Temple University

Annual Progress Report: 2007 Formula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Formula Grant Overview

Temple University received $1,957,901 in formula funds for the grant award period January 1, 2008 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Molecular Mechanisms of Calcium Entry in Cancer Cells - Numerous studies have identified altered Ca^{2+} signaling in cancer cells; recent work has led to the discovery and characterization of a previously unknown molecular regulator of Ca^{2+} signals named STIM1. Although cancer cells generally exhibit some changes in their Ca^{2+} responses, rhabdosarcoma cells exhibit loss of STIM1 expression; we propose to assess the impact of this on changes on Ca^{2+} signals, cancer cell growth and survival. This work will not only enhance our understanding of this specific type of cancer but may also lead to generally applicable new treatment strategies.

Duration of Project

7/1/2008 - 06/30/2010

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. 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 Transcriptional Elements Controlling Expression of DDH1 in Lung and Liver Cancer Cells - Dihydrodiol dehydrogenases (DDH) are a family of aldo-keto reductases involved in the de novo detoxification of xenobitoics. Expression profiles have indicated increased expression of DDH1 in human lung, liver and esophageal tumors as well as in carboplatin- and cisplatin-resistant human ovarian and lung cancer cells. This increase in DDH protein expression was associated with the alterations in the transcription of the DDH gene suggesting that the promoter region of the DDH gene plays an important role in controlling its expression. This study aims to decipher the precise genetic elements and its associated transcription factor(s) that control the induction of DDH1 gene in human lung and liver cancer

cells. Identification of the transcriptional controls of DDH expression will allow designing strategies to control its expression and thus interfere with the process of carcinogenesis as well as development of tumor cell resistance to anticancer drugs.

Duration of Project

7/1/2008 - 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 Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 3: Project Title and Purpose

Mercury-Induced Cell Death: a Source of Autoantigen? - Recent work in the field of autoimmunity has focused on apoptotic cells as a possible source of autoantigen. In the setting of murine mercury-induced autoimmunity, mercury-induced cell death, which differs from apoptosis, may be the source of autoantigen. This study will aim to characterize the Hg-induced cell death process and its effects on fibrillarin, the self antigen most specifically targeted in murine Hg-induced autoimmunity.

Duration of Project

7/1/2008 - 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 Annual C.U.R.E. 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

Inhibition of HIV Infection Targeting cFMS Signaling Pathways - Once infected with HIV, cells known as tissue macrophages are resistant to the effects of current drugs that are used to treat HIV infection. Infected macrophages likely exert detrimental effects on the immune system and contribute to neurologic abnormalities associated with HIV infection. The studies proposed here investigate pathway(s) whereby HIV infection, by its upregulation of Macrophage Colony Stimulating Factor production in the infected cell, orchestrates the long-term survival of the infected macrophages. The studies should help develop strategies that can be used to help eliminate virus infected cells and help to clear HIV infection in combination with current therapies.

Duration of Project

7/1/2008 - 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 Annual C.U.R.E. 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

Mechanism of JCV Involvement in Brain Tumors - The human polyomavirus, JCV, infects greater than 80% of the human population worldwide and remains in a latent state throughout life. Under certain physiological conditions such as immunosuppression, JCV becomes reactivated and induces the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML) in the brain. In addition, JCV has been shown to possess oncogenic activity in several experimental animals and has been detected in a significant number of human brain tumors including medulloblastomas and glioblastomas. The purpose of this study is to unravel the underlying molecular events associated with tumorigenesis of JCV and translate the knowledge from these studies toward the development of therapeutic strategies.

Duration of Project

7/1/2008 - 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 Annual C.U.R.E. 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

Immune Regulation and Reactivation of JC Virus in the Demyelinating Disease, PML - Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease caused by JC virus which has recently occurred in patients with autoimmune disorders being treated with the powerful immunosuppressive therapy, rituximab. Our project will lay a foundation for determining the mechanisms involved in immune regulation of JC virus and investigate how rituximab may promote JC virus infection through soluble immunomodulators.

Duration of Project

7/1/2008 - 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 Annual C.U.R.E. 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

The Role of ICOS in Mercury-Induced Autoimmunity - An inducible co-stimulatory molecule (ICOS) is a molecule expressed on the surface of white blood cells. It plays an important role in the immune response and blocking antibodies to ICOS are promising new treatments for conditions such as autoimmune diseases. We want to understand the role of ICOS in a mouse model of heavy metal-induced autoimmune disease and to fully assess its role as a potential therapeutic target.

Duration of Project

7/1/2008 - 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 Annual C.U.R.E. 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

Role of Excess Ca^{2+} Influx in Cardiac Dysfunction after Myocardial Infarction - Congestive heart failure (CHF) is a devastating syndrome with 50% mortality within five years. It develops after the heart is challenged with hemodynamic stress imposed by hypertension, cardiac attack and genetic alterations. The current view on CHF is that the myocyte (heart cell), the basic component responsible for heart contraction, is weaker than normal. In contrast, there is an emerging concept that the loss of working heart cells plays a critical role in the progression of CHF. In this study, we will use a transgenic mouse model with heart specific overexpression of the L-type calcium channel (Cav1.2) to determine whether the increase of contractility by overexpressing Cav1.2 will rescue heart failure induced by myocardial infarction (heart attack) or worsen CHF development by inducing myocyte loss.

Duration of Project

9/16/2008 – 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 Annual C.U.R.E. Reports on the Department's

Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 9: Project Title and Purpose

Loss of Wilms' Tumor Suppressor 1 Regulates STIM1-mediated Ca^{2+} Entry - Wilms' Tumor is one of the most common pediatric tumors, occurring 1/10,000 people in North America. It is thought to result from the loss of a transcriptional regulator known as Wilms' Tumor Suppressor 1 (WT1). Preliminary studies in our laboratory have linked WT1 with a Ca^{2+} entry pathway known as store-operated Ca^{2+} entry. This is important, because changes in Ca^{2+} concentration are linked to cell growth, differentiation and cell death. Hence, our goal is to gain a better understanding of Ca^{2+} signals, how the loss of WT1 causes Wilms' Tumor and how to design new treatment strategies.

Duration of Project

9/16/2008 – 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 Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 10: Project Title and Purpose

The Role of Osteoactivin in Osteoblast Development and Function - Osteoactivin (OA) has recently emerged as an important factor in osteogenesis. The identification of novel anabolic agents in bone and, perhaps even more importantly, gaining insights into their mechanisms of action, are subjects of intense clinical interest. Systemic or localized forms of bone loss are caused by a variety of diseases or conditions, including aging, and the resulting osteopenia is accompanied by an increased incidence of fracture. Treatment of patients with osteoporosis is a major health care challenge and many pharmaceutical companies are focused on identifying novel therapeutic agents that can selectively stimulate new bone formation.

Duration of Project

9/16/2008 – 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 Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 11: Project Title and Purpose

Hyperhomocysteinemia and Thrombosis Formation - The purpose of this project is to identify the mechanistic links between HHcy and thrombosis in homocysteinemia animal model.

Duration of Project:

9/16/2008 – 5/10/2009

Summary of Research Completed

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

Research Project 12: Project Title and Purpose

Identification of the Cis- and Trans- Elements Required for Stress-Mediated Induction of Gadd45B - Gadd45B is a small nuclear protein which is implicated in modulating the cellular response to physiological stresses. Gadd45B mRNA levels are robustly induced in mammalian cells following treatment with a variety of different stress agents, which either directly or indirectly damage DNA. The mechanism of this induction is unknown. Therefore, we plan to determine the extent to which this induction is regulated transcriptionally and post-transcriptionally, and identify cis elements and corresponding transcription factors required for induction. Knowledge of this mechanism is important in understanding how Gadd45B can become deregulated, and thus lead to a greater propensity to tumor growth and cancer.

Duration of Project

9/16/2008 – 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 Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 13: Project Title and Purpose

A Novel Approach for Engineering Neovasculature for Stem Cell Therapy - Most current pharmacological and/or invasive therapies aim to treat heart disease. During the past few years, however, there has been much excitement and interest in developing regenerative approaches for curing heart disease. Research is aimed at restoring the contractile function of the heart through engineering replacement myocardium and its supporting microenvironment using approaches such as cell-based therapy. Recent attempts at rebuilding the myocardium using stem cells have yielded disappointing results. The overall goal of this study is to develop the technology to

enhance the morphology and function of post-infarct neovasculature, prior to scar formation, and to establish the optimal time post-myocardial infarction (MI) when proangiogenic interventional strategies could result in maximal in situ renewal of myocardial tissue which has been lost to MI.

Anticipated Duration of Project

1/30/2009 – 12/31/2011

Project Overview

The overall goal of this study is to develop the technology to enhance the morphology and function of post-infarct neovasculature, prior to scar formation, and to establish the optimal time post-myocardial infarction (MI) when proangiogenic interventional strategies could result in maximal in situ renewal of myocardial tissue. The specific aims of this study are to: 1) Develop immunoliposomes containing pro-angiogenic compounds (vascular endothelial growth factor (VEGF) and/or basic fibroblast growth factor (bFGF)) and determine their biodistribution in MI animals; 2) Selectively deliver pro-angiogenic compounds to the infarct region by targeting MI upregulated adhesion molecules in microvessels bordering on the infarct site, and quantify improvements in vascularity, perfusion, and cardiac function; and study the efficacy of combining stem cell therapy with targeted pro-angiogenic therapy to determine if this combinational therapy can significantly improve vascularity, perfusion, and cardiac function as compared to stem cell therapy alone.

