Thomas Jefferson University

Annual Progress Report: 2005 Formula Grant

Reporting Period

July 1, 2009 – December 31, 2009

Formula Grant Overview

The Thomas Jefferson University received $4,262,076 in formula funds for the grant award period January 1, 2006 through December 31, 2009. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Regulation of the Immune Response to Colorectal Cancers - A subgroup of colorectal cancers caused by deficiencies of a DNA repair mechanism called DNA mismatch repair is characterized by dense lymphocytic inflammation surrounding invading tumor cells. The biological behavior of this subgroup of tumors is typically less aggressive than the usual colorectal cancer. This project attempts to elucidate the molecular mechanism underlying the inflammatory response by analyzing different types of cells in and around the tumor. Up - and down-regulation of molecules involved in the inflammatory response at the transcription level will be studied using expression microarrays. Co-culture experiments will address the effect of the different types of cells on each other's growth. An understanding of the inflammatory host response could help to develop immune-modulatory treatment.

Duration of Project

1/1/2006 – 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 2: Project Title and Purpose

Characterization of FFR Function in Obesity, Diabetes Mellitus and Heart Diseases - The ability to modulate lipid metabolism can have profound clinical implications in humans because a variety of human diseases are associated with abnormal lipid metabolism, such as obesity, diabetes mellitus, and cardiovascular diseases. FFR is a newly discovered gene that has been shown to impair lipid metabolism when it is mutated. The mechanisms involved in FFR mutation and its role in lipid metabolism are poorly understood. This project will use molecular,

biochemical, and animal models to better understand the mechanisms that regulate lipid metabolism through manipulating FFR levels and functions. The long-term objective of this project is to provide novel therapeutic strategies for preventing or treating obesity, diabetes mellitus, and cardiovascular diseases.

Duration of Project

1/1/2006 - 12/31/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Infrastructure Project 3: Project Title and Purpose

Core Equipment for Physiological Research – A Research Facilities Renovation Project - The purpose of this project is to establish a core facility containing state-of-the-art analytical equipment to facilitate the research objectives of the Department of Physiology of Jefferson Medical College.

Duration of Project

1/1/2006 – 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 4: Project Title and Purpose

Characterization and Modulation of DNA Damage Response in Vertebrates (Zebrafish) - This project focuses on novel approaches to define at the molecular level the response of cells to DNA damaging agents including radiation or chemotherapy, both commonly employed modes of cancer therapy. The proposed studies will benefit cancer patients by guiding efforts to maximize tumor control while minimizing damage to normal tissues associated with anti-cancer therapies.

Duration of Project

1/1/2006 - 4/21/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 5: Project Title and Purpose

The Role of Cyclin D1 and Androgen Receptor (AR) Mutations in Prostate Cancer - The goals of this project are to determine the mechanisms of escape from cyclin D1 repression by mutant ARs found in patients with prostate cancer. The possibilities are systematically examined, including regulation of AR transactivation, nuclear translocation and DNA binding. As DHT-induced AR activity is linked to direct acetylation it is proposed that cyclin D1 may directly inhibit acetylation of wild type AR, but fail to inhibit the mutant ARs. This project aims to determine the role of cyclin D1 and androgen receptor (AR) mutations in prostate cancer cellular growth.

Duration of Project

1/1/2006 – 9/30/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure

Research Project 6: Project Title and Purpose

Caveolin-1 in Prostate Cancer: Translational Research Employing Mouse Models - The long-term objective of this project is to understand the role of caveolae and caveolin-1 in 1) advanced prostate cancer and 2) metastasis. Caveolae are "little caves" at the surface of cells that function both in vesicular trafficking and signal transduction. Caveolin-1 is the principal oligomeric structural protein of caveolae membranes that are found in most cell types.

Duration of Project

1/1/2006 – 6/15/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 7: Project Title and Purpose

Molecular Mechanisms that Govern Cell Differentiation of Breast Cancer - The project goal is to identify molecular mechanisms that govern cellular differentiation of human breast cancer. Loss of differentiation is required for progression from solitary tumors to metastases, and the mechanistic knowledge gained from this project will be used to develop new breast cancer therapies.

Duration of Project

1/1/2006 - 12/31/2009

Project Overview

More than one million new cases of breast cancer are diagnosed each year worldwide, and an estimated 370,000 women die each year. The majority of breast cancer fatalities are caused by metastatic spread of the primary tumor. Unfortunately, progression of cancer from primary tumor to metastasis remains a complex and poorly understood process. Identifying the molecular changes that lead to metastatic progression will be critical for development of better strategies to prevent metastatic spread and for therapeutic intervention of advanced breast cancer. While it is generally accepted that loss of differentiation and epithelial-to-mesenchymal transition are critical elements required for breast cancer metastasis, efforts to reverse de-differentiation of breast cancer have been limited.

The Principal Investigator has recently uncovered novel evidence that transcription factor Stat5a acts as a suppressor of invasive characteristics of breast cancer. Restoring active Stat5a, and identifying the gene programs and mediators of Stat5a-induced re-differentiation could open new avenues for prevention of disease progression and could lead to therapeutic strategies to reverse progression-related changes in human breast cancer. The proposed studies will also facilitate a general understanding of control of epithelial-to-mesenchymal de-differentiation of breast cancer, and is expected to lead to more effective and individualized therapeutic options with an emphasis on novel differentiation therapies.

