University of Pittsburgh

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2013 – December 31, 2013

Formula Grant Overview

The University of Pittsburgh received $9,897,440 in formula funds for the grant award period January 1, 2010, through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Application of Adult Stem Cells for Tissue Regeneration - Degenerative skeletal diseases like osteoarthritis are the primary cause of disability, particularly in the elderly. The purpose of this research project is to develop novel tissue regenerative approaches for the treatment of such diseases. The included studies will investigate the regenerative activities of human adult stem cells, specifically the nature of the optimal physical and biological microenvironment that supports their activities. This knowledge will guide the design of biomaterial scaffolds and the selection of bioactive factors for effective stem cell-based therapy for the engineering, regeneration, and functional restoration of diseased or injured skeletal tissues.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

Degenerative skeletal diseases are the primary cause of disability in the elderly. Cell-based therapy is a promising treatment approach for the engineering and regeneration of the diseased or injured tissues. Adult stem cells, known as mesenchymal stem cells (MSCs), possess the ability to differentiate into musculoskeletal cells and represent a promising cell type for skeletal tissue engineering. Successful application of MSCs in cell-based therapy requires a full understanding of the mechanisms that regulate their biological activities, specifically proliferation and differentiation. Three interconnected projects are described here. The first project focuses on elucidating the mechanism of action of selected genes previously identified as potential "stemness genes" (i.e., genes that encode proteins that act to maintain the potency of the MSCs). These genes include both secreted factors and extracellular matrix components and will be analyzed in terms of their control of MSC proliferation and differentiation and their cellular signaling pathways. Functional molecules identified in this study will be considered as ligands for incorporation into a three-dimensional (3D) matrix (e.g., using nanostructured biodegradable

polymer fibers to construct a bioactive scaffold for MSCs). Using bioreactor technology, culture conditions will be optimized to induce the cell-seeded constructs to differentiate into a hyaline cartilaginous tissue with biological and mechanical characteristics approximating those of native articular cartilage. The utility of the engineered constructs for cartilage repair will be tested in animal models of traumatically induced lesions in the femoral condylar articular cartilage. In summary, these studies will identify the key molecules important for MSC activity and exploit the identified pathways to produce optimal 3D scaffolds for cartilage engineering that will be tested for cartilage repair in clinically relevant animal models. Positive results from these proof-of-concept studies will yield a rational basis for the design of clinical trials using this technology.

The project will have the following specific aims:

Aim 1 – To analyze the molecular mechanism of action of a specific "stemness gene" previously identified and associated with the maintenance of proliferative and differentiation-readiness of MSCs (specific attention will be paid to identifying molecular domains or moieties that may be applied as reagents to generate active MSCs)

Aim 2 – To apply the reagents generated in Aim 1 as ligands for incorporation into and/or conjugated onto biodegradable polymeric nanofibers (produced via electrospinning) to construct bioactivated scaffolds that will be seeded with MSCs; the efficacy of such MSC-seeded constructs to produce improved engineered cartilage will be tested and optimized *in vitro.*

Aim 3 – To test the ability of the engineered cartilage construct to repair cartilage lesions produced via supraphysiological impact in medium (rabbit) and large (goat) animal models.

Principal Investigator

Rocky S. Tuan, PhD
Arthur J. Rooney Sr. Professor of Sports Medicine
University of Pittsburgh,
Department of Orthopaedic Surgery
450 Technology Drive, 2nd Floor, Room 221
Pittsburgh, PA 15219

Other Participating Researchers

Natasha Baker, PhD; Solvig M. Diederichs, PhD; Lauren Y. Statman, BS; Karen L. Clark, MA; Veronica Ulici, PhD; Heidi Hofer, BS – employed by University of Pittsburgh

Expected Research Outcomes and Benefits

The new discipline of regenerative medicine aims to restore form and function to the patient via promotion of the regenerative activities of tissues and organs. Cell-based therapy is one of the most promising approaches in regenerative medicine. Adult human mesenchymal stem cells (MSCs) are able to differentiate into multiple cell types and represent a highly promising cell

type in cell-based therapy. The studies included in this project take an integrated approach to probe the molecular mechanisms that maintain MSCs in an active state and ready for differentiation. The information gained from identifying the signaling pathways and bioactive factors involved presents a platform to design and/or select specific molecular moieties for MSC activation. In the tissue engineering study, by incorporating selected moieties as ligands to functionalize an electrospun nanofibrous polymeric matrix, a bioactive three-dimensional (3D) scaffold for MSCs will be constructed. Upon chondrogenic induction, these MSC-seeded constructs are expected to yield structurally and biologically improved engineered cartilage tissue. The preclinical study will use articular cartilage injury animal models to directly assess the use of engineered cartilage in repairing trauma-induced cartilage degeneration. The investigation, thus, has the potential to yield findings that will lead, with further clinical trials, to the development of an innovative clinical approach—based on autologous MSCs and bioactive scaffolds—to treat degenerative joint diseases like osteoarthritis, which are a primary cause of disability.