In Specific Aim 1, we will use clinically relevant drug carriers (immunoliposomes) bearing mAbs to adhesion molecules that are upregulated on the vasculature of infarct tissue to show that particles can be targeted to infarct tissue and to optimize particle design and drug loading & release profiles in vitro. We will quantify the level and time course of the upregulation of E- and P-selectin, ICAM-1, and $\alpha v\beta 3$ that are known to be upregulated in MI tissue to determine the best molecular target(s) and time point(s). In Specific Aim 2, functional significance of pro-angiogenic therapy will be assessed in terms of neovascular formation, oxygen delivery capacity, and cardiac function. Combinatorial effects of no treatment, targeted pro-angiogenic therapy, and systemic pro-angiogenic therapy will be investigated. To bypass the potential side effects of systemic administration of proangiogenic compounds, we will use clinically relevant drug carriers (immunoliposomes) to deliver VEGF and/or bFGF to the infarct region via upregulated adhesion molecules. We will also investigate the enhancing effects of targeted pro-angiogenic therapy on marrow stromal cell treatment of myocardial infarction. A combinatorial design will be used to study the effects of stem cell therapy, targeted pro-angiogenic therapy, and systemic pro-angiogenic therapy on neovascular formation, generation of cardiomyocytes, and cardiac function. These findings will then be used to quantify the beneficial effects of combinational stem cell + targeted pro-angiogenic therapy to determine if targeted pro-angiogenic therapy can augment stem cell treatment. Infusion of these cells into the tail vein will approximate the clinical scenario of autologous intravenous cell therapy.

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Expected Research Outcomes and Benefits

Myocardial infarction often leads to congestive heart failure and is a leading cause of death in the U.S. and other industrialized countries. In addition to the lost muscle mass, a transmural MI also involves the deterioration of the microenvironment through the proteolysis of extracellular matrix, vasculature, and nerves. Subsequent tissue repair usually does not involve a significant regeneration of the microenvironment and the microvasculature. Bold innovative strategies are needed to prevent the appearance of chronic cardiac failure following MI. Engineering replacement myocardium and its supporting vasculature, in approaches such as bone marrow-derived stromal cell (MSC) therapy or muscle cell transplantation, may represent such initiatives. Recent attempts at rebuilding the myocardium using stem cells have yielded disappointing results. The lack of a supporting vasculature which provides oxygen and nutrients for the differentiating stem cells may in part explain these disappointing findings. However, concerns over possible side effects have hampered attempts at revascularizing the infarcted myocardium using systemic delivery of pro-angiogenic compounds such as VEGF and bFGF. Recently, we have developed a novel approach for preferentially delivering drug-carrying immunoliposomes to infarct tissue by targeting cell adhesion molecules which are upregulated in the vasculature of infarcted myocardium. We seek to develop a novel methodology to enhance the morphology and function of infarct neovasculature, prior to scar formation, and to establish the optimal time post-MI when interventional strategies, e.g. MSC therapy, could result in maximal, in situ renewal of myocardial tissue lost to MI. Our preliminary findings indicate that local delivery of pro-angiogenic compounds to the infarct region could initiate the regrowth of neovasculature supporting regeneration of myocardial tissue from stem cells, which in turn could lead to improved cardiac function. The long-term goal of this project is to develop a combined pro-angiogenic/stem cell therapy for restoring myocardial function in a clinical setting.

Summary of Research Completed

Development of Dual Targeted Drug Carriers: Previously we have shown that model drug carriers (2μm in diameter) conjugated to anti-ICAM-1 or anti-E-selectin alone show similar attachment to TNF-α activated endothelial cells in a flow chamber under shear flow. However, particles conjugated to both anti-E-selectin and anti-ICAM-1 (1,000 anti-ICAM1 ligands/particle + 1,000 anti-E-selectin ligands/particle) show significantly higher levels of adhesion under shear

flow as compared to particles conjugated to anti-E-selectin alone (2,000 anti-E-selectin ligands/particle), see Figure 1, n=3 experiments per data point. Please note that the total number of ligands/particle is the same for both single-ligand and multi-ligand conjugated particles and that the fold increase in adhesion level should be close to unity if conjugating particles to multiple ligands does not offer an advantage. Furthermore, the increase in adhesion with multi-ligand conjugated particles is observed over a large range of physiological shear flows (0.5-4 dynes/cm2). This data clearly supports the hypothesis that multi-targeted delivery of drug carriers significantly enhances their delivery to targeted tissue.

Assessing Molecular Changes in The Tissue Matrix using FTIR: To fully understand the mechanism that underlies the process of competent tissue regeneration, assessment of molecular features of the repair tissue is required. The technique of Fourier transform infrared (FTIR) spectroscopy is a powerful tool to study molecular changes in connective tissues. The frequency at which a molecule absorbs infrared radiation is sensitive to its conformation and can be used to obtain information on its secondary structure and on orientation of specific molecular bonds. A novel infrared spectroscopic technique that can be used to evaluate the quality of tissue at a molecular level is Fourier transform infrared imaging spectroscopy (FT-IRIS). The coupling of an FT-IR spectrometer to an optical microscope with an array detector provides a unique opportunity to study the relative amount, molecular nature, distribution, and orientation of the components of connective tissues at a spatial resolution of ~6.25 microns. This data, combined with microscopic visualization of the tissue, essentially allows for "infrared imaging" of the sample. Thus, micron-resolution maps of the molecular components of histological sections of tissues can be obtained. In contrast to histology studies, infrared imaging studies permit visualization of molecular structure information, in addition to component distribution. Preliminary studies on one VEGF-treated and one non-treated post-MI rat heart shown in Figure 2 indicate differences in collagen quantity, quality, and glycosylation.

Collagen Deposition in VEGF treated and Untreated Hearts: Gomori's trichrome stain (Richard Allen Scientific) is a one-step stain used to differentiate collagen (blue) from muscle tissue (red). Cross-sections of fresh frozen heart tissue sliced 9 um thick were obtained on polylysine coated slide. Heart sections were fixed with Bouin's solution at 56 ºC for 1 hour, and then stained according to the manufacturer's directions. Stained heart sections were dehydrated using various grades of alcohol and xylenes, and then mounted with glass slides. Images were obtained using a Nikon Digital Sight color camera and Nikon Element software. A full mosaic image was created using Adobe Photoshop CS software. Image J was used to measure the length of the blue infarct scar, normalized to the circumference of the heart, and the area of the collagen region, normalized to the total area of the heart.

Gomori's trichrome staining revealed a thick, collagenous scar (stained blue) along the anterior wall of the left ventricle in both targeted VEGF treated and untreated animals (Figure 3.A, n=6 animals per group). The scar length, as a percentage of the total circumference of the heart, was significantly shorter in hearts treated with targeted VEGF than untreated MI hearts (Figure 3.B). Likewise, the area in the anterior wall which was composed of the collagen scar tissue was significantly smaller in hearts treated with targeted VEGF therapy, compared to untreated MIs (Figure 3.C).

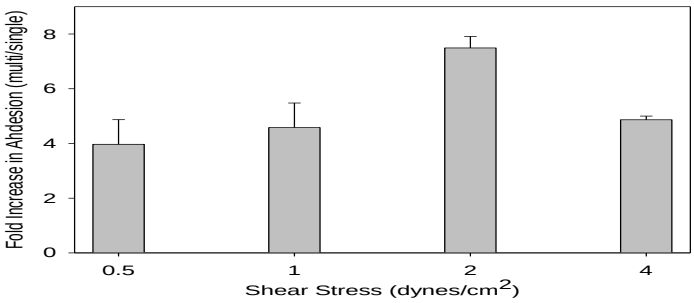

Figure 1: The bars represent the number of adhering particles bearing ligands to both E-selectin and ICAM-1 (multi) divided by the number of particles bearing ligands to only E-selecting (single). Particles bearing both ligands show significantly enhanced adhesion at all shear levels tested.

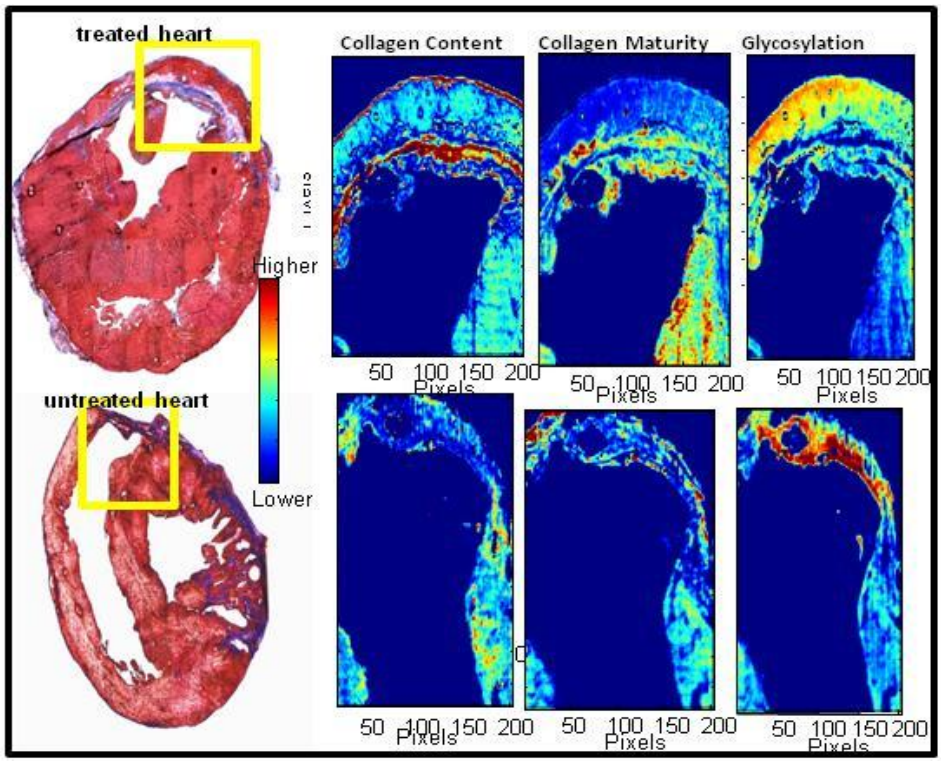

Figure 2. FT-IRIS evaluation demonstrates that targeted VEGF therapy modifies collagen quantity and quality, as well as glycosylation quantity. 1 pixel = 25 microns