Principal Investigator

Hallgeir Rui, MD, PhD
Thomas Jefferson University
Department of Cancer Biology
Bluemie Life Sciences Building
233 South 10th Street, BLSB 330
Philadelphia, PA 19107

Other Participating Researchers

None

Expected Research Outcomes and Benefits

This project will explore the role of Stat5a as a major regulatory molecule in breast cancer differentiation to derive new strategies for breast cancer differentiation therapy. Stat5-based strategies may reverse epigenetic changes associated with de-differentiation and invasive behavior. A novel and improved mouse model that will be critical for more reliable in vivo testing of the pro-differentiation effect of PRL-Stat5 on human breast cancer will also be developed.

Summary of Research Completed

Four research articles supported by this grant have been published during the current reporting period.

Tran TH, Utama FE, Lin J, Yang N, Sjolund AB, Ryder A, Johnson KJ, Neilson LM, Liu C, Brill KL, Rosenberg AL, Witkiewicz AK, Rui H. Prolactin Inhibits Expression of the Proto-oncogene BCL6 in Breast Cancer through a Stat5 Dependent Mechanism. Cancer Research 70; 1711–21, 2010.

Utama, FE, Tran TH, Ryder A, LeBaron MJ, Parlow AF, Rui H. Insensitivity of human prolactin receptors to non-human prolactins: Relevance for experimental modeling of prolactin receptor-expressing human cells. Endocrinology, 150, 1782-90, 2009.
Web link to article: http://endo.endojournals.org/cgi/content/full/150/4/1782

Plotnikov A, Li Y, Tran TH, Tang W, Palazzo JP, Rui H, and Fuchs SY. Oncogene-mediated inhibition of glycogen synthase kinase 3β impairs degradation of prolactin receptor. Cancer Research, Cancer Research, 68, 354-61, 2008.
Web link to article: http://cancerres.aacrjournals.org/cgi/content/full/68/5/1354

Neilson LM, Zhu J, Xie J, Malabarba MG, Sakamoto K, Wagner K-U, Kirken RA, Rui H. Coactivation of Jak1 Positively Modulates Prolactin-Jak2 Signaling in Breast Cancer: Recruitment of ERK and Stat3 and Enhancement of Akt and Stat5a/b Pathways. Mol Endo, 21, 2218-2232, 2007.
Web link to article: http://mend.endojournals.org/cgi/rapidpdf/me.2007-0173v1

Novel progress showing prolactin inhibits BCL6 expression in breast cancer through a Stat5a-dependent mechanism.
BCL6 is a transcriptional repressor that recognizes DNA target sequences similar to those recognized by signal transducer and activator of transcriptions 5 (Stat5). BCL6 disrupts differentiation of breast epithelia, is downregulated during lactation, and is upregulated in poorly differentiated breast cancer. In contrast, Stat5a mediates prolactin-induced differentiation of mammary epithelia, and loss of Stat5 signaling in human breast cancer is associated with undifferentiated histology and poor prognosis. Here, we identify the mammary cell growth factor prolactin as a potent suppressor of BCL6 protein expression in human breast cancer through a mechanism that requires Stat5a, but not prolactin-activated Stat5b, MEK-ERK, or PI3K-AKT pathways. Prolactin rapidly suppressed BCL6 mRNA in T47D, MCF7, ZR75.1, and SKBr3

breast cancer cell lines, followed by prolonged reduction of BCL6 protein levels within 3 hours. Prolactin suppression of BCL6 was enhanced by overexpression of Stat5a but not Stat5b, was mimicked by constitutively active Stat5a, but did not require the transactivation domain of Stat5a. Stat5 chromatin immunoprecipitation demonstrated physical interaction with a BCL6 gene regulatory region, and BCL6 transcript repression required histone deacetylase activity based on sensitivity to trichostatin A. Functionally, BCL6 overexpression disrupted prolactin induction of Stat5 reporter genes. Prolactin suppression of BCL6 was extended to xenotransplant tumors in nude mice in vivo and to freshly isolated human breast cancer explants ex vivo. Quantitative immunohistochemistry revealed elevated BCL6 in high-grade and metastatic breast cancer compared with ductal carcinoma in situ and nonmalignant breast, and cellular BCL6 protein levels correlated negatively with nuclear Stat5a ($r = -0.52$; $P < 0.001$) but not with Stat5b. Loss of prolactin-Stat5a signaling and concomitant upregulation of BCL6 may represent a regulatory switch facilitating undifferentiated histology and poor prognosis of breast cancer.

Novel progress showing that human prolactin receptors on breast cancer samples are insensitive to mouse prolactin and bovine prolactin, pointing out the need for better experimental models for in vitro and in vivo studies of human breast cancer.

