1], K.I. Pennington[1], R.D. McComb[2], and J.D. Shull[1,2,3]. [1]Eppley Institute for Research in Cancer, [2]Department of Pathology and Microbiology, [3]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198-6805

The female ACI rat is a unique and physiologically relevant animal model for the study of breast cancer etiology in that it is highly susceptible to the development of estrogen-induced mammary cancer, but rarely develops mammary cancer spontaneously. The purpose of the present study was to investigate whether dietary energy restriction inhibits mammary carcinogenesis induced by 17beta-estradiol (E2) in female ACI rats. To test this hypothesis, we examined the incidence of mammary tumors and precancerous lesions in E2 treated female ACI rats fed a 40% energy restricted diet. The effects of dietary energy restriction and estrogen on mammary tumor latency, multiplicity and size were also examined. To address the mechanisms through dietary energy restriction might exert its inhibitory effects on E2-induced mammary cancer development, we examined progesterone receptor expression and cell proliferation in the mammary epithelium of E2 treated rats fed the energy restricted diet. The data presented herein, indicate that dietary energy restriction inhibits E2-induced mammary cancer development in female ACI rats apparently by inhibiting the progression of precursor lesions into carcinomas. The potential involvement of progesterone receptor expression and mammary cell proliferation in the ability of dietary energy restriction to inhibit E2-induced mammary carcinogenesis will also be discussed.
Supported by grant 97A146 from the American Institute for Cancer Research and grants CA68529 and CA77876 from the NIH.

3. CATENIN AND GLI SIGNALING IN THE MAMMARY GLAND

Sarah Hatsell, Tracey Rowlands, Suzzette Arnal & Pam Cowin

We have produced a transgenic mouse expressing MMTV-?N89 ß-Catenin. This mouse shows mild ductal retardation and precocious mammary development undergoing lobuloalveolar development in virgin mice. Involution is incomplete and all female mice rapidly develop adenocarcinomas. This phenotype differs significantly from the MMTV-Wnt-1 induced phenotype in that no hyperbranching occurs. A comparison of the two strains shows that Wnt-1 signals to myoepithelial cells inducing a dramatic upregulation of K14 and K5 expression. MMTVß-catenin, which is restricted to ductal cells does not produce this effect suggesting that Wnt-1 is acting in a direct paracrine fashion on myoepithelial cells. Crossing ?N89ß-catenin mice to cyclin D1 null mice does not reduce the incidence of tumor formation suggesting that ß-catenin's oncogenic function acts through other proliferative pathways in mammary gland. Examination of the pattern of promoter activation of Gli 1 suggests that this protein does not play a significant role patterning the mammary epithelium but is expressed in other elements of the gland.

4. Role of the homeobox gene HSIX1 in mammary development and neoplasia

Heide Ford, Departments of Obstetrics and Gynecology and Biochemistry, UCHSC

Both tumorigenesis and normal development involve alterations in cell proliferation and differentiation, alterations in cell death, neovascularization, cell motility, and invasion of surrounding tissue. Therefore, homeobox genes involved in normal developmental processes may contribute to tumorigenesis if misexpressed. The human HSIX1 homeobox gene may fit this category. HSIX1 is upregulated in 44% of primary breast cancers and 90% of metastatic lesions, and its overexpression leads to a significant increase in tumor burden in a nude mouse model. We have demonstrated that overexpression of HSIX1 attenuates the DNA damage-induced G2 cell cycle checkpoint, representing the only known involvement of a homeobox gene in G2 checkpoint control in mammary carcinoma cells and providing a mechanism through which it may affect tumorigenesis. However, the molecular means by which HSIX1 affects this checkpoint is still unknown. As HSIX1 is a homeobox gene and is likely involved in normal development through its role in activating or repressing transcription, we have examined the expression of HSIX1 in the normal mammary gland by in situ hybridization. Preliminary data suggest that HSIX1 is differentially expressed during mammary gland development. Models being developed to identify the targets of HSIX1, as well as to determine the in vivo function of HSIX1 in normal mammary gland development and in tumorigenesis will be discussed.

5. Effects of vitamin A intake on adolescent mammary gland development and MNU-induced carcinogenesis susceptibility in the Sprague-Dawley rat.

Metz, R. P., Kaeck, M., Stacewicz-Sapuntzakis, M., Mitrenga, T., McCarty, H. and P. Schedin.