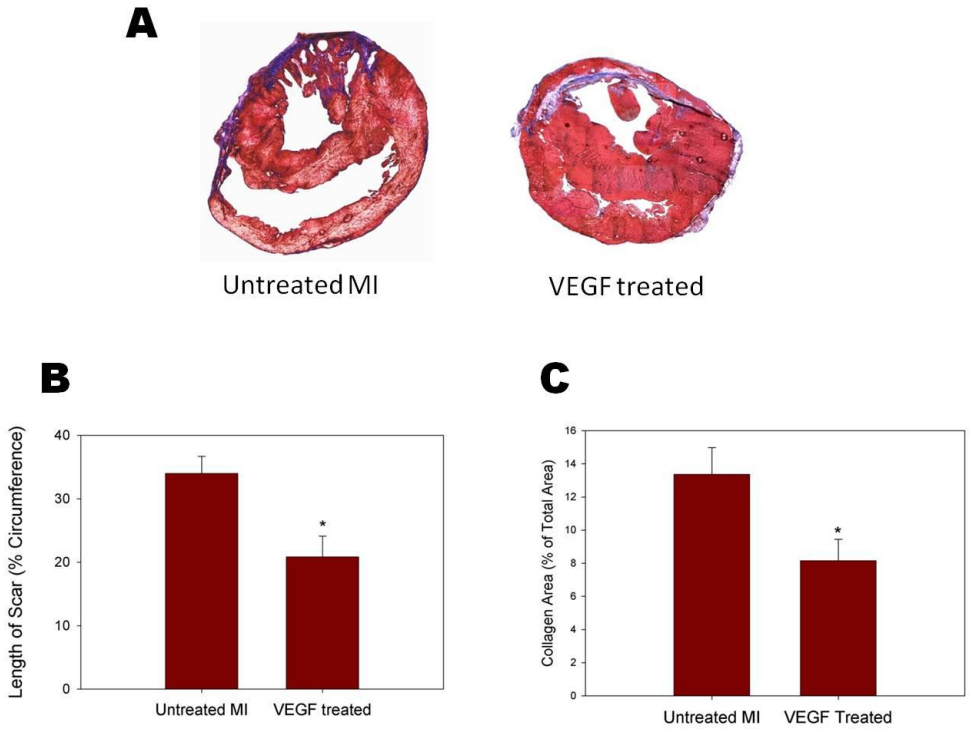

Figure 3: A: Trichrome staining of targeted VEGF treated and untreated MI rat hearts distinguishes collagen scar tissue (blue) from muscle tissue (red). B: Collagen scar length as a percentage of the heart circumference. Hearts treated with targeted VEGF therapy show a significant decrease in the length of the infarcted tissue. C: Hearts treated with targeted VEGF therapy showed a decrease in the total cross-sectional area that is composed of scar tissue. * $p < 0.05$ for targeted VEGF treated vs. untreated MI.

Research Project 14: Project Title and Purpose

Omega-3 Fatty Acids as Therapeutic Anti-inflammatory Agents - In recent years inflammation has emerged as an essential underlying process in diseases of various etiologies such as Alzheimer's disease, cardiovascular diseases and cancer, which joined classical inflammatory/autoimmune disorders such as arthritis, periodontal disease, septic shock, inflammatory bowel diseases, multiple sclerosis, lupus erythematosus, etc. Although both steroidal and nonsteroidal anti-inflammatory therapies have been developed, there is a pressing need for new therapeutic anti-inflammatory agents with fewer side effects and better efficacy.

Duration of Project

1/30/2009 – 6/30/2011

Project Overview

Omega-6 and omega-3 fatty acids obtained solely from food are essential for human health. Omega-6 fatty acids are abundant in land animals, whereas omega-3 fatty acids are preponderant in marine mammals and fatty fish. Preponderance of fish in the diet is associated with reduced incidence of inflammatory and cardiovascular diseases, and dietary supplements containing mixtures of the major omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have a beneficial effect in several human inflammatory conditions.

In the present project we will investigate the effects of the omega-3 fatty acid DHA in models of inflammatory bowel disease (IBD). Human IBD are chronic, relapsing disorders, characterized by inflammatory reactions to microbial antigens. Despite recent advances in research and therapeutic approaches, IBD patients are often resistant to treatment, justifying the search for new therapies. Murine IBD models have been used extensively for mechanistic studies and proof of concept in therapeutic interventions. We propose to use the TNBS colitis model, a model for human Crohn's disease, to assess the anti-inflammatory effects of DHA and to investigate some of the molecular mechanisms involved in the therapeutic effect.

1. *DHA effects on disease*. We will assess DHA effects on disease prevention, on established disease and on disease recurrence. We expect that DHA will have a protective effect, by reversing established disease and preventing recurrence.
2. *Effects of DHA on colonic cellular composition and function*. The protective effects might be due to reduced infiltration of inflammatory cells. Colons will be examined for macroscopic and histology scores, myeloperoxidase activity (reflective of neutrophil infiltration), and T cell, monocyte/macrophages, and dendritic cell infiltration. Colonic expression of proinflammatory cytokines and chemokines will be determined.
3. *Effects of DHA on T cell differentiation.* DHA might affect T cell differentiation. If this is the case, we will observe changes in the numbers of IFNγ+, IL-4+, IL-17+ T cells. Mesenteric lymph node cells will be analyzed in terms of T cell proliferation and for intracellular and/or secreted cytokines characteristic for the effector T cell subsets Th1, Th2, Th17 and Treg.

Principal Investigator

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Other Participating Researchers

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Expected Research Outcomes and Benefits

The project will provide information on the anti-inflammatory role of omega-3 fatty acids in experimental models of inflammatory bowel diseases, and will contribute to the understanding of the cellular/molecular mechanisms involved in the protective and/or therapeutic effects of omega-3 fatty acids. These results will provide supporting evidence for future clinical trials using omega-3 fatty acids and their derivatives in gastrointestinal disorders of an inflammatory nature. Since patients suffering from such disorders are often resistant to existing therapies, this project could have a significant impact on the improvement of human health.

Summary of Research Completed

In this funding period we addressed research related to Aim 3 - *Effects of DHA on T cell differentiation.*

Since in previously reported experiments we observed an effect of DHA administration on the generation of the proinflammatory T cell subsets Th1/Th17, we continued with in vitro studies which indicated that DHA inhibited T cell proliferation by arresting T cells in the G0/G1 cycle and prevented T cell differentiation into proinflammatory Th1/Th17 cells. These results were published in Brain, Behavior and Immunity, 2011, 25: 872-882, in the article entitled "Docosahexaenoic acid prevents dendritic cell maturation, inhibits antigen-specific Th1/Th17 differentiation and suppresses experimental autoimmune encephalomyelitis" by W. Kong, J-H Yen and D. Ganea. The results are summarized in the Abstract below:

ABSTRACT
Docosahexaenoic acid (DHA), the most abundant essential n-3 polyunsaturated fatty acid in the CNS, emerged recently together with eicosapentaenoic acid (EPA) and DHA/EPA metabolic derivatives as a major player in the resolution of inflammation. Protective anti-inflammatory effects of DHA were reported in clinical studies and animal models of colitis, sepsis, and stroke. Here we report for the first time a beneficial effect of dietary n-3 fatty acids in experimental autoimmune encephalomyelitis (EAE), a model for human multiple sclerosis. In the present study we investigated the effects of DHA on the function of bone marrow-derived dendritic cells (DC) in $CD4^+$ T cell stimulation and differentiation. Pretreatment of DC with DHA prevented LPS-induced DC maturation, maintaining an immature phenotype characterized by low expression of costimulatory molecules and lack of proinflammatory cytokine production (IL-12p70, IL-6 and IL-23). DHA-treated DC were poor stimulators of antigen-specific T cells in terms of proliferation and Th1/Th17 differentiation. This was associated with an increase in p27(kip1), a cell cycle arresting agent, and with decreases in Tbet, GATA-3 and RORγt, master transcription factors for Th1, Th2, and Th17. In contrast, T cells co-cultured with DC-DHA express higher levels of TGFβ and Foxp3, without exhibiting a functional Treg phenotype. Similar to the in vitro results, the beneficial effect of DHA in EAE was associated with reduced numbers of IFNγ- and IL-17-producing $CD4^+$ T cells in both spleen and CNS.

These results were also summarized in a review paper published in Clinical Lipidology (2011, 6:277-291) and entitled "Modulation of dendritic cell function by PGE2 and DHA: a framework

for understanding the role of dendritic cells in neuroinflammation" by D. Ganea, V. Kocieda, W. Kong and J-H. Yen.

Research Project 15: Project Title and Purpose

Angiocidin Induces Stem Cell Activation and Differentiation - Our laboratory has discovered a protein which we call angiocidin. When the protein is injected into mice that have cancer, it keeps the cancer from growing and spreading. The purpose of our project is to see if angiocidin inhibits cancer growth by inhibiting the growth and spread of cancer stem cells, a subset of cancer cells that are thought to cause cancer growth and spread.