Summary of Research Completed

I. AIM 1: Maintenance of Proliferative and Differentiation-Readiness of MSCs

Project (1) *Characterization of Molecular Control of Stemness and Proliferative/Differentiative State of Adult Stem Cells*

A. *Caveolin-1 Regulation of Proliferation and Differentiation of Human Mesenchymal Stem Cells*

A critical requirement for the regenerative application of adult stem cells is their maintenance in a proliferative and differentiation competent state. Our goal in this project is to decipher the underlying cellular mechanism regulating these processes and how they may be optimally manipulated as a result. Our previous findings strongly indicate that caveolin-1, as a component of the caveolae, cholesterol-rich lipid subdomains of the plasma membrane, serves a functional role in regulating the proliferation and differentiation capabilities of human mesenchymal stem cells (MSCs). Specifically, our data suggest involvement of a signaling pathway via partial inhibition of Akt phosphorylation, which may operate downstream of, or in collaboration with, integrin focal adhesion signaling to induce differentiation through β-catenin activation. We also established that MSC osteogenic differentiation and PI3K/Akt signaling are sensitive to perturbations in membrane cholesterol. Furthermore, we found that Akt is present in non-caveolar lipid rafts and may move to caveolae when activated (phosphorylated) under osteogenic conditions.

To further study the relationship between Cav-1 and Akt, we performed co-immunoprecipitation studies of chemically cross-linked proteins from MSCs stimulated with control medium or osteogenic medium. Meanwhile, to study the possible interconnected roles of membrane cholesterol, Cav-1, and adhesion signaling such as PI3K/Akt signaling in MSC differentiation, we perturbed cholesterol and PI3K/Akt signaling in MSCs subject to three-dimensional (3D) substrate-driven stimulation. The 3D culture system we used for this was composed of gelatin of

photocrosslink-controlled stiffness. We chose this because substrate rigidity has been shown by others to direct MSC differentiation and behavior, possibly via Cav-1/caveolae endocytosis of integrins. In addition, we have also performed pilot studies to observe the adhesive characteristics of MSCs in relation to donor source age, because this variable could potentially affect the outcome of our future experiments with 3D substrate stimuli.

For co-immunoprecipitation experiments, growth medium (GM) and osteogenic medium (OM) cultures were treated with the chemical crosslinker dithiobis[succinimidylpropionate] (DSP) before harvesting and immunoprecipitation of Cav-1 protein using a polyclonal Cav-1 antibody (BD Biosciences) and immunopreciptation kit (Pierce). Immunoprecipitates were then western blotted for Cav-1 and Akt. For 3D culture studies, MSCs were pre-cultured in GM, then detached and re-suspended in gelatin containing a light-activatable photocrosslinker. MSC/gelatin suspensions were then exposed to light for varied lengths of time to generate 3D cultures with soft and stiff gelatin substrate rigidity. In addition, subsets of MSCs were either: (1) treated with the PI3K/Akt signaling inhibitor, LY294002, after photocrosslinking; (2) pre-treated for one hour with the cholesterol chelator methyl-β-cyclodextrin (MβCD, to disrupt lipid rafts) before dilution in gelatin; (3) pre-incubated in cholesterol-containing medium for 24 hours before dilution in gelatin; or (4) treated with a short peptide mimetic of part of the Cav-1 protein (Cavtratin) after photo-crosslinking. Medium was changed 24 hours after photo-crosslinking (with LY294002 or Cavtratin where necessary) and cells viewed by calcien-am staining after a further 24 hours.

Finally, the adhesive characteristics of MSCs isolated from six young (20 yrs- 45 yrs) and six old (75 yrs- 84 yrs) donors were observed after passage three in GM, using an ACEA RTCA-DP xCELLigence analyzer.

Our results showed that after chemical cross-linking, Akt was found to co-immunopreciptate with Cav-1 protein in cells that were treated with OM, and to a lesser extent in cells incubated in GM. Cell culture in soft and stiff 3D gelatin scaffolds was associated with morphological differences in MSCs. Those in stiff gels adopted a rounded morphology, while those in soft gels were lengthened and more spindle shaped/branched. Pre-culture in cholesterol or treatment with LY294002 enhanced this morphology in soft scaffolds, and appeared to induce it in stiff scaffolds. Treatment with cavtratin induced an unusual rounded/polygonal morphology irrespective of substrate rigidity, but was apparently not cytotoxic at the time point examined.