Retinoids have been shown to protect against mammary cancer in rodents. Epidemiological evidence suggests that retinoids also provide protect against breast cancer in women. However, this effect is slight and only observed in premenopausal women. Research has confirmed that the mammary gland is more susceptible to carcinogenic insult during adolescence than at any other developmental stage. Based on these observations, we hypothesize that the developing adolescent mammary gland represents the optimal target for retinoid chemotherapeutic intervention.

To test this, female Sprague-Dawley rats were fed a purified diet that was vitamin A deficient, adequate (2.2mg retinyl palmitate/kg diet), or supranutritional (16mg retinyl palmitate/kg diet) from 21 to 63 days of age, the period of adolescent mammary gland development in rats. At 73 days of age, rats were given 25 mg MNU/kg bw i.p. and monitored for mammary tumors over the next six months. Tumors appeared earlier and more frequently in rats fed either vitamin A deficient or supplemented diets. Vitamin A deficiency during adolescence was associated with alveolar mammary gland development and precocious milk protein expression, while vitamin A supplementation was associated with ductal gland development and suppressed milk protein expression. Differences in circulating estradiol or in mammary estrogen receptor alpha mRNA were not observed, suggesting that effects of dietary vitamin A on mammary gland development and carcinogenesis are estrogen-independent. Mammary expression of two other steroid hormone receptors known to regulate lobuloalvealor development, the progesterone and glucocorticoid receptors, was also unaffected

6. Phagocytosis by mammary epithelial cells

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The process by which a fully functional, lactating mammary gland regresses to a quiescent, resting gland is known as mammary involution. The morphological changes in the whole gland, and in the milk-secreting, alveolar cells during this process have been described by many investigators, although there is disagreement in the literature as to whether the immune system plays an integral role in mammary involution. Most often, macrophages have been implicated in clearance of residual milk, milk fat and apoptotic cells. However, removal of apoptotic cells during mammary involution is known to be mediated in part by neighboring mammary epithelial cells, themselves destined to die and be cleared. Here we begin to describe the mechanisms utilized by cultured mammary epithelial cells (MECs) to bind and engulf apoptotic cells. We find that MECs use many of the receptors known to be involved in macrophage binding of apoptotic cells and stimulation of engulfment. Additionally, we find that MECs respond to apoptotic cells by releasing TGF-_1, an anti-

- inflammatory and growth regulatory cytokine. This implies that the MECs themselves may contribute to an immunosuppressive environment during involution of the mammary gland.

7. Effect of Recombinant Human Protein C Expression on Mouse Mammary Gland Development

Carol Palmer, Roman Drews, Timothy K. Lee, Henryk Lubon

Transgenic livestock animals are considered the future in the production of recombinant proteins. Before moving on to a larger production animal, transgenic mice are useful models to predict how a transgene will perform in a chosen tissue, and if expression of a foreign gene will affect the animal's physiology. Human protein C (HPC), a vitamin K-dependent plasma protein, has been expressed in the milk of transgenic mice using a construct containing the 4.2 kb mouse whey acidic protein (WAP) promoter, the 9.0 kb HPC gene, and 0.4 kb of the HPC gene 3' flanking sequence (WAP/HPC). Five lines of mice transgenic for WAP/HPC lactate normally and secrete recombinant human protein C (rHPC) at a concentration of 0.1 to 0.7 mg/ml. A slight phenotype is observed in hemizygote mammary glands as compared to control animals. They have less distended alveoli, with centrally positioned nuclei in thicker epithelial cells. To determine whether production of recombinant human protein C could be increased in a transgenic system, mice homozygous for the WAP/HPC transgene were created. Homozygous WAP/HPC mice had normal growth, activity and fertility, but homozygous females had problems with lactation and were unable to raise normal litters of pups. Histological analyses of lactating mammary glands from homozygous females showed barely distended alveoli filled with dense-staining milk. The mammary epithelial cells had distinct, centrally located nuclei and contained large cytoplasmic lipid droplets. Northern blot hybridization analysis using RNA from hemizygous and homozygous mice showed that homozygous mice from the 6.4 line expressed the transgene at higher levels than hemizygous animals, but the 4.2 homozygous line expressed the transgene at lower levels than the hemizygote line. At the protein level, the 6.4 homozygous line also had increased rHPC levels in the milk as revealed by Western blot analysis. For the milk proteins WAP, b-casein, and a-lactalbumin, decreased and/or delayed mRNA expression was observed in all transgenic lines. In addition, the WAP/HPC transgene was induced before endogenous WAP. In the milk, the major milk protein bands were expressed at similar levels in all animals. There were, however, elevated levels of expression of three high molecular weight proteins in transgenic samples. These proteins were identified by microsequencing as glucose-regulated protein 94, glucose-regulated protein 78, and calreticulin. These proteins are all members of a soluble group of endoplasmic reticulum proteins known as reticuloplasmins. From theses results, it is concluded that expression of the WAP/HPC transgene affects mouse mammary gland development.