Duration of Project

1/30/2009 – 12/31/2010

Project Overview

We hypothesize that angiocidin differentiates THP-1 leukemia cells as well as other tumor cells into a more normal phenotype by activating and differentiating stem cells present in the tumor cell population to a more normal phenotype that is not tumorigenic. We base our hypothesis on the observation that angiocidin-treated THP-1 cells up-regulated CEA-CAM-1, a major stem cell marker, discovered by comparing the microarray profiles of cells treated with angiocidin with that of controls. Additionally, in a collaboration with Dr. John Wong, CEO of Moraga Biotech Corporation (http://www.moragabiotech.com), we observed that angiocidin activated and differentiated normal blood stem cells into a fibroblast-like phenotype when cultured on collagen. These cells not only expressed CEA-CAM-1 but were also stimulated to proliferate in response to angiocidin - an effect that was more potent than that obtained with growth factors such as basic fibroblast growth factor. Temple University is filing a provisional patent application seeking patent protection for the observation that angiocidin can activate and stimulate stem cell differentiation. The benefits of this research relate not only to angiocidin-mediated cancer treatment but also to angiocidin-mediated stimulation of tissue regeneration and wound healing.

Based on these observations, we propose to test directly our hypothesis that angiocidin can activate cancer stem cells and differentiate them into a more normal phenotype. We have obtained a human melanoma stem cell line from Dr. Meenhard Heryln of the Wistar Institute. These cells are highly tumorigenic but can be differentiated into normal melanocytes. In this project, we propose to culture these cells in the presence and absence of angiocidin. We will compare the gene expression profiles of control and angiocidin-treated cells using microarray analysis and PCR arrays. We anticipate that the angiocidin-treated cells will up-regulate melanocyte markers and down-regulate markers characteristic of melanoma. The in vitro functional activity of control and angiocidin-treated cells will also be evaluated in proliferation, adhesion and migration assays. The in vivo tumorigenic activity of control and angiocidin-treated cells will be evaluated in athymic mice injected subcutaneously with either control or angiocidin-treated cells. We anticipate that the angiocidin-treated cells will lose their ability to form tumors. The results of these studies will provide proof-of-concept that angiocidin activates

and differentiates cancer stem cells making them less tumorigenic.

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Other Participating Researchers

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Expected Research Outcomes and Benefits

We hope to show that angiocidin, a protein that our laboratory discovered and has been studying for its anti-cancer activity, has a direct inhibitory effect on the ability of cancer stem cells to grow and spread as tumors. We will evaluate the effect of angiocidin on melanoma stem cells but also other cancer stem cells isolated from human cancers. We will analyze the gene composition of cancer stem cells treated with angiocidin and hope to show that angiocidin causes cancer stem cells to express fewer cancer genes and more normal genes. Our studies should directly benefit cancer patients by providing them a cancer therapy that targets cancer stem cells. The potential advantage of this type of therapy is that once patients are treated with stem cell targeting agents such as angiocidin, the cancer is less likely to come back since cancer stem cells are thought to be responsible for the recurrence of cancer. There is currently no cancer therapeutic that effectively targets cancer stem cells.

Summary of Research Completed

There is extensive evidence demonstrating that angiogenesis and tumor growth are regulated by a variety of host and tumor derived factors. While overproduction of proteins associated with angiogenesis such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are necessary for tumor development, expression of angiogenic inhibitors such as angiostatin, and endostatin are also required for efficient regulation of tumor growth. Many of these angiogenic inhibitors have been recently developed as cancer therapeutics. Our laboratory cloned a tumor-associated, secretory protein angiocidin, which has potent anti-tumor and anti-angiogenic activities. We previously postulated that angiocidin's anti-tumor and anti-angiogenic activities are through its ability to modulate cell matrix interactions. However, we recently made an unexpected discovery of an angiocidin-mediated activity that may provide an alternative mechanism for the anti-tumor activity of angiocidin as well as suggest new therapeutic applications for the protein. We discovered that angiocidin induces the human acute myeloid leukemia (AML) cell line, THP-1, to differentiate into a normal macrophage-like phenotype. The angiocidin-treated THP-1 cells expressed macrophage markers and behaved phenotypically like macrophages acquiring an adherent phenotype and the ability to phagocytize. The angiocidin-differentiated cells up-regulated, CEA-CAM-1, a major AML stem cell marker. Up-regulation of

CEA-CAM1 was discovered by comparing the microarray profiles of cells treated with angiocidin with that of controls; as well as several other AML stem markers such as CD123 (a major marker found on most leukemic stem cells). These results suggested that *angiocidin could be activating and differentiating a small subset of cancer stem cells present in our THP-1 cultures.* Additionally in a collaboration with Dr. John Wong, CEO of Moraga Biotech Corp. (http://www.moragabiotech.com), we observed that angiocidin activated *in vitro* normal quiescent stem cells circulating in human peripheral blood which predisposed the activated stem cells to differentiate into cells representing all three germ layer lineages when cultured on a collagen matrix. These cells not only expressed CEA-CAM-1 but were also stimulated to proliferate in response to angiocidin- an effect that was more potent than that obtained with growth factors such as basic fibroblast growth factor and epidermal growth factor (personal communication with Dr. John Wong). From these experiments we conclude that angiocidin induces AML leukemic cells as well as other tumor cells to differentiate to a more normal phenotype by activating and differentiating cancer stem cells. Additionally, in other cancer systems such as melanoma, breast and glioma, angiocidin inhibits cancer stem cell proliferation by its ability to bind and neutralize basic growth factors such as basic fibroblast growth factor and nerve growth factor (NGF). This activity can also stimulate neurite outgrowth suggesting that angiocidin could also play a role in neurogenesis. The following are some of the data that support our conclusions.

Angiocidin Differentiates THP-1 Leukemia Cells

We have discovered that angiocidin is able to differentiate a human acute myeloid leukemia (AML) cell line, THP-1, to an adherent macrophage-like normal phenotype Figure 1 illustrates the observed phenotypic and some of the biochemical changes induced by angiocidin.
In Figure 2 we show by microarray analysis the specific leukemia stem cell markers up-regulated in angiocidin treated cells as compared to controls. Carcinoembryonic antigen-related cell adhesion molecule 1 (CEA-CAM1), a novel leukemic stem cell marker was up-regulated 13 fold . C-type lectin-like molecule-1 CLL-1 another novel AML stem cell marker identified in the CD34 CD38- AML stem cell population of 77/89 AML patients was up-regulated 3.4 fold. Furthermore, other major AML stem cell markers identified by such as cyclin D1, alpha subunit of the interleukin-3 receptor or CD123, LIM domain kinase 2, and neurotropic tyrosine kinase receptor 3 (NTRK3) were up-regulated 15, 5, 2.3, 2.3 fold, respectively. These data suggest that our angiocidin treated adherent THP-1 cells may contain a putative subpopulation of leukemia-associated stem cells.

Additionally, angiocidin was shown to directly proliferate and activate blastomere-like stem cells (BLSCs) isolated from human blood. BLSC are totipotent stem cells isolated from peripheral blood by a proprietary method developed by Moraga Biotech Corporation. In vitro induction of BLSCs results in differentiation of these unusually small cells (< 2 microns) into cells representing tissues of all three germ layer lineages as well as spermatogonia. Dr. John Wong, CEO of Moraga, has shown that angiocidin activates and induces these stem cells into an adherent phenotype shown in Fig. 3 (a state prior to cells exiting the cell cycle towards terminal differentiation). Angiocidin induction of BLSC results in the cells undergoing a transition state with an increase in cell size concomitant with the cells becoming more refractile when examined by phase contrast microscopy. When unactivated BLSC are cultured in vitro they are found to proliferate in a suspension culture consisting of undifferentiated spheroids. After 24 hours in the

presence of 10 microgram per ml of angiocidin, the majority of cells adhere to a collagen-coated tissue culture dish and show enhanced growth potential accumulating in large aggregates as shown by the arrow in figure 3A. The growth and differentiation potential of angiocidin is greater than that of bFGF, a potent stem cell growth factor, that only shows mild induction and proliferative activity with no evidence of aggregation (Fig. 3B) when 20 ng/ml of bFGF is added to the cultures. Angiocidin, however, showed comparable activity when cells were treated with both bFGF and epidermal growth factor (EGF) at concentrations of 10 ng/ml (Fig. 3C).

Based on these observations we hypothesize that angiocidin induces AML leukemic cells as well as other tumor cells into a more normal phenotype by activating and causing a putative subpopulation of cancer stem cells present in the tumor to become a more terminally differentiated phenotype that reduces their metastatic as well as their tumorigenic potential. Thus, angiocidin may serve as a novel therapeutic reagent that induces the quiescent cancer stem cell to progress to a more differentiated and less tumorigenic phenotype and pave the way for additional therapeutic applications involving angiocidin-mediated activation and differentiation of stem cells.

Angiocidin Inhibits Glioma Stem Cell Growth

Since neurite outgrowth and stem cell renewal is dependent on growth factors such as NGF and FGF-2, we investigated whether angiocidin binds to these growth factors. We developed a simple binding assay in which the growth factors are adsorbed to the wells of a micotiter dish and the adsorbed factors are allowed to bind with biotinylated angiocidin. The bound biotinylated angiocidin was detected with streptavidin-coupled horse radish peroxidase and developed with the colorimetric substrate ultra 3,3´,5,5´-tetramethylbenzidine (TMB) (Figure 4A). To determine that biotinylated angiocidin binds specifically, we were able to show that binding was competed with unbiotinylated angiocidin (Figure 4B).

Angiocidin Binds Basic Fibroblast Growth Factor (FGF-2) and Nerve Growth Factor (NGF)

Solutions containing various concentrations of angiocidin were added to the wells. The 96 well plate was incubated at room temperature with shaking for two hours. Remaining protein solutions were aspirated and then the plate was washed with tris-buffered saline containing 0.1% Tween 20 (TBST) three times. An aliquot of 100 μl of a 0.05 μg/ml streptavidin horseradish peroxidase solution dilution in TBST was added for 30 minutes with shaking. Remaining reagent was then aspirated and the wells were washed three times with TBST. Ultra 3,3´,5,5´-tetramethylbenzidine (TMB) was added in each well for 15 minutes and 100 μl of 0.5M H_2SO_4 was added to stop the reaction and the plate read in an ELISA plate reader at 450nm. For competition experiments shown in Figure 3B, non-biotinylated protein was added to 100 ng/ml biotinylated angiocidin and binding was performed in either FGF-2 or NGF-coated plates as described above.