The adhesion dynamics of biologically old and young MSC sources were not statistically different.

In summary, our co-immunopreciptation results suggest that Akt physically associates with Cav-1 and this association may increase in the presence of osteogenic supplements. In addition, cell culture in soft and stiff 3D gelatin scaffolds induced morphological changes that have been associated with substrate-induced cell differentiation by others. A branched morphology has been associated with MSC neurogenesis in soft 2D culture, while a rounded morphology has been associated with MSC osteogenesis in stiff 3D gels. However, the differentiation of cells in our experiment was not measured here. As LY294002, which inhibits osteogenic PI3K/Akt

signaling, enhanced MSC morphology in soft substrates and disrupted it in stiff substrates, it is possible that PI3K/Akt signaling contributes to the MSC morphological response positively in stiff substrates and negatively in soft substrates. Meanwhile, as cholesterol appeared to have similar effects to LY294002, it is possible that excess membrane cholesterol in 3D negatively affects PI3K/Akt signaling and/or negatively affects stiffness-induced adhesion dynamics. We postulate that this may be due to over-amplification of integrin adhesive forces that causes rupture of the adhesion contacts. We hypothesize that cholesterol, Cav-1 and caveolae act in combination to modulate MSC sensitivity to substrate stiffness through regulation of PI3K/Akt signaling, and this will be the basis of our future funding applications.

Finally, our preliminary results suggest that MSC age does not affect MSC adhesive characteristics. Though further work is required, this suggests that MSC cholesterol status may be more important than MSC age in determining adhesion responses.

II. AIM 2: Tissue Engineering Application of MSCs

Project (2) *Application of Human MSCs to Enhance Innervation, Mobility, and Joint Function*

A. *Neural Differentiation of MSCs for Peripheral Nerve Repair*

In regenerative medicine research, the harsh and complex environment in diseased, dysfunctional, or injured tissues must be taken into consideration in attempting to augment and enhance the healing abilities of native cells and tissues. We seek to understand this non-ideal environment and the potential of cells exposed to trauma and its physiological consequences, chiefly inflammation. Traumatized tissue-derived mesenchymal progenitor cells (MPCs) are a relatively new and promising source of easily obtained stem-like cells. Their presence in damaged tissues of blast-injured victims and similarities to the most commonly studied MSCs suggest an important role in wound healing. We predict that these cells act in concert with neighboring cells to affect their proliferation, migration, and function, in accordance to the inflammatory environment. We have therefore investigated MPC and endothelia cell (EC) interaction.

First, MPC promotion/inhibition of angiogenesis, specifically vessel recruitment, was investigated using an avian chorioallantoic membrane (CAM) model. MPCs and their conditioned media were encapsulated in methacrylated-gelatin and incubated for two days on the CAM. Our results showed that MPCs serve to recruit blood vessels from the surrounding vasculature, the activity being stronger than the conditioned media alone, suggesting that it is the dynamic response of the living MPCs to the invading vessels that contributes to successful vessel recruitment.

Next, we investigated the attenuation of mesenchymal differentiation of MPCs under inflammatory conditions and the role of ECs in rescuing those phenotypes. Preliminary data shows that EC-conditioned media, regardless of the presence of the pro-inflammatory cytokines, TNF-α and IL1-β, increased the early but not the late osteogenic differentiation capacity of bone marrow-derived MSCs and MPCs (alkaline phosphatase expression).

We finally assessed the combined MPC and EC support of nerve growth. As detailed in previous reports, MPC- and EC-conditioned media, and to a lesser extent, MPC- and EC- cell combinations, increased the length of surviving neurite extensions of chick embryonic dorsal root ganglia (DRG). This difference was significant over single cell type-conditioned controls and was strongest when ECs and MPCs were grown on tissue culture plastic vs. poly-caprolactone nanofibers.

In summary, with the goal of enhanced understanding of tissue repair, we have generated model systems that allow a more thorough investigation of physiologically-relevant post-trauma cell interactions that may lead to the development of more nuanced regenerative medicine approaches.

Research Project 2: Project Title and Purpose

Targeting Nrf2 for Cancer Prevention - Cancer prevention involving reduction or elimination of human exposure to environmental carcinogens may not always be possible. The overriding goal of this project is to provide the mechanistic framework to facilitate the efficient translation of the most effective small molecule activators of the Nrf2 signaling pathway into use as protective agents in human populations exposed to environmental toxicants like aflatoxins. To facilitate this goal, we need better understanding of the molecular mechanisms of action of the chemopreventive agents, further validation of intermediate biomarkers, and assessment of efficacy in animal models with close relevance to human carcinogenesis.