8. THE SEARCH FOR BREAST CANCER SUSCEPTIBILITY GENES USING A MOUSE MODEL

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High-penetrance mutations such as BRCA1 & 2 are well established but infrequent causes of increased breast cancer susceptibility. In most cases, breast cancer is etiologically heterogeneous and multiple genetic traits such as low-penetrance mutations and natural genetic polymorphisms are likely involved. Polymorphisms and low-penetrance mutations affecting DNA repair genes are attractive candidates as they could contribute to breast cancer susceptibility by increasing genomic instability and mutability of cells and their progeny. Previous investigations in our lab using cell dissociation and ductal dysplasia assays have demonstrated that BALB/c mice are more susceptible to mammary cancer development after exposure to ionizing radiation (IR) than are C57BL/6 mice or other strains of mice. This susceptibility is associated with increased cytogenetic instability, as well as decreased tissue concentrations and kinase activity of the DNA repair enzyme DNA-dependent protein kinase (DNA-PK). Two BALB/c-specific point mutations have been identified within coding regions of the gene for the catalytic subunit of DNA-PK, DNA-PKcs (Prkdc). We hypothesize that one or both of these mutations plays a causative role in BALB/c's susceptibility to IR-induced mammary cancer. Preliminary studies on F2 backcross mice have demonstrated an association between the Prkdc genotype, genetic instability, and ductal dysplasias. However, phenotypic variability observed among animals with persistent dysplasias would suggest that modifier genes in the BALB/c genome could also be involved in the development of persistent ductal dysplasia and its progression to cancer. We aim to determine the contribution of the variant DNA-PKcs gene, PrkdcBALB, to susceptibility to radiation-induced mammary carcinogenesis. Congenic mouse strains (mice that differ only at a specific gene locus/allele of interest) are being generated as follows: C.B6-Prkdc, (C57BL/6 Prkdc allele on the BALB/c background), and B6.C-PrkdcBALB, (BALB Prkdc allele on a C57BL/6 background). These congenic strains will allow us to evaluate the degree of association between the variant gene and the development of mammary tumors. It is anticipated that although some degree of association will be observed, results will indicate that modifier genes in the BALB/c genome are also involved. Mapping strategies and gene expression studies will help us identify these genes.

9. Transduction of the Mammary Epithelium with Adenoviral Vectors

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Up the teat microinjection of adenoviral vectors presents a non-invasive, non-inflammatory delivery system to study gene expression in the mammary epithelium. An E1/E3 deleted adenoviral vector (human adenovirus type 5) encoded with either LacZ or GFP reporter genes was injected into the fourth (LacZ) or third (GFP) mammary gland of mice at various stages of mammary gland development. 14C-sucrose was injected intraductally on the day of sacrifice to test the status of tight junctions and glands were excised to examine evidence of mastitis. Doses of 107 pfu (fourth mammary gland) or 2.6 x 106 pfu (third mammary gland) injected into day 17 pregnant mice showed minimal inflammation after 3 days. However, significant mastitis resulted after 7-10 days even with optimal doses of the adenovirus. Up to 40% of alveoli can be transduced at day 17 of pregnancy and up to 25% of the total epithelium can be transduced in the portion of the gland proximal to the teat. Although adenovirus can transduce the epithelium at any stage of mammary gland development, transduction gives the least inflammation in the late pregnant animal and can be maintained into early lactation. These findings demonstrate that up the teat microinjection of adenoviral vectors provides a versatile method of changing gene expression in cells of the mammary epithelium. EFF was supported by NCI Cancer Education Grant R25 CA49981 and ACS Colorado Division, Brooks Trust. TDR, NEB and MCN were supported by grants from the Department of Defense.