To determine if angiocidin growth factor complex plays any role tumor cell proliferation, breast cancer cells, which are known to possess receptors for NGF and C were treated for 48 hours with either serum-free media containing 1 mg/ml BSA, or media-free media containing 1 mg/ml BSA with either 10 μg/ml of angiocidin, 10 μg/ml angiocidin plus 100 ng/ml NGF, or 100 ng/ml NGF. Total viable cells were then determined using the Almar blue assay as previously described in Sabherwal Y., Rothman, V.L., Svetoslav, D, L'Heureax D. Z., Marcinkiewicz, C.,

Sharma, M., and Tuszynski, G.P.**,** Integrin $\alpha 2\beta 1$ mediates the anti-angiogenic and anti-tumor activities of angiocidin, a novel tumor-associated protein, Experimental Cell Research, 312: 2443-2453, 2006. The data in Figure 5 show that NGF and angiocidin induce growth arrest and cell death after 48 hours.

Angiocidin and NGF Induce Cell Arrest and Apoptosis in MDA MB231 Breast Carcinoma Cells

Briefly, these data show that angiocidin in the presence of NGF promotes differentiation of PC12 cells and induces cell death of breast cancer cells. Additionally, angiocidin causes growth arrest of glioma stem cells presumably by its ability to complex FGF-2. Since angiocidin binds NGF and FGF-2, we propose that angiocidin-growth factor complex inhibits stem cell growth by withdrawing growth factor from the cells while angiocidin NGF complexes promote cell differentiation while promoting tumor cell growth arrest and apoptosis.

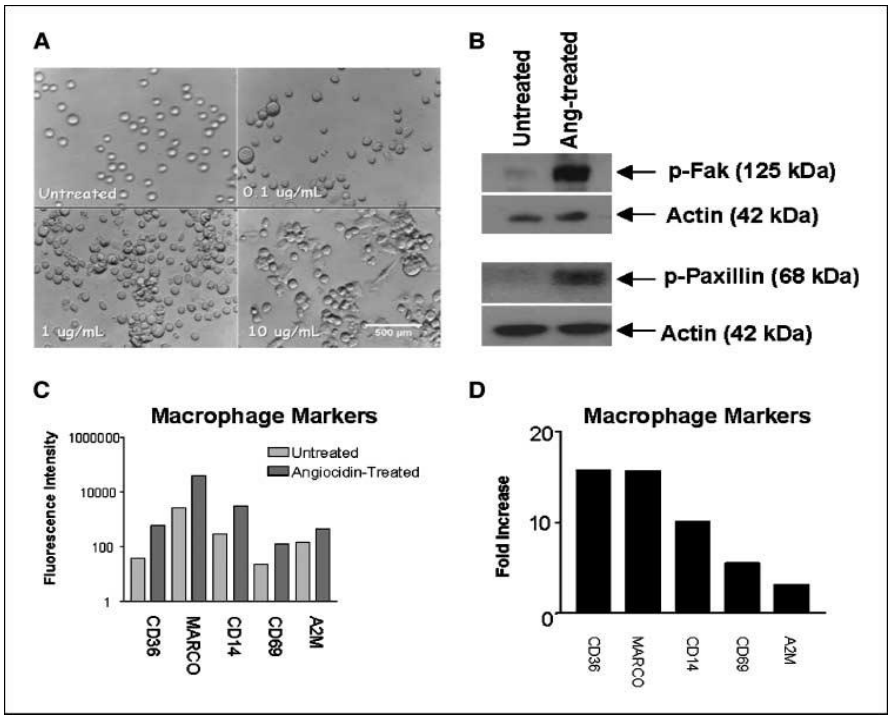


Figure 1 – Angiocidin Induces Focal Adhesion-Dependent Changes in THP-1 Cell Adhesion and Morphology. THP-1 cells were treated in a dose-dependent manner with concentrations of angiocidin ranging from 0 – 10 μg/ml. After 24 hours, wells were examined using Hoffman optics and photographed. For Immunoblot analysis, THP-1 cells were treated with or without 10 μg/ml angiocidin and left to activate for 24 hours. Blots were probed with an anti-phospho-FAK antibody or an anti-phospho-paxillin antibody at a 1:1000 dilution in 5% BSA/PBS and developed. Molecular weights in the blots were estimated from molecular weight markers run simultaneously on the same gel. For microarray analysis, cells were treated with or without 10 μg/ml angiocidin for 24 hours. The following day, total RNA was collected from cells using Trizol Reagent according to the manufacturer's protocol. (A) Photomicrographs of THP-1 cells treated with different concentrations of angiocidin, 40X magnification. The experiment was repeated numerous times, and the data presented represents a typical experiment. (B) Angiocidin-induced phosphorylation of focal adhesion kinase (FAK) and paxillin. The experiment was repeated three times, and the data presented represents a typical experiment. (C) Total fluorescence intensity values for macrophage markers as assessed by microarray analysis. The data represent an average of at least two determinations obtained from the Agilent Chip. (D) Fold increase in fluorescence intensity for each macrophage marker as assessed by microarray analysis.

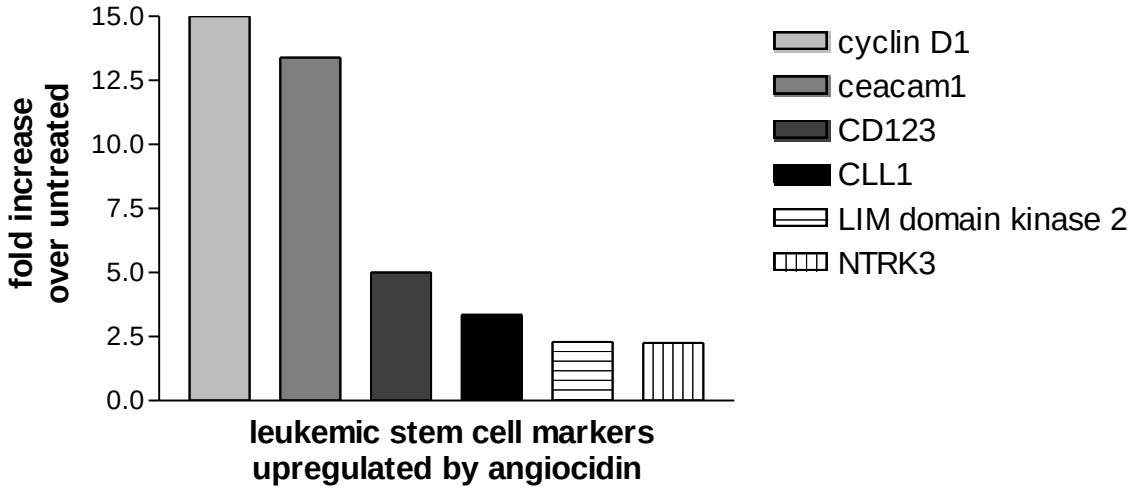


Figure 2 Angiocidin up-regulates leukemic stem cell markers in THP-1 Leukemic Cells treated with angiocidin. THP-1 Leukemic cells were treated with 10 μg/ml for 24 hours. Microarray of treated and untreated cells revealed the up-regulation of several markers specific to leukemic stem cells.

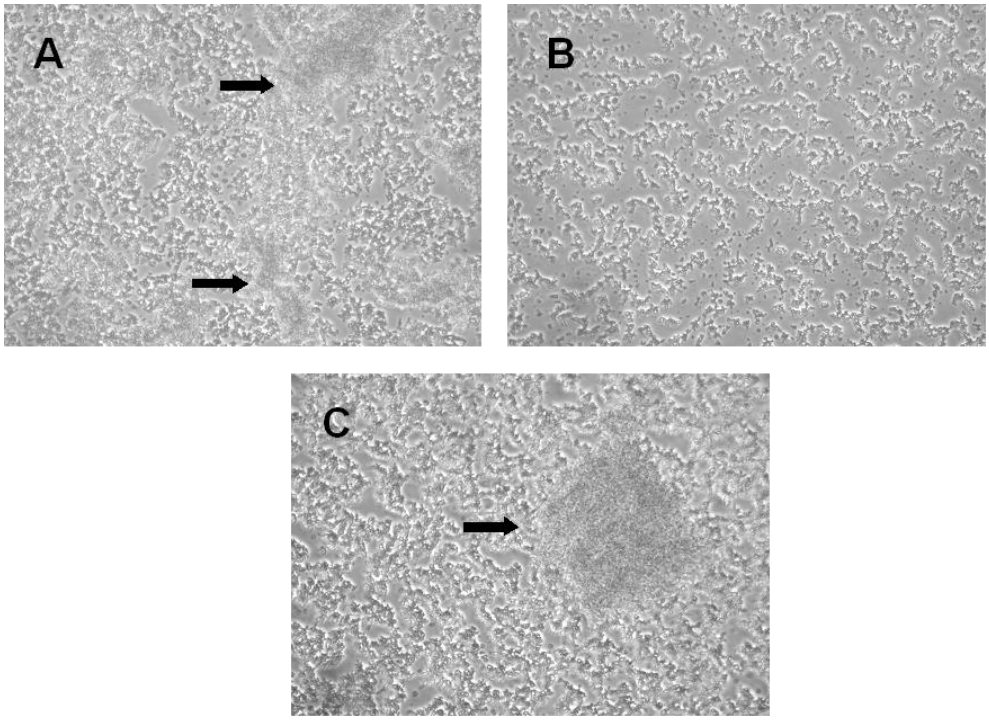
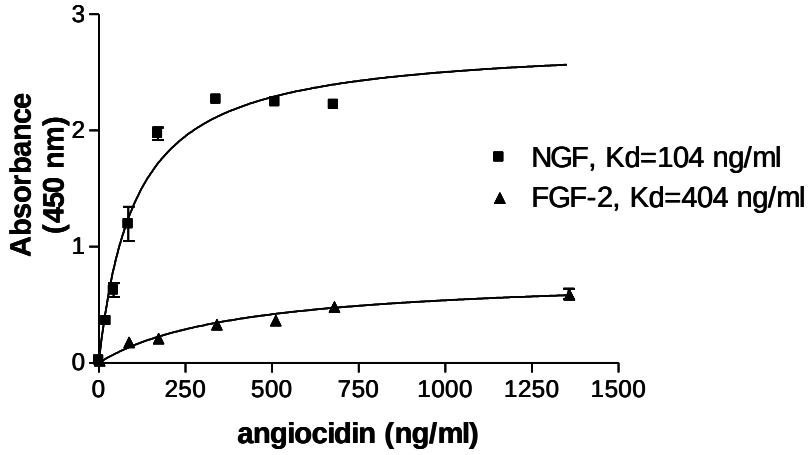


Figure 3 The effect of angiocidin and growth factors on the differentiation and proliferation of Blastomere-Like Stem Cells (BLSCs) isolated from Human Blood.