Duration of Project

1/1/2010 - 12/31/2013

Project Overview

Inhibiting cancer development by administering anticarcinogenic agents may offer practical alternatives for reducing human cancer burden. The Keap1-Nrf2-ARE signaling pathway has been identified as a target for chemoprevention. The Nrf2 transcription factor regulates an integrated cell survival response that can be triggered by multiple classes of cancer chemopreventive agents (e.g., dithiolethiones, isothiocyanates, triterpenoids). In this project, both pharmacologic and genetic approaches will be used to probe the molecular mechanisms of action of these chemopreventive agents, assess their efficacy in animal models with close relevance to human carcinogenesis, and use these interventions to validate intermediate biomarkers. Two complementary aims will be pursued.

First, advanced methods of isotope-dilution mass spectrometry will be used for quantitation of biomarkers of the human carcinogen aflatoxin in liver, blood, urine, and feces to develop for the first time a comprehensive mass balance for the fate of the ultimate carcinogen, exo-aflatoxin-epoxide, in rats. Unique to this project, the predictive value of these biomarkers for individual risk of liver cancer will be assessed. Biomarkers will be quantified longitudinally during a bioassay for protection against hepatocarcinogenesis by an exceptionally potent triterpenoid

activator of Nrf2 signaling. We will also evaluate the predictive value of monitoring DNA circulating in plasma for mutations in target oncogenes using quantitative short oligonucleotide mass spectrometry.

In the second aim, we will evaluate the similarities and distinctions of chemical and genetic activation of Keap1-Nrf2 signaling by comparing the gene expression patterns in rodent liver following treatment with chemical Nrf2 activators with the gene expression patterns in liver of mice in which either Nrf2 or its repressor, Keap1, has been genetically disrupted. The effect of these genetic and pharmacologic interventions on aflatoxin disposition will be determined using the mass spectrometry-based analysis of its biomarkers. Collectively, these studies will further the goal of effectively using activators of Nrf2 signaling as protective agents in human populations exposed to environmental and endogenous carcinogens.

Principal Investigator

Thomas W. Kensler, PhD
Professor of Pharmacology and Chemical Biology
University of Pittsburgh
E1352 Thomas E. Starzl Biomedical Science Tower
Pittsburgh, PA 15261

Other Participating Researchers

Nobunao Wakabayashi, PhD; Li Yang, PhD – employed by University of Pittsburgh
John D. Groopman, PhD; Patricia A. Egner, MS – employed by Johns Hopkins University

Expected Research Outcomes and Benefits

This project will provide a comprehensive analysis of the molecular and biochemical actions of two distinct chemical classes (isothiocyanates, triterpenoids) of anticarcinogenic compounds. Members of these chemical classes have been utilized in clinical trials in humans, so there are realistic possibilities for continued development as practical cancer preventive agents. These studies will also rigorously establish the utility of targeting the Nrf2 transcription factor pathway as a means for cancer prevention. Central to the project, highly quantitative, exquisitely sensitive, mass spectrometry methods will be used for analyzing candidate biomarkers reflecting both the fate and disposition of an important environmental carcinogen, aflatoxin. In addition, mass spectrometry in the form of "short oligonucleotide mass analysis" will be used to measure genetic changes in tumor DNA shed into the circulation using biospecimens provided to the study team from long-term animal bioassays. Collectively, these studies will provide a longitudinal evaluation of biomarkers during the course of cancer development induced by exposure to environmental agents. Results from this project will provide an analytical template for the assessment of early indices of environmental carcinogenesis in humans at risk for exposures to food-, air- and water-borne carcinogens.

Summary of Research Completed

The principal accomplishments during the final half year of this project were the completion and submission of two manuscripts for publication detailing the modulation of DNA adducts by chemopreventive agents in *in vitro* and *in vivo* models. Both subprojects – and manuscripts – relied extensively on the mass spectrometry capabilities supported by this grant for the quantitative measures of DNA adduct burden.