10. Deletion of downstream effectors of PRLR signaling affect mouse mammary gland development

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Normal development of the mammary gland results from a precise balance of proliferation and cell death. Proliferation occurs predominantly during pregnancy and the size of the gland is maintained during lactation. After weaning, through a process of programmed cell death or apoptosis, the gland goes through involution and returns to a state of pre-pregnancy. We are studying the downstream signaling molecules of the prolactin receptor to determine the effect of each of the signaling pathways on mammary gland development. The tumor suppressor PTEN is a phospholipid phosphatase that regulates the activation of the anti-apoptotic serine/threonine protein kinase Akt as well as a number of other signaling molecules including Shc and Fak. We observe a delay in involution in the mammary glands of mice that have a heterozygous deletion of PTEN. The Src-like kinases are non-receptor tyrosine kinases that are potentially upstream of both the Akt and MAP kinase pathways in prolactin receptor signaling. We have examined the mammary glands from mice with either a heterozygous deletion of Src or a homozygous deletion of Fyn. The Src deletion may result in an increased rate of involution and some disorganization of the gland during pregnancy, while, so far, we have not identified a phenotype in the Fyn knockout mice.

11. Microarray Analysis of Secretory Activation in the Mouse Mammary Gland

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Department of Physiology and Biophysics

The molecular agents that regulate activation of copious milk secretion in the mammary gland after parturition remain largely unknown. Hormonal regulation of this switch requires progesterone withdrawal in the presence of prolactin and glucocorticoid. In order to define the genes and pathways involved in this process, we used Affymetrix(tm) microarrays to examine gene expression in the mouse mammary gland at days 12 and 17 of pregnancy and lactation days 1, 2, and 9. Preliminary analysis of microarray results showed that secretory activation between pregnancy day 12 and lactation day 2 was accompanied by highly significant changes in the expression patterns of 1385 genes. As expected, expression of the milk proteins genes was significantly upregulated. Additional upregulated genes include the prolactin receptor, AKT, glucocorticoid receptor, and homeobox genes A4, D3, A2 and B6. Conversely, SOX4 (a known progesterone responsive gene), HOX D8, IGF binding proteins, the TGF-ß pathway and members of the WNT pathway were significantly downregulated. We conclude that secretory activation is a developmentally regulated switch that potentially represents a major change in cell fate.

12. The Role of Claudins in Tight Junction Closure in the Mouse Mammary

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During pregnancy the tight junctions in the mammary gland are highly permeable, allowing molecules as large as immunoglobulins to pass, where as during lactation they mammary tight junctions are highly impermeable. The transition between open and closed tight junctions is brought about by the withdrawal of progesterone around partutition. We examined the gene expression of components of the tight junction finding that most components (e.g. occludin, JAM, ZO1) did not change significantly. However, we found that the membrane protein claudin 7 was expressed at high levels and claudin 8 increased more than 3 fold during secretory activation and continued to increase during the course of lactation. The expression of the genes for these two proteins was verified by real time RT-PCR. Claudin-7 expression correlated well with the expression of keratin 19 suggesting that it is a marker for luminal epithelial cells. This conclusion was verified by in situ hybridization. Both claudin 7 and 8 are highly expressed in mouse mammary tumor models. We are currently characterizing antibodies to these two proteins to test the hypothesis that these proteins are components of mammary tight junctions and plan to overexpress claudin 8 to determine whether it plays a role in tight junction closure during secretory activation. Supported by DOD Breast Cancer Research Program DAMD17-01-1-0211.

13. New Transcriptional Regulators of Progesterone Receptors Discovered by cDNA Microarray Methods

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By examining progesterone regulated genes in progesterone receptor (PR) positive T47D breast cancer cells using microarray analysis, we have found that members of the Kruppel-like zinc finger, homeobox, and winged helix domain family of transcription factors are upregulated by PR after 6 hrs of hormone treatment. These transcription factors were not previously known to be progesterone regulated. We have confirmed the regulation of these factors by PR, using RT-PCR, northern or western blot analysis. PR directly regulates these transcription factors in the presence of cycloheximide at early time points (1.5 and 3.0 hrs) of progesterone treatment. Other transcripts encoding proteins already known to bind to PR and affect their activity were also found to be regulated by PR in our study. Therefore, to test the theory, that the immediate early transcription factors which we have found to be PR regulated might in turn affect PR activity, we examined their effects on transcription of PRE2tata, MMTV, and other progesterone-responsive gene promoters linked to a luciferase reporter. One of the Kruppel-like factors strongly upregulates basal transcription but inhibits transcriptional activity of progesterone occupied PR on PRE2tata-luc in a dose-dependent manner by 85%. The winged helix domain protein, on the other hand, increases PR-dependent transcriptional activity 8-10-fold, without altering basal transcriptional activity. These data establish the interesting principle, that factors which are regulated by progesterone, can in turn regulate progesterone's activity. They underscore an important use of array data. We are analyzing the role of these new coregulatory proteins in the normal and malignant breast.
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14. Construction of Improved Adenovirus Vectors for Delivery In Vivo