Prolactin (PRL) receptors are expressed in a broad range of human cell types and in a majority of human breast and prostate cancers. Experimentally, normal and malignant human cells are typically cultured *in vitro* in media containing bovine prolactin (bPRL) from fetal calf serum, or as xenotransplants *in vivo* in the presence of murine prolactin (mPRL). The biological efficacy of bPRL toward hPRL receptors (hPRLR) is controversial, and hPRLR are insensitive to mPRL but the mechanism is not known. To clarify limitations of current *in vitro* and *in vivo* experimental model systems for studies of hPRLR-expressing cells, we tested recombinant human and relevant subprimate prolactins in multiple hPRLR bioassays. bPRL and oPRL were 10-fold less potent hPRLR agonists than hPRL although maximal responses at high ligand concentrations (efficacies) equaled that of hPRL. mPRL and rPRL had >50-fold lower potencies toward hPRLR than hPRL and had 50% reduced efficacies. In fact, mPRL and rPRL were less effective hPRLR agonists than murine growth hormone. Unexpectedly, mPRL was an effective competitive inhibitor of hPRL binding to hPRLR, with a k_i of 1.3 nM and showed partial antagonist activity in two different bioassays. Collectively, the low bioactivity of bPRL and mPRL to hPRLR suggest that existing laboratory cancer cell lines grown in 10% bovine serum-supplemented media or in mice are selected for growth in the absence of exogenous hPRLR agonist activity. The biology and drug responsiveness of existing human cell lines may therefore not be representative of clinical cancers that are sensitive to circulating PRL.

Novel progress showing that the prolactin receptors in human breast cancers are stabilized by oncogenic signaling.

Prolactin receptors (PRLr) expressed in a majority of breast cancer are activated by prolactin and growth hormone. The PRLr is commonly stabilized in human breast cancer due to decreased phosphorylation of residue Ser349, which, when phosphorylated, recruits the βTrcp E3 ubiquitin ligase and facilitates PRLr degradation. Here, we show that constitutive oncogenic signaling downstream of ErbB2 and Ras stabilizes PRLr via inhibitory phosphorylation of glycogen synthase kinase-3β (GSK3β) on Ser9. Importantly, inactivation of GSK3β correlates with elevated levels of PRLr protein in clinical human breast cancer specimens. Additional studies using pharmacologic, biochemical, and genetic approaches reveal that GSK3β is a bona fide

PRLr kinase that phosphorylates PRLr on Ser349 and is required for the recognition of PRLr by βTrcp, as well as for PRLr ubiquitination and degradation.

Novel progress showing for the first time that prolactin receptors activate the tyrosine kinase Jak1, in addition to previously recognized Jak2, in human breast cancer cell lines.
Prolactin receptors (PRLR) have been considered selective activators of the tyrosine kinase Jak2 but not Jak1, Jak3 or Tyk2. We now report marked PRL-induced tyrosine phosphorylation of Jak1, in addition to Jak2, in a series of human breast cancer cell lines, including T47D, MCF7, and SKBR3. In contrast, PRL did not activate Jak1 in immortalized, non-cancerous breast epithelial lines HC11, MCF10A, ME16C, and HBL-100, or in CWR22Rv1 prostate cancer cells or MDA-MB-231 breast cancer cells. However, introduction of exogenous PRLR into MCF10A, ME16C, or MDA-MB-231 cells reconstituted both PRL-Jak1 and PRL-Jak2 signals. *In vitro* kinase assays verified that PRL stimulated enzymatic activity of Jak1 in T47D cells, and PRL activated Jak1 and Jak2 with indistinguishable time and dose-kinetics.

Overexpression of Jak2 did not interfere with PRL activation of Jak1. Instead, PRL activated Jak1 through a Jak2-dependent mechanism, based on disruption of PRL activation of Jak1 following Jak2 suppression by 1) lentiviral delivery of Jak2 shRNA; 2) adenoviral delivery of dominant-negative Jak2, and 3) AG490 pharmacological inhibition. Finally, suppression of Jak1 by lentiviral delivery of Jak1 shRNA completely blocked PRL activation of ERK and Stat3, and suppressed PRL activation of Jak2, Stat5a, Stat5b, and Akt, as well as tyrosine phosphorylation of PRLR. The data suggest that PRL activation of Jak1 represents a novel, Jak2-dependent mechanism that may serve as a regulatory switch leading to PRL activation of ERK and Stat3 pathways, while also serving to enhance PRL-induced Stat5a/b and Akt signaling.

Key Research Accomplishments

1) Determination that the differentiation-suppressing oncogene, BCL6, is repressed by prolactin via a Stat5-dependent mechanism in breast cancer.

2) Determination that human prolactin receptors on breast cancer samples are insensitive to mouse prolactin and bovine prolactin, pointing out the need for better experimental models for in vitro and in vivo studies of human breast cancer.

3) Determination that oncogenic signaling in breast cancer stabilizes the prolactin receptor

4) Determination that prolactin receptors activate the tyrosine kinase Jak1, in addition to previously recognized Jak2, in human breast cancer cell lines. This key progress supports the work on this grant by providing a novel enzymatic target, Jak1, for therapeutic targeting in breast cancer of signals downstream of the prolactin receptor.