In experimental animals and humans, aflatoxin B_1 (AFB_1) is a potent hepatic toxin and carcinogen. The synthetic oleanane triterpenoid 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), a powerful activator of Keap1-Nrf2 signaling, protects against AFB_1-induced toxicity and preneoplastic lesion formation (GST-P positive foci). The current studies were undertaken to assess and mechanistically characterize the chemoprotective efficacy of CDDO-Im against AFB_1-induced hepatocellular carcinoma (HCC). A lifetime cancer bioassay was undertaken in F344 rats dosed with AFB_1 (20 µg/rat/day) for four weeks and receiving either vehicle or CDDO-Im (three times weekly), one week prior to and throughout the exposure period. Weekly, 24-hour urine samples were collected for analysis of AFB_1 metabolites. In a subset of rats, livers were analyzed for GST-P foci. The comparative response of a toxicogenomic RNA expression signature for AFB_1 was examined. CDDO-Im completely protected against AFB_1-induced liver cancer compared to a 96% incidence observed in the AFB_1 group. With CDDO-Im treatment, integrated level of urinary AFB_1-N^7-guanine was significantly reduced (66%) and aflatoxin-N-acetylcysteine, a detoxication product, was consistently elevated (300%) after the first AFB_1 dose. In AFB_1-treated rats, the hepatic volume of GST-P positive foci increased substantially (0% to 13.8%) over the four weeks but was largely absent with CDDO-Im intervention. The toxicogenomic RNA expression signature characteristic of AFB_1 was absent in the AFB_1 + CDDO-Im treated rats. The remarkable efficacy of CDDO-Im as an anticarcinogen is established even in the face of a significant aflatoxin adduct burden. Consequently, the absence of cancer requires a concept of a threshold for DNA damage for cancer development.

Sulforaphane (SFN), an isothiocyanate isolated from cruciferous vegetables, is a potent inducer of detoxication enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione *S*-transferases (GST) via the Kelch-like ECH-associated protein 1 (Keap1) - NF-E2-related factor 2 (Nrf2) signaling pathway. NQO1 reduces the carcinogenic estrogen metabolite, catechol estrogen-3,4-quinone, while GSTs detoxify it through conjugation with glutathione. These 3,4-quinones can react with DNA to form depurinating DNA adducts. Thus, SFN may alter estrogen metabolism and, thus, protect against estrogen-mediated DNA damage and carcinogenesis. Human breast epithelial MCF10A cells were treated with either vehicle or SFN and either estradiol (E2) or its metabolite 4-OHE2. 4-Hydroxy derived estrogen metabolites and depurinating DNA adducts formed from E2 and its interconvertable metabolite estrone (E1) were analyzed by mass spectrometry. Levels of the depurinated adducts, 4-OHE1/2-1-N3Adenine and 4-OHE1/2-1-N7Guanine, were reduced by 60% in SFN treated cells, while levels of 4-OCH3E1/2 and 4-OHE1/2- glutathione conjugates increased. To constitutively enhance the expression of Nrf2-regulated genes, cells were treated with either scrambled or siKEAP1 RNA. Following E2 or 4-OHE2 treatments, levels of the adenine and guanine adducts dropped 60% -

70% in siKEAP1 treated cells while 4-OHE1/2-glutathione conjugates increased. However, 4-OCH3E1/2 decreased 50% after siKEAP1 treatment. Thus, treatment with SFN or siKEAP1 has similar effects on reduction of depurinating estrogen-DNA adduct levels following estrogen challenge. However, these pharmacologic and genetic approaches have different effects on estrogen metabolism to O-methyl and glutathione conjugates. Activation of the Nrf2 pathway, especially elevated NQO1, may account for some but not all of the protective effects of SFN against estrogen-mediated DNA damage.

Publications:

Johnson, N.M., Egner, P.A., Baxter, V.A., Sporn, M.B., Wible, R.S., Sutter, T.R., Groopman, J.D., Kensler, T.W. and Roebuck, B.D. (2013) Complete protection against aflatoxin B1-induced liver cancer with a triterpenoid: DNA adduct dosimetry, molecular signature and genotoxic threshold. *Cancer Prevention Res.,* submitted

Yang, L., Zahid, M., Liao, Y., Rogan, E.G., Cavalieri, E.L., Davidson, N.E., Yager, J.D., Visvanathan, K., Groopman, J.D., Kensler, T.W. (2013) Diminished formation of estrogen depurinating-DNA adducts by sulforaphane or KEAP1 disruption in human mammary epithelial MCF-10A cells. *Carcinogenesis* 34:2587-92.

Research Project 3: Project Title and Purpose

Establishing New Biobehavioral Paradigms for Translational Research in Cancer Prevention and Control - The possibility that "mind-body" effects may contribute to the risks of developing cancer and experiencing debilitating cancer- and treatment-related symptoms has been examined in numerous studies over the past three decades with mixed results and considerable controversy. These desultory results stand in stark contrast to the steady progress that has been made in biomedical cancer research, which is grounded in a translational approach where basic laboratory findings from tissue culture and animal models are brought to bear on critical clinical issues. The purpose of this project is to bring this translational approach to mind-body research. Both arms of the project use preclinical models; one will investigate vomiting and other aversive effects of chemotherapy, while the other explores animal and tissue culture models of human breast cancer progression.