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We are attempting to improve adenovirus vectors for in vivo delivery two ways: first, by deleting adenovirus early region genes; and second, by incorporating adenovirus gene products that inhibit inflammation. A cell line that efficiently complements growth of adenoviruses mutated in the preterminal protein (pTP) and DNA polymerase (Pol) genes has been constructed and used to construct an adenovirus deleted for the pTP and Pol genes. The pTP/Pol deletion mutant chromosome has been introduced into the plasmid AdEasy1 to facilitate construction of transducing vectors. We are now attempting to construct pTP/Pol deletion mutant vectors encoding the reporter genes GFP and LacZ.
In contrast to natural infection with wild-type adenovirus, transduction in vivo with adenovirus vectors induces strong inflammation in a manner dependent on the amount of vector used and the level of adenovirus gene expression. Two regions of the virus that are typically deleted in gene therapy vectors, E1 and E3, encode functions that are important for inhibiting inflammatory responses. The E3 region encodes proteins that inhibit the effects of TNF-?. E1A proteins inhibit the function of interferons, including IFN-?. Since E1A proteins are the only viral gene products that inhibit transcriptional activation due to treatment with IFN-?, we have tested the effects of expression of the E1A and E3 proteins on inhibition of inflammation after transduction of mouse ears. Our preliminary evidence indicates that the presence of both E1A and E3 functions leads to a dramatic reduction in inflammation.

15. Differential Gene Regulation by Glucocorticoids and Progestins

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Glucocorticoid receptor and progesterone receptor are hormone-activated receptors sharing many structural and functional similarities including virtually identical DNA recognition specificity. Nonetheless, the cognate hormones mediate very distinct biological functions. To understand the mechanisms that engender biological specificity, we have identified 31 genes differentially regulated by the two hormones by >3-fold in the human breast cancer cells T47D/A1-2 using an affymetrix microarray analysis. To explore the mechanisms underlying the differential regulation, we have further investigated one of these genes, the RGS2 gene. This gene encodes a Gq(inhibitor and is specifically induced by glucocorticoids but not by progestins. In neither transient nor stable transfection assays could promoter regions of the RGS2 gene confer hormone regulation to reporter gene. This suggests that the regulation might be dependent on the endogenous chromatin environment and genomic locus, as seen for the stably integrated MMTV-luciferease gene in the same cells. DNaseI hypersensitivity assays revealed four nuclease hypersensitive sites in the RGS2 promoter. However, the DNaseI hypersensitivity at these sites is not regulated by either hormone. Due to the presence of a CpG island in the promoter, three sets of experiments were performed to test the role of DNA methylation in the regulation of RGS2 gene. None suggested that DNA methylation is involved in either basal expression or hormone regulation. Chromatin immunoprecipitation analyses were performed to assess the loading of receptors, RNA polymerase II and coactivators, as well as the level of histone acetylation and phosphorylation in the endogenous RGS2 promoter. The results demonstrated that the differential hormone regulation is not due to the different abilities of GR and PR to induce histone acetylation or to recruit RNA polymerase II binding in the proximal promoter region.

16. Jun dimerization protein-2 functions as a progesterone receptor N-terminal domain coactivator.

Suzanne E. Wardell, Dean P. Edwards, Dept. of Pathology and Program of Molecular Biology, University of Colorado Health Sciences Center, Denver, CO