A



B

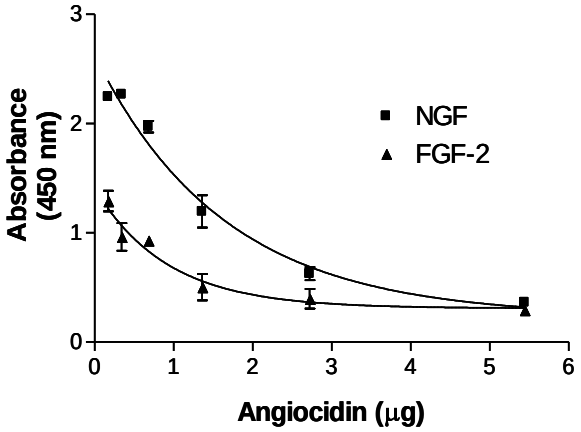


Figure 4: Direct binding of angiocidin to adsorbed NGF and FGF-2. Briefly, In a 96-well plate, 1 ng of NGF or FGF-2 in a 20mM HEPES buffer was adsorbed to the well in duplicate overnight at 4°C. Remaining buffer was aspirated and the wells were washed with phosphate-buffered saline (PBS). Then, the plate was blocked in 1% (w/v) BSA in PBS for 30 minutes without shaking. Aliquotes in a 20mM HEPES buffer containing various dilutions of biotinylated angiocidin prepared according to Sabherwal Y., Rothman, V.L., Svetoslav, D, L'Heureax D. Z., Marcinkiewicz, C., Sharma, M., and Tuszynski, G.P., Integrin $\alpha 2\beta 1$ mediates the anti-angiogenic and anti-tumor activities of angiocidin, a novel tumor-associated protein, Experimental Cell Research, 312: 2443-2453, 2006.

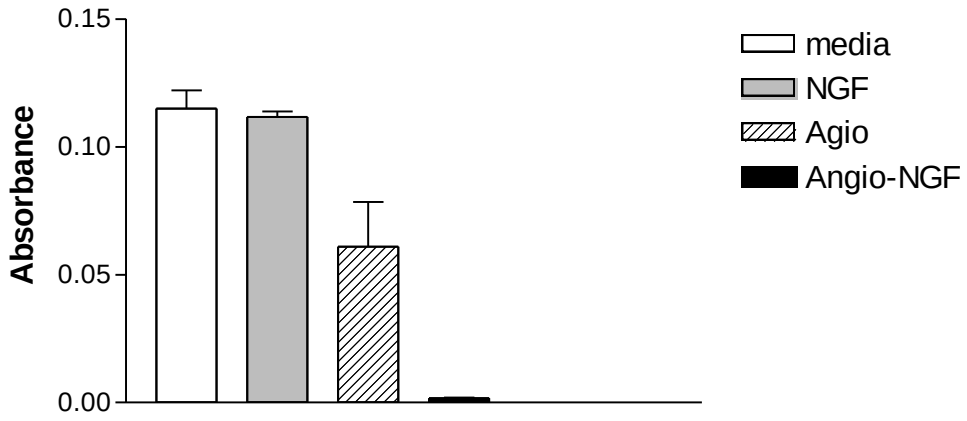


Figure 5: The effect of angiocidin and NGF on MDA MB231 Breast Carcinoma proliferation.

Research Project 16: Project Title and Purpose

Diversification of Streptococcus pyogenes during Persistence - The bacteria *Streptococcus pyogenes* causes many human diseases including pharyngitis (strep throat), impetigo (skin infection), streptococcal toxic shock syndrome and necrotizing fasciitis. In addition, after these acute diseases have been cured, post-streptococcal sequelae can develop that affect the heart (rheumatic fever) and kidneys (glomerulonephritis). To cause this myriad of diseases, *S. pyogenes* produces many virulence factors. When *S. pyogenes* is isolated from a patient, the strains are often not the same. This diversity of strains makes it difficult to determine which virulence factors are contributing to which diseases. The goal of this project is to determine, using laboratory models, how *S. pyogenes* diversify into unique strains during slow growth in stationary phase or inside human (eukaryotic) cells.

Duration of Project

1/30/2009 – 3/29/2010

Summary of Research Completed

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

Research Project 17: Project Title and Purpose

Extended Chemosensitization with a Novel Small-Interfering RNA (siRNA) Sustained Release Nanosystem - The overall purpose of this project is to use the recent advances in nanotechnology to develop an effective and safe siRNA-based therapeutic strategy for drug-resistant ovarian cancers. Ovarian cancers are frequently resistant to standard chemotherapy. siRNA are a new

class of therapeutic molecules that may provide a novel means to improve the effectiveness of chemotherapy; however, current use of siRNA for clinical purpose is largely limited by its short action, potential toxicity and inefficient delivery. This project will develop a novel platform that is capable of substantially prolonging the siRNA activity with reduced adverse effects. This may help translate this promising new treatment into a clinically useful form of treatment for a cancer that is normally refractory to the current standard drug therapy.

Duration of Project

1/30/2009 – 12/31/2009

Summary of Research Completed

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

Research Project 18: Project Title and Purpose

Using an IVR-Cellular Telephone System to Improve Outcomes in Chronic Obstructive Pulmonary Disease - We intend to extend our findings of reducing the incidence and severity of Chronic Obstructive Pulmonary Disease (COPD) exacerbations by using a PDA-computer based management system to one that uses an *interactive bidirectional cell or land-based phone technology*.

Anticipated Duration of Project

1/30/2009 – 12/31/2011

Project Overview

In this project, we intend to extend our findings of reducing the incidence and severity of COPD exacerbations by using a PDA-computer based management system to one that uses an *interactive bidirectional cell or land-based phone technology*. Our primary specific aim is that that this method will decrease the time to the first COPD exacerbation. Our secondary specific aims are that we believe that this method will: 1) enhance patient acceptance of self monitoring, 2) improve communication between patients and providers, and 3) provide a platform for the generalization of our management paradigm to the COPD patient population at large. Finally, we intend to use the information from this pilot project as preliminary data to: 1) submit an application via the NIH RO1 funding mechanism to demonstrate that this method decreases the frequency of COPD exacerbations in a multicenter prospective and randomized 1- year trial and; 2) prepare a proposal to the PA-DOH Medical Assistance program to pilot this program as a disease management program for COPD patients having repeated hospital admissions, thereby reducing the costs and morbidity of repeated COPD exacerbations.

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Expected Research Outcomes and Benefits

Although this project is designed as a proof-of-concept study, we will track all hospital admissions and exacerbations of either COPD as a measure of efficacy. Data from previous studies in our center on similar populations will provide a basis for historical controls. Based on our Web-based telemedicine studies, we expect to find a reduction of hospital days and a reduction of acute exacerbations of COPD.

Summary of Research Completed

The focus for this reporting period was participant recruitment from our outpatient clinics. Since the inception of the program we have enrolled 109 patients into the $COPD_{phone}$ system. In the last year twenty-nine patients were enrolled; 18 in the $COPD_{phone}$ study and eleven using the system as part of their clinical care. Enrollment figures are presented in Table 1 below.

All but three of the study participants have completed the initial assessment survey. These three have the survey and will return it at their next visit. Two participants have completed the 2-year follow-up survey, but there is insufficient data available to begin a useful analysis. The remaining study patients will submit the final assessments over the next few months after which we will actively begin complete data analysis. The daily symptom reports should provide the most useful information and permit us to look at how using the system affects exacerbation rates, hospitalizations, and need for other services. The surveys for those using the phone system are being refined and will provide important additional information.

The two-year follow-up period was chosen to provide a robust dataset and to demonstrate patient acceptance of the system over an extended period. However, the two-year reporting period has had the unintended consequence of limiting patient enrollment. Many patients who were otherwise qualified for the study were reluctant to commit to two years of symptom reporting. Because of this, each was offered the opportunity to use the telemedicine system as part of their clinical care.

There are 52 active users of the telemedicine system. All patients report their symptoms, which

are reviewed daily by the study coordinator. COPD exacerbations are identified through symptom reports and interventions are made as clinically necessary. There are two differences between the clinical care patients and those enrolled in the study. First, the clinical care patients do not complete quality of life questionnaires or other study-related surveys. Second, the regularly scheduled office visits required by the study are replaced by office visits at intervals determined by the physician's clinical judgment.

Many of the patients who did not want to participate in the $COPD_{phone}$ study because of the two-year commitment to use the reporting system have been using the system for a year or longer. Anecdotally we have learned that 3 of the patients were able to travel outside of the United States because they felt that they had a lifeline in daily reporting and that someone was monitoring their health. Other individuals have reported that they are much more comfortable in traveling outside of the Philadelphia area. Also, we had 3 patients who enrolled in the program who stopped reporting for a time, but asked to re-enter the program because it was a comfort to them.