Duration of Project

1/1/2010 - 12/31/2011

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

Clinical Trials in Multiple Myeloma - The University of Pittsburgh Cancer Institute's (UPCI) Clinical Research Service (CRS) provides valuable resources for researchers and clinicians seeking to improve clinical research as a means to elevate patient care standards and treatment efficacy. This project will examine two therapeutic trials in patients with multiple myeloma: (1) a safety and efficacy trial to determine a maximum dose of bendamustine combined with lenalidomide to establish a treatment modality for relapsed, refractory multiple myeloma; and (2) a randomized clinical trial to determine whether there is ultimately a need for autologous peripheral blood stem cell transplant in patients with newly diagnosed myeloma who are receiving lenalidomide and low-dose dexamethasone.

Duration of Project

1/1/2010 - 12/31/2011

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

Improving the Therapy of Women with Metastatic Breast Cancer - While great advances in breast cancer therapy have been made in recent years, therapy for metastatic breast cancer (MBC) is not adequate. We propose three trials that can have a direct impact on MBC treatment. The first trial seeks to determine whether changes in positron emission tomographic (PET) scanning very early in the treatment of MBC can be used to predict response and outcome to therapy. The second and third trials investigate the activity of a novel agent (ABT-888), which inhibits a protein active in deoxyribonucleic acid (DNA) repair in response to stress, in the treatment of BRCA 1/2 (breast cancer genes 1 and 2) positive metastatic breast cancer and other cancers.

Duration of Project

1/1/2010 – 12/31/2011

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 6: Project Title and Purpose

Relocation of a 9.4 Tesla Magnetic Resonance Imager - The project involves the relocation of a 9.4 Tesla magnetic resonance (MR) imaging unit to provide a University of Pittsburgh team of structural biology and biophysics researchers a cohesive research space in which they can improve and advance their collaborative efforts. Existing, unoccupied space in the Biomedical Science Tower 3 (BST3) that is adjacent to the structural biology magnet facility will be renovated to accommodate the MR imager. After these preparations have been made, the MR unit will be moved from its current location in the McGowan Institute for Regenerative Medicine to the BST3, about 2.3 miles away.

Summary of Research Completed

This project was deleted. No health research grant funds were spent on this project.

Research Infrastructure Project 7: Project Title and Purpose

Research Infrastructure: Neurological Surgery Laboratory Renovations - Neurological surgery research is a growing area of translational research and is rapidly expanding at the University of Pittsburgh. This infrastructure project will facilitate the renovation of neurological surgery laboratories in Scaife Hall on the main campus of the University of Pittsburgh. These new laboratories will enable more efficient use of space for the expanding department and will promote collaboration and shared resources both within and outside the Department of Neurological Surgery.

Duration of Project

3/16/2011 – 10/13/2012

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 8: Project Title and Purpose

Racial Disparities in Associations between Chronic Airflow Obstruction and Cardiovascular Risk – Cardiovascular disease (CVD) is the leading cause of morbidity in individuals with chronic obstructive pulmonary disease (COPD). COPD, which is characterized by airflow obstruction, is associated with a greater than two-fold increase in CVD mortality, independent of smoking. This association is amplified by CVD risk factors and may be related to a link between COPD and inflammation. We have shown that in Western Pennsylvania, CVD risk factors and inflammation are more prevalent in blacks than in whites. This disparity may explain why, in Pennsylvania, the median age of death from chronic lung disease is 6.5 years younger in blacks.

This project will identify racial disparities in associations among airflow obstruction, CVD risk factors, and inflammation. Anticipated findings will direct strategies toward reducing racial differences in the link between chronic airflow obstruction and CVD.

Duration of Project

8/1/2011 – 12/31/2013

Project Overview

Racial disparities exist in CVD, with blacks disproportionately affected compared to whites. We have shown that these disparities may be related, in part, to racial differences in inflammation and abnormal arterial vasoreactivity, both of which are more prevalent in blacks. These associations have also been noted in COPD and may help explain why COPD is associated with high rates of CVD morbidity and mortality. However, the influence of race on COPD may actually be underestimated, as blacks with COPD may be more likely to die from CVD than from end-stage lung disease. This observation is particularly relevant because the presence of CVD risk factors that are more prevalent in blacks (e.g., hypertension) is associated with a nearly three-fold increase in CVD mortality over patients with COPD. We will determine whether racial disparities exist in associations among lung function, CVD risk factors, and inflammation. Specifically, we will study a cohort of western Pennsylvanians between the ages of 50 and 80 years to: (1) identify racial disparities in the prevalence of chronic airflow obstruction and (2) investigate race-related differences in the interrelationship among chronic airflow obstruction, CVD risk factors, and inflammation, all of which are associated with increased CVD morbidity and mortality. This study will be conducted in 1,000 participants (approximately 43 percent black) in the ongoing Heart Strategies Concentrating on Risk Evaluation (Heart SCORE) study. Heart SCORE is a community-based participatory research study of 2,000 participants (43 percent black) that began in 2003 to study the epidemiology and mechanisms of racial disparities in CVD. Heart SCORE follows participants annually and has collected a wealth of biological and psychosocial data, markers of subclinical atherosclerosis, and sleep assessment data. The new study will: (1) assess lung function by spirometry and (2) correlate measurements, such as forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, and mid-expiratory flow (FEF25-75), with assessments of CVD risk factors (e.g., blood pressure, lipid levels, glucose) and measured levels of inflammation (high sensitivity C-reactive protein) in 1,000 Heart SCORE participants.