Progesterone receptor (PR) activates transcription of target genes through two activation domains, ligand-dependent AF-2 in the C-terminal ligand binding domain (LBD) and ligand-independent AF-1 in the N-terminus. The steroid receptor coactivators (SRCs) interact with and mediate the activity of AF-2. Interacting proteins that mediate activity of AF-1 are not well understood, yet AF-1 participates in tissue specific responses, synergizes with AF-2, and mediates partial agonist activity of the antagonist RU486. By a yeast two-hybrid screen, we identified Jun dimerization proteins (JDP)-1 and -2 as PR interacting factors. JDP-1 and -2 were recently described as repressive members of the AP-1 family of transcription factors. PR and JDPs interact directly in vitro and in vivo as demonstrated respectively by GST pull-down assays and co-immunoprecipitation assays of transfected mammalian cells, and in both cases, PR-JDP interaction was not significantly affected by ligand. The JDP interaction site maps to the DNA binding domain (DBD) of PR. JDP-2 forms a ternary complex with PR on DNA, and this interaction does not significantly influence PR-DNA binding. In cell transfections, JDP-2, but not JDP-1, strongly enhanced (6-10 fold) ligand-dependent PR transactivation of target reporter genes, either a minimal response element or the more complex MMTV promoter. JDP-2 also enhances transactivation by either the complete N-terminal region or a minimal AF-1 (aa 456-546) linked to the DBD, independent of the LBD. Although the PR-DBD is sufficient for JDP-2 binding in vitro, JDP-2 does not enhance transactivation of a PR-DBD-VP16 construct, suggesting the adjacent N-terminus is necessary for JDP-2 effects on PR function. In the absence of PR, JDP-2 interacts directly in vitro with general coactivators CBP and pCAF, but not with SRCs. Coexpression of JDP-2 resulted in a five-fold induction of reporter gene expression by RU486; no induction by RU486 was observed in cells cotransfected with a vector control or SRC-1. These results suggest that JDP-2, through binding to the DBD, recruits and/or stabilizes a novel coactivator complex distinct from the SRC complex that is capable of mediating transcription through the N-terminus and potentiating agonist activity of partial antagonists.

17. Using adenovirus to explore the role of focal adhesion kinase in mammary epithelial cell differentiation

Harriet Watkin, Hazel Weir* and Charles Streuli,
University of Manchester and *Astra Zeneca

Signals regulating cell proliferation, differentiation and survival come from a variety of sources including the extracellular matrix and soluble ligands. In the mammary gland lactogenic hormones and growth factors are not the sole mediators of epithelial cell differentiation, but act in concert with signals provided by a laminin-rich basement membrane. Integrins are activated upon binding extracellular matrix ligands and this induces the formation of focal adhesions. To further elucidate the molecular mechanisms underlying this cross-talk between signals from the extracellular matrix and prolactin, we have developed a method using adenovirus-mediated gene transfer that allows us to express heterologous proteins in primary mammary epithelial cells. High expression levels can be obtained (over 90 % of cells expressed the LacZ reporter gene). Focal adhesion kinase (FAK) plays a critical role in integrin mediated signaling and we have generated recombinant-adenoviruses expressing dominant negative FAK in order to investigate whether FAK is important for b-casein synthesis in primary mammary epithelial cells.

Student Name: Sarah C. Roemer

Mentor/Principal Investigator: Dean Edwards, Ph.D.

Progress Report:

The steroid receptor, progesterone receptor (PR), has been implicated in the development and progression of both breast and uterine cancer. Previous work from the Edwards' lab has shown that the chromatin associated high mobility group box proteins 1 and 2 (HMGB-1/-2) enhance the affinity of PR for specific target gene DNA *in vitro*, and the transcriptional activity of PR *in vivo*. HMGB-1/-2 is also an important coregulatory protein for estrogen receptor (ER) and other steroid hormone receptors. Additionally, the level of HMGB-1/-2 expression in breast cancer cells can significantly alter the pharmacology of the anti-estrogen tamoxifen, thus, implicating HMGB-1/-2 as a factor involved in determining tumor response to tamoxifen therapy. Over the past year, Sarah Roemer has continued to work on defining the mechanism of enhancement of PR-DNA binding by HMGB-1/-2. An answer to this problem will require structure analysis to precisely determine how HMG interacts with PR. To obtain a suitable complex for X-ray crystallography trials, the minimal domains of PR and HMGB-1 required for interaction must first be determined. GST pull down assays were utilized to determine that DNA binding domain of PR is the region of the receptor that makes protein interaction with HMGB-1. The site within PR-DBD required for interaction was further mapped to the C-terminal extension (CTE), a region of ~40-50aa on the C-terminal side of the core zinc finger modules. Previous studies from the Edwards' lab have shown that the CTE of steroid receptors is also responsible for mediating the functional effects of HMGB-1 on PR-DNA binding and transcriptional activity indicating that the CTE is a meaningful site. The HMGB-1 construct that contains both DNA binding domains, termed box A and B, but lacks the C-terminal acidic tail was determined to be the minimal region of HMGB-1 required for protein interaction with the PR-DBD. However, a ternary complex between PR, HMGB-1, and DNA has not been detected in electrophoretic mobility shift assays (EMSA), suggesting that DNA may dissociate protein interaction between HMGB-1 and PR. As direct evidence of this, addition of specific target DNA greatly diminished the protein-protein interaction between HMGB-1 and PR-DBD, while the addition of a non-specific DNA had no effect. These results suggest that a ternary complex between HMGB-1, PR, and DNA may not exist, and that HMGB-1 affects PR function through a "hit and run" protein interaction mechanism. This information has been applied to making PR and HMGB-1 constructs that will be used in a protein complex for upcoming crystallography trials to determine the structural bases for HMGB-1 interaction with PR.