The unsolicited reports made by telemedicine users has prompted us to take steps to ensure that we do not lose the information generated by patients using the system as part of clinical care. We are developing protocols that will be submitted to the Temple University IRB to retrospectively review the daily electronic diary reports, as well as clinical and demographic information from these patients. All of this information currently exists as part of the patient's medical record and will be extracted and analyzed as de-identified data. We have also developed a survey to collect patient satisfaction with the system. Participation in this survey will be voluntary.

Table 1.

Enrollment in the $COPD_{phone}$ Telemedicine Program			
	Last year	**Since inception**	**Current active users**
$COPD_{phone}$ study	18	21	19
$COPD_{phone}$ clinical care	11	88	33
Total	29	109	52

Research Project 19: Project Title and Purpose

Immunotherapeutic Strategies for Alzheimer's Disease - A promising and understudied animal model of Alzheimer's disease (AD) is the cholesterol-fed rabbit. In the last three years, we replicated the demonstration that a rabbit model of AD carries a number of AD neuropathologies and is impaired in associative learning and extended the AD rabbit model to the domain of therapeutics by demonstrating efficacy of galantamine (Razadyne™) in ameliorating learning impairment in these rabbits. Whereas drugs such as Razadyne™ treat cognitive impairment in AD, their efficacy is modest. Immunotherapy may have the potential to prevent the development of AD in later life as well as to treat and reverse symptoms. Using AD model rabbits, we aim to test immunotherapeutic strategies that will maximize humoral (antibody) immune responses while minimizing proinflammatory responses.

Duration of Project

1/30/2009 – 12/31/2010

Project Overview

Our initial data indicate that immunization of cholesterol-fed and control rabbits with β-amyloid (Aβ) had efficacy when administered beginning 6 or 8 weeks after the cholesterol/copper diet was initiated. However, the data on the timing of onset of Aβ and other Alzheimer's disease (AD) neuropathology in the AD rabbit model are limited. We need to determine if we can prevent or reduce Aβ and other AD neuropathology using Aβ immunotherapy. Therefore, *Specific Aim 1* will characterize the development of neuropathology over a 10-week period of cholesterol/copper diet. *Specific Aim 2* will determine whether a noninvasive behavioral measure is associated with onset and development of AD neuropathology. *Specific Aim 3* will focus on the development of efficacious immunotherapeutic strategies as well as the optimal timing of immunotherapy using behavioral- and neuropathological-dependent measures. Our goal is to design an immunization strategy that promotes antibody responses through preferential differentiation and enhanced survival of helper T cells (T_H2) in lieu of T_H1 cells that produce proinflammatory cytokines.

Alzheimer's Disease Rabbit Model. Young (3-4 months) male New Zealand white specific pathogen free (SPF) rabbits and female retired breeder will be tested. *Dietary Regime.* Rabbits will be fed for 10 weeks (or the number of weeks specified in the research design) with 160 g/day of a commercially produced diet (Test Diet 7520) of 2% cholesterol added to Purina Mills High Fiber Diet. Control rabbits will be fed 160 g/day of Purina Mills High Fiber Diet. For experimental rabbits, 0.12 mg/liter copper sulfate will be added to distilled drinking water. Control rabbits will receive distilled drinking water.

Specific Aim 1. A total of 40 rabbits (20 young adult males, 20 retired breeder females a minimum of 24 months old) will be studied to characterize AD pathology development during the period that the animals are fed the cholesterol/copper diet. Brains will be examined for pathology in sections in frontal, temporal, parietal, and occipital cortex, hippocampus, and cerebellum and stained with H & E and immunostained for Aβ, tau, apoptosis, and gliosis.

Specific Aim 2. The same 40 rabbits used in Aim 1 will receive five days of training in eyeblink classical conditioning five days before they are euthanized. Training will follow procedures approved by the Temple IACUC and reported previously for AD model rabbits. After training is completed, rabbits are euthanized, perfused through the heart, and brains are collected for histology as described above.

Specific Aim 3. Based upon the neuropathology profile of young male and retired breeder female AD model rabbits (Aim 1), we will begin immunizing cohorts of AD model rabbits with β-amyloid 1-42 peptide (Aβ1-42). Animals will be divided into two experimental adjuvant cohorts to allow us to compare: (1) conventional Freund's adjuvant; and (2) VIP anti-inflammatory neuropeptide. Moreover, we will assess the efficacy of prophylactic immunotherapy using these adjuvant regimes as well as immunotherapy efficacy at two time points post-pathology onset.

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Expected Research Outcomes and Benefits

Immunotherapy may have the potential to prevent the development of AD in later life as well as to treat and reverse some of its symptoms in AD patients. The rabbit model of AD, arguably the most valid of all existing animal models of AD, has not been used to test immunotherapeutic strategies, yet the rabbit has a close amino acid sequence to human Aβ. The concurrent risk of AD and cerebrovascular dementia, cardiovascular disease, diabetes, and other age-related chronic diseases make the rabbit model of AD a promising shared resource among investigators at Temple University School of Medicine. In addition to studies of immunotherapy, tissues from AD model rabbits could be used as a Core bioresource for a Program Project proposal focusing on Alzheimer's disease and related disorders. Thus, this proof-of-concept proposal using the AD rabbit model has significant translational potential for treatment and prevention of AD in humans. By extension, this potentially high impact work would position Temple University as a major contributor to the global research effort to quell the morbidity and mortality associated with neurodegenerative diseases such as AD.

Summary of Research Completed

During this reporting year when all data collection has been completed, we addressed the three specific aims of the project by analyzing data and preparing presentations at scientific meetings. Development of neuropathology in the hypercholesterolemic rabbit is the focus of Aim 1, and we have an abstract accepted at the Alzheimer's Association International Congress of Alzheimer's Disease (AAICAD) on this topic. Our presentation, entitled *"Progression of Alzheimer's Disease Pathology in Hypercholesterolemic Rabbits"* is scheduled on July 17, 2011 at the AAICAD meeting in Paris, France. We will also report data from Aim 2 on eyeblink classical conditioning during pathology development in this presentation. A second presentation at AAICAD, also on July 17, 2011 addresses Aim 3 of this project – the effect of immunization in this animal model of AD. That presentation is entitled, "*Immunotherapy in the Hypercholesterolemic Rabbit Model of Alzheimer's Disease*."

Progression of Alzheimer's Disease Pathology in Hypercholesterolemic Rabbits

The major aim of this research was to characterize further development and progression of AD neuropathology over 12 weeks of ingestion of a cholesterol/trace copper diet that causes AD neuropathology to develop in normal rabbits free from pathology before diet onset. Rabbits have a closer phylogenetic proximity to primates than do rodents, and this closer relation is expressed in the amino acid sequence of beta amyloid (Aβ), which is identical to the human sequence. Also, rabbits have an extensively characterized profile on a measure of learning and memory that closely parallels human performance and that is impaired in human patients diagnosed with AD: Eyeblink classical conditioning (EBCC). Normal older adults are impaired in EBCC compared to young adults, but all age groups of normal, non-demented adults, including adults in their 80s and 90s, show clear evidence of associative learning. In probable AD, there is very limited EBCC in this 30 min test. EBCC has utility in identifying patients with AD early in disease onset. In human AD and in AD model rabbits, EBCC is impaired beyond the level of normal controls. In two separate studies, we examined the effect of various durations of the cholesterol/trace copper diet on the integrity of the blood brain barrier (BBB) and on the development of intracellular Aβ, and extracellular Aβ plaques as well as EBCC. Our aims were to determine when the BBB became compromised and to assess the magnitude of brain Aβ disposition in the hippocampus at various time intervals after cholesterol/trace copper diet onset.

Method: For BBB studies there were four 4-mo SPF New Zealand white rabbits tested 4 weeks after cholesterol/trace copper diet onset and four rabbits tested after 10 weeks on the diet. Changes in BBB permeability were assessed using the fluorescent tracer, sodium-fluorescein (Na-F). To assess BBB permeability, the tissue was homogenized using a 40 ml Dounce tissue grinder. The homogenate was centrifuged for 10 min at 13000xg and the supernatant was neutralized with 5 M NaOH (1:0.8). Measurement of Na-F fluorescence was determined at excitation/emission wavelengths of 440/525 nm using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA). Fluorescent dye content was calculated using external standards (range of 10-200 ng/ml), and data were expressed as amount of tracer per gram of tissue. For intracellular brain Aβ and Aβ plaque determination, the research design is shown in Table 1. Rabbits received the cholesterol copper diet in periods varying from 2 to 12 weeks. Immunohistochemistry was used to evaluate brain pathology.

Results: The integrity of the BBB was compromised in AD model rabbits treated for 4 weeks as well as at 10 weeks. On average, there was about a 1.6 fold increase in permeability in animals treated for 4 weeks compared to controls. Over four times the amount of sodium fluorescein was detected in the brains of AD model rabbits treated for 10 weeks on the diet. Our results with 8 rabbits indicate progressive impairment of BBB over the 10-week course of diet administration, with a 1.6 fold increase in permeability at 4 weeks and over a 4 fold increase in permeability at 10 weeks. Intracellular brain Aβ also increased with disease progression as shown in Figure 1. Intracellular Aβ was significantly higher in hippocampus in rabbits treated for 12 weeks than for rabbits treated for 2 weeks. In spite of the developing neuropathology, EBCC was not different in rabbits exposed longer to the diet.

Conclusions: The BBB is impaired in AD model rabbits, and brain Aβ content increases with longer time on the cholesterol/trace copper diet. Despite increases of Aβ pathology and BBB impairment, there were no changes in EBCC performance.