Principal Investigator

Steven E. Reis, MD
Professor of Medicine
University of Pittsburgh
3550 Terrace Street
Pittsburgh, PA 15261

Other Participating Researchers

Patrick J. Strollo, MD – employed by the University of Pittsburgh

Expected Research Outcomes and Benefits

Racial disparities exist in chronic diseases such as CVD, which is more prevalent in blacks than whites, and COPD, which is more prevalent in whites than blacks. In Pennsylvania, blacks who die from COPD or CVD are 6.5 and 10.4 years younger, respectively, than whites who die from the same conditions. Many of the chronic diseases that exhibit race-related differences in prevalence, morbidity, and mortality have common pathophysiologic manifestations (e.g., inflammation) and are linked with respect to clinical outcomes. For example, CVD is a major cause of mortality in COPD, and both of these diseases are associated with systemic inflammation. However, racial differences in the relationship between CVD and COPD have not been explored in detail. The goal of this project is to prospectively investigate race-related differences in the link among CVD, chronic airflow obstruction, and inflammation in 1,000 participants in our extensively characterized and ongoing Heart SCORE study. From a public health perspective, we anticipate to find that for any degree of airflow obstruction, blacks will have a greater burden of CVD risk factors and higher levels of systemic inflammation, both of which are associated with CVD events. These findings may serve as the basis for the development of a more personalized approach to the treatment of airflow obstruction that considers race, concomitant CVD risk factors, and markers of inflammation and may provide mechanistic insights into racial disparities in chronic lung disease. Each participant will receive a lung function assessment that can be reviewed with his/her health care provider, who can then incorporate the data into an individualized care plan.

Summary of Research Completed

During this final reporting period we enrolled 34 participants before closing the study. Statistical analyses were initiated during this reporting period. The results of these analyses are reported below.

This study included 1,338 participants, which exceeded the proposed cohort size of 1,000 participants. Statistical analysis was restricted to Heart SCORE participants of Caucasian or African-American race, and those with complete data for measures of both FEV1 and FEF25-75. This resulted in a cohort of 1,299 participants, of whom 529 (40.7%) were African American. Demographic data stratified by race and gender indicate that black participants were significantly younger than white participants and had significantly lower educational attainment, higher body mass index, higher blood pressure, and higher fasting glucose levels. In contrast, blacks presented with lower triglycerides and, in women, lower cholesterol levels than Whites.

Aim 1. To identify gender and racial disparities in the prevalence of chronic airflow obstruction as measured by spirometry.

In univariate analyses, our results indicate that black males have more airflow obstruction than

white males manifested by significantly lower percent predicted FEV1, and a trend towards lower FEF25-75, both of which are objective measures of airflow obstruction (Figures 1, 2). Similarly, black females had significantly lower predicted FEV1 than white females, yet in a paradoxical manner, higher predicted FEF25-75.

Using white males as the comparison (reference) group, Table 1 demonstrates a lower percent predicted FEV1 among both black males and black females. Similar analyses using white males as the comparison (reference) group demonstrate lower percent predicted FEF25-75 among black males and white females. In multivariable analysis adjusting for body mass index and current smoking status, Table 2 indicates that black males have lower adjusted percent predicted FEV1. In contrast to assessment of predicted FEV1, Table 3 indicates that white females have lower adjusted values of FEF25-75.

The above data indicate that both racial and gender differences exist in objective measurements of airflow obstruction.

Aim 2. To investigate race-related differences in the interrelationship among airflow obstruction, CVD risk factors, and inflammation, all of which are associated with increased CVD morbidity and mortality.

For this analysis, percent predicted FEV1 was used as the primary outcome variable. For the total cohort, high sensitivity C-reactive protein level was not associated (correlated) with predicted FEV1 (r=-0.02, p=0.65). As seen in Table 4, higher body mass index was associated with significantly lower percent predicted FEV1, whereas higher LDL cholesterol was associated with better function. The category of black male remained a strong independent predictor of lower percent predicted FEV1.