Student Name: Steven Rosinski

Mentor/Principal Investigator: Ian McNiece, Ph.D.

Progress Report:

Five-year relapse free survival for metastatic breast cancer following high-dose chemotherapy and hematopoietic stem cell rescue is 45%. A possible direction to improve relapse free survival is the development of strategies to utilize the immune system to eliminate the residual tumor after transplant. Immunodeficiency due to insufficient T cell recovery remains a significant barrier to the development of effective immunotherapy in patients that have undergone cytoreductive therapy. Mouse experiments suggest that thymus independent mechanisms maybe responsible for a component of T cell reconstitution in adults following cytoreductive therapy. Over the past year, Steve Rosinski has tested this hypothesis by enumerating T cell and three distinct blood dendritic cell levels from patients with metastatic breast cancer before and sequential times post autologous transplant. Preliminary analysis in the majority of patients shows a return of CD8+ T cell levels within 30 days, however, CD4+ T cell levels have not returned for a year or longer following transplant an observation consistent with previous reports. Additionally, naïve CD8+ T cell levels but not CD4+ T cells return after transplant. Also, a V-beta analysis on the reconstituted CD4 and CD8 T cell populations has shown a skewing of the repertoire further analysis is ongoing. Patients that have deficient CD4+ T cell levels have CMRF44+ DC levels that are ten fold lower then patients who have reconstituted their CD4+ T cell levels. This suggests a role for CMRF44+ DC in thymus independent T cell maintenance. The ability of blood dendritic cells to reconstitute CD4+ T cells has major implications for the development of immunotherapuetic strategies directed at preventing infectious complications and improving relapse free survival.

Over the last year this project has yielded several discoveries with respect to T cell levels and relapse free survival (RFS) for patients with breast cancer who undergo an autologous stem cell transplant (ASCT). We have shown before and after transplant that CD4+ T cell levels correlate with RFS. Specifically, CD4+CD45RO memory T cells and the CD4+CD45RA-CD62L- correlate with RFS. In addition, these correlations are clinically relevant with patients with higher levels of these T cells demonstrating a higher percentage of RFS. Finally, these correlations are independent of the stage of disease, Her2 neu status, and other T cell levels. Our results suggest that T cells, and specifically the memory subset CD4+CD45RA-CD62L-, may play a role in eliminating residual disease post transplant. These observations, if confirmed, provide the rationale for studies testing post-transplant immune strategies in ASCT recipients for BC.

Student Name: Jason D. Prescott

Mentor/Principal Investigator: Arthur Gutierrez-Hartmann, M.D./Ph.D.

Progress Report:

ESX is a novel member of the proto-oncogenic Ets factor family, a diverse group of transcription factors involved in differentiation, cell cycle control, and development.

Specifically, ESX action has been linked to multiple epithelium specific processes, including keratinocyte differentiation and epithelial involution. ESX expression has also been implicated in human breast carcinogenesis. ESX over-expression is detected in 40% of human breast ductal carcinomas in situ. Further, we have shown that stable expression of recombinant ESX protein imparts a transformed phenotype to the normally nonmalignant, ESX negative MCF12A human breast cell line.

Interestingly, studies conducted during my second year of DOD-supported research suggest that the ESX-mediated transformation demonstrated in the above cell line is due to extranuclear ESX activity. Specifically, data involving a GFP-ESX fusion protein not only demonstrate that stable ESX expression in the MCF12A cell line is cytoplasmic but also suggest that prolonged nuclear ESX expression is cytotoxic. Further, the cytoplasmic localization of ESX observed in stably transfected MCF12A cells appears to be a general phenomenon: five additional cell lines engineered to stably express GFP-ESX also demonstrate cytoplasmic restriction of the GFP-ESX protein. Taken together, these data indicate that the ESX protein has cytoplasmic functions and that these cytoplasmic functions mediate cell transformation. This hypothesis is of particular interest because ESX is a known transcription factor and as such was presumed to mediate transformation at the level of gene expression. Finally, I am now generating defined deletion mutations of the ESX portion of the GFP-ESX protein in an attempt to identify those ESX protein domains required for cellular transformation.