Immunotherapy in the Hypercholesterolemic Rabbit Model of Alzheimer's Disease

From the standpoint of the Aβ proteins that are the central pathogenic cause of neurodegeneration in AD, rabbit models have a definite advantage. The rabbit's closer phylogenetic relation to primates is expressed in the amino acid sequence of Aβ, which is almost identical to the human sequence. As a species for cognitive assessment, rabbits are unique among AD animal models because of the large body of parametric data on the classically conditioned eyeblink response. Disruption of the brain cholinergic system in AD links this dementing disease to the model system of eyeblink conditioning in mammals, including humans. The well-documented impairment of delay eyeblink classical conditioning in AD may reflect medial-temporal lobe atrophy and associated central nervous system cholinergic dysfunction that occurs early in the disease progression. The cholesterol-fed rabbit model of AD with its close parallels to human genetics and physiology, along with its validity as a model of human AD from molecular to cognitive levels, provides an interesting vehicle for testing immunotherapies for AD. Experiment 1 (E-1) addresses immunotherapy, brain Aβ and learning assessed with eyeblink classical conditioning (EBCC) and Experiment 2 (E-2) focuses on the potential of an immunotherapeutic regimen to prevent accumulation of brain Aβ and impairment in EBCC. In both studies hypercholesterolemic rabbits were tested in 750 ms trace and 750 ms delay EBCC eyeblink classical conditioning procedure in the last two weeks of the cholesterol/trace copper AD pathology-inducing diet.

Method: In E-1 and E-2, rabbits were fed a cholesterol/trace copper diet for 10 weeks. In E-1, 4 groups of rabbits were immunized 5X (Table 2). In E-2, rabbits were inoculated 4X over a 10-week period before the cholesterol/trace copper diet (Table 3). Rabbits were immunized with $A\beta_{1-42}$ peptide coupled to KLH administered sc and im (7 injections, 100μg in 0.1 ml). CFA was used as adjuvant in the primary injection. IFA was used in all subsequent injections. Rabbits were bled one day prior to each injection and at euthanasia. Plasma was analyzed by ELISA for anti-Aβ antibodies. Brains were rapidly removed, fixed, and immunostained for Aβ (Clone 6E10, Covance SIGNET line) in 4 μm sections from frontal, temporal, parietal, and occipital cortex and cerebellum. A PaxCam3 Digital Camera System interfaced with a Nikon LabPhot microscope (20x objective) and PC was used to carry out optical digital analysis of specified saturation and pixel densities (PaxIt Enhanced Measurement/Image Analysis Module). The amount of pathology (intercellular Aβ, extracellular Aβ plaques) in a representative selected field of equal size for each rabbit was assessed. Analyses were carried out blind with regard to treatment. After quantification, data were saved in Excel, transferred to SPSS, and statistically analyzed by treatment group.

Results: Results of ELISAs for both E-1 (Figure 2) and E-2 (Figure 3) indicate that cholesterol-fed rabbits treated with Aβ immunotherapy generated anti-Aβ responses. In E-1 concentration of plasma anti-Aβ antibodies in inoculated young male rabbits was lower than in non-inoculated young male rabbits. In older female rabbits anti-Aβ antibodies increased with and without inoculation. The increase in plasma anti-Aβ antibodies was numerically greater in young male than retired breeder inoculated rabbits. Comparison of the acquisition of conditioned responses (CRs) between inoculated and non-inoculated AD model rabbits in E-1 indicated that the inoculations did not affect behavior for either young male or retired breeder female rabbits. Previously we have demonstrated that statistically significant age differences in acquisition of CRs. This result also occurs in AD model rabbits, with younger male AD model rabbits learning

more rapidly and to a higher asymptote than retired breeder female AD model rabbits. Male and female rabbits of the same age perform equally well on eyeblink conditioning, so we interpret the differences between young male and older female AD model rabbits as an age rather than a gender effect. Even with inoculations occurring in advance of pathology in E-2, there was not a significant difference in acquisition of CRs in inoculated rabbits. Comparisons between brain Aβ plaques in immunized and non-immunized rabbits in E-1 indicate that there is a numerical reduction in inoculated female retired breeder AD model rabbits but not in inoculated young male AD model rabbits. The amount of brain Aβ in inoculated female retired breeder rabbits is about 86% of the brain Aβ in older female rabbits that were not inoculated. The amount of brain Aβ in inoculated young male rabbits actually exceeded the amount in non-inoculated rabbits. A major difference between the present study and a previous study in which we observed significant differences in brain Aβ in inoculated young male AD model rabbits was the interval between inoculations. Brain Aβ was reduced when we inoculated biweekly, whereas weekly inoculations in the present study did not reduce brain Aβ in young males. The longer interval between inoculations appears to be a key factor in optimizing treatment, at least in the young male AD model rabbits. In E-2, with longer intervals between inoculations that were given before the AD pathology-inducing diet, reductions in Aβ were observed.

Conclusions: Inoculation of young male AD model rabbits with $A\beta_{1-42}$ peptide coupled to KLH resulted in an increase in plasma anti-Aβ antibodies in E-1 and E-2, whereas in older female rabbits anti-Aβ antibodies increased with and without inoculation. The increase in plasma anti-Aβ antibodies was numerically (but not significantly) greater in young male than retired breeder inoculated rabbits. Aβ plaques in inoculated retired female breeder AD model rabbits were reduced numerically. Longer intervals between inoculations appear to increase the magnitude of brain Aβ reduction. Acquisition of conditioned eyeblink responses was not different between inoculated and non-inoculated young and older rabbits either in E-1 or E-2. These results with a behavioral assessment of learning that is of documented impairment in human AD are consistent with recent studies in Aβ-inoculated human AD patients and canines. Reduction of brain Aβ resulting from immunization in humans and canines did not ameliorate impaired cognitive processes. These initial studies suggest a number of additional avenues of research using the hypercholesterolemic rabbit model. At this early stage of research using this model we can conclude that the cholesterol-fed rabbit model of AD with its close parallels to human genetics and physiology, along with its validity from molecular to cognitive levels as a model of human AD, provides a promising vehicle for development of immunotherapies.

Table 1. *Research Design - Number of Rabbits Beginning Cholesterol/Trace Copper Diet by Week During the Study*

Week in the Study	Number of Rabbits Beginning Diet	Control Rabbits Fed Normal Diet (n = 2)
1	0	
2	3	
3	2	
4	3	
5	2	
6	3	
7	2	
8	3	
9	2	Euthanized 2 Control Rabbits
10	0	
11	0	
12	3	
13	Euthanized 23 AD Model Rabbits	

Table 2. *E-1: Immunotherapy, Brain Aβ and Classical Eyeblink Conditioning*

Rabbit Group	***n***	**1st Inoculation (CFA)[1]**	**4 Boosts (IFA)[2]**	**EBCC**	**Euthanized**
4 mo Male AD model	13	4 wks after diet onset	Every wk	wk 9-10	11 wks
4 mo Male AD model	14	None	None	wk 9-10	11 wks
32 mo Female AD model	13	4 wks after diet onset	Every wk	wk 9-10	11 wks
32 mo Female AD model	13	None	None	wk 9-10	11 wks

[1] CFA – Complete Freund's adjuvant; [2] IFA – Incomplete Freund's adjuvant

Table 3: *E-2: Immunotherapy Before Neuropathology for Prevention of AD*

Rabbit Group	***n***	**1st Inoculation (CFA)[1]**	**3 Boosts (IFA)[2]**	**EBCC**	**Euthanized**
4 mo Male AD model	10	10 wks before diet onset	Biwkly	wk 19-20	21 wks
4 mo Male AD model	10	None	None	wk 19-20	21 wks

[1] CFA – Complete Freund's adjuvant; [2] IFA – Incomplete Freund's adjuvant

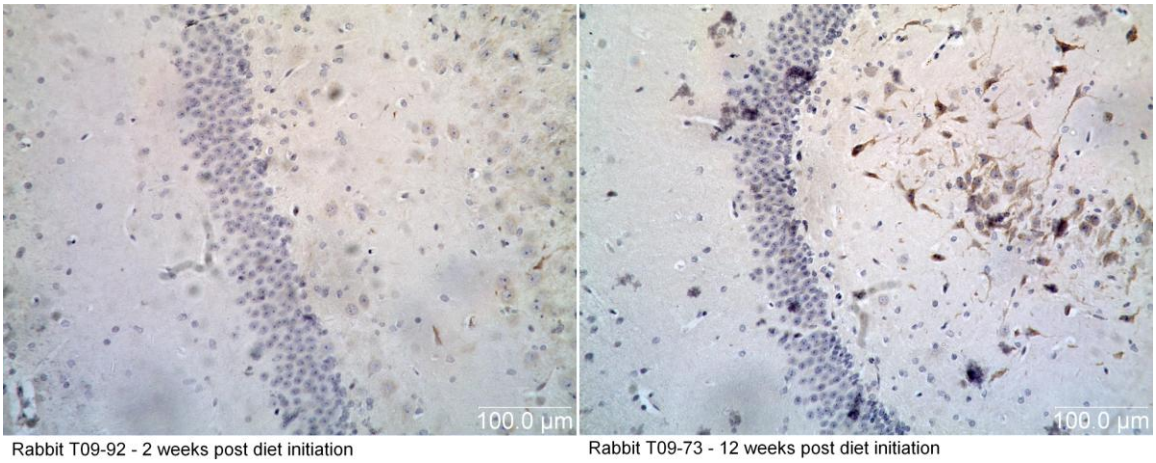

Figure 1. Staining with antibody 6E10 against Aβ in hippocampus of a rabbit treated for 2 weeks on the cholesterol/trace copper diet (left) and a rabbit treated for 12 weeks on the diet (right). Increased staining of Aβ is evident in the rabbit treated for 12 weeks.