Given the predominant finding of black males having significantly lower predicted FEV1, analyses were conducted to examine possible interactions with other risk factors that might explain this association. This analysis was conducted in two ways – comparing black males to white males, and comparing black males to all females and white males together.

There was no evidence of interaction in relation to lower predicted FEV1 between black male and body mass index (p=0.29, p=0.50), fasting glucose (p=0.33, p=0.24), systolic blood pressure (p=0.60, p=0.52), LDL cholesterol (p=0.72, p=0.83), or C-reactive protein (p=0.92, p=0.51). Thus, the finding of black males having significantly lower predicted FEV1 could not be explained through differences in conventional CVD risk factors.

Figure 1. Percent predicted FEV1 by race and gender.

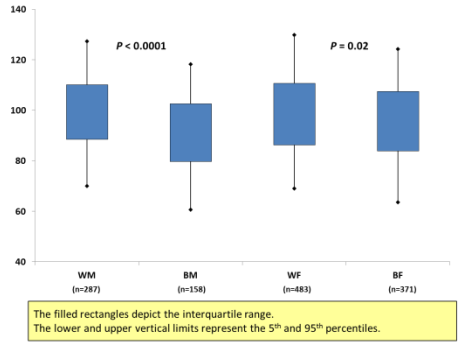

Figure 2. Percent predicted FEF25-75 by race and gender.

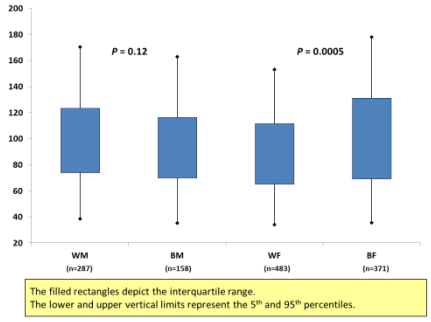

Table 1. Univariate Association Between Race/Gender and Percent Predicted FEV1

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Squared Partial Corr Type II**
Intercept	1	99.02230	1.09874	90.12	<.0001	.
Black male	1	-8.57477	1.84394	-4.65	<.0001	0.01642
White female	1	-0.51381	1.38729	-0.37	0.7112	0.00010592
Black female	1	-3.32473	1.46326	-2.27	0.0232	0.00397

Table 2. Multivariate Association Between Race/Gender and Percent Predicted FEV1

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Squared Partial Corr Type II**
Intercept	1	113.37406	2.79876	40.51	<.0001	.
Current smoker	1	-1.11179	2.45728	-0.45	0.6510	0.00015965
Former smoker	1	0.43697	1.05876	0.41	0.6799	0.00013285
BMI	1	-0.49001	0.08797	-5.57	<.0001	0.02363
Black male	1	-7.96128	1.83124	-4.35	<.0001	0.01453
White female	1	-1.27357	1.37509	-0.93	0.3545	0.00066866
Black female	1	-1.91608	1.48085	-1.29	0.1959	0.00130

Table 3. Multivariate Association Between Race/Gender and FEF25-75

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Squared Partial Corr Type II**
Intercept	1	4.42862	0.07667	57.77	<.0001	.
Current smoker	1	-0.08477	0.06731	-1.26	0.2081	0.00124
Former smoker	1	0.01244	0.02900	0.43	0.6680	0.00014354
BMI	1	0.00301	0.00241	1.25	0.2119	0.00122
Black male	1	-0.09445	0.05016	-1.88	0.0599	0.00276
White female	1	-0.10551	0.03767	-2.80	0.0052	0.00608
Black female	1	-0.01968	0.04056	-0.49	0.6276	0.00018359

Table 4. Factors Associated with Percent Predicted FEV1.

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Squared Partial Corr Type II**
Intercept	**1**	118.12810	5.82053	20.30	<.0001	.
Current smoker	**1**	0.19118	2.66474	0.07	0.9428	0.00000460
Former smoker	**1**	0.71147	1.12323	0.63	0.5266	0.00035842
Body mass index	**1**	-0.44613	0.09868	-4.52	<.0001	0.01794
Glucose	**1**	-0.04789	0.02700	-1.77	0.0764	0.00280
Systolic Blood Pressure	**1**	-0.05052	0.03863	-1.31	0.1912	0.00153
LDL cholesterol	**1**	0.04421	0.01635	2.70	0.0069	0.00650
Black male	**1**	-7.24687	1.97178	-3.68	0.0002	0.01193
White female	**1**	-1.91496	1.46906	-1.30	0.1927	0.00152
Black female	**1**	-2.12325	1.57644	-1.35	0.1783	0.00162