Student Name: Suzanne Wardell

Mentor/Principal Investigator: Dean Edwards, Ph.D.

Progress Report:

As a member of the nuclear receptor super-family of ligand-dependent transcriptional activators, progesterone receptor (PR) activates gene transcription in response to progesterone binding. PR possesses a C-terminal ligand binding (LBD), a centrally located DNA binding (DBD) domain, and an unstructured N-terminal region. PR activates transcription through two activation functions (AFs). Ligand-dependent AF-2 in the LBD recruits steroid receptor coactivators (SRCs) and other known coactivators. Ligand-independent AF-1 in the N-terminal domain has not been well characterized, and protein interactions that mediate AF-1 activity are not known. These interactions are important to know because the N-terminal region mediates many cell and promoter-specific PR activities, as well as partial agonist activity of the PR antagonist RU486. Suzanne's project has focused on a novel PR-interacting factor, Jun dimerization protein 2 (JDP-2). JDP-2 is a repressive member of the AP-1 family of transcription factors, but has an unexpected activity as a coactivator of PR-mediated gene transcription. As demonstrated by *in vitro* GST pull-down assays, JDP-2 interacts directly with the DBD of PR to form a ternary complex with PR and DNA. JDP-2 also physically associates with PR in mammalian cells, as demonstrated by co-immunoprecipitation (co-IP) assays, and is recruited with PR by progesterone responsive promoters *in vivo*, as demonstrated using

chromatin immunoprecipitation (ChIP) assays. In transient transfection assays with mammalian cell lines, JDP-2 stimulates ligand-dependent PR transcriptional activation of target reporter genes. JDP-2 can enhance AF-2 activity, but more strongly affects constitutive activation by the N-terminal AF-1 domain. JDP-2 also enhanced AF-1 dependent partial agonist activity of the PR antagonist RU486, further demonstrating a role for JDP-2 in enhancing transactivation by the N-terminal domain. While the PR DBD is the minimal site of JDP-2 interaction, the DBD is necessary, but not sufficient for JDP-2 coactivation. JDP-2 enhancement of N-terminal domain activity requires the DBD and AF-1 together.

The goal of Suzanne's project is to determine the mechanism by which JDP-2 mediates transcriptional activity of PR AF-1. Linker scanning mutagenesis will be used to determine the precise PR N-terminal sequences required for maximal coactivation of the N-terminal AF-1 domain. JDP-2 lacks an intrinsic activation domain and binds directly to the general coactivators CBP and pCAF, but not to SRCs. These data suggest that JDP-2 acts as a docking factor and through interaction with PR DBD acts to recruit coactivators to the N-terminal AF-1 domain independent of SRCs. Components of a putative JDP-2-coactivator complex will be studied using *in vitro* interaction assays as well as co-IP assays of endogenous cellular proteins. Alternatively, JDP-2 binding to PR DBD may induce the DBD to exert a conformational change in the N-domain. The N-domain of receptors is unstructured and has been reported to consist of random coil. Recently, protein interactions have been demonstrated to cause a structural change in the N-domains of glucocorticoid receptor and estrogen receptor. Such a change in conformation mediated by JDP-2 might allow recognition and binding of other coactivators with AF-1. Using circular dichroism (CD) spectroscopy, secondary structural changes of the N-domain will be analyzed in the presence and absence of JDP-2.

Student Name: Katherine Lobel-Rice

Mentor/Principal Investigator: Gary Johnson, Ph.D.

Progress Report:

MEKK2 and MEKK3 are serine/threonine kinases that regulate the c-Jun N-terminal kinase (JNK), ERK5 and p38 mitogen-activated protein kinase pathways. Targeted gene disruption was used to inhibit the expression of MEKK2 and MEKK3. It was demonstrated that MEKK2 was required for FGF2 and EGF activation of ERK5 and contributed to JNK activation in response to growth factors. MEKK2 knockout mice are viable and fertile. In contrast, MEKK3 knockout was embryo lethal. MEKK3 regulates FGF2 activation of ERK5 and p38 in trophoblasts and appears to be critical for the development of the cardiovascular system. These findings indicate that MEKK2 and MEKK3 both regulate ERK5 activity in different cell types and differentially regulate JNK and p38 in response to growth factors and angiogenic factors including FGF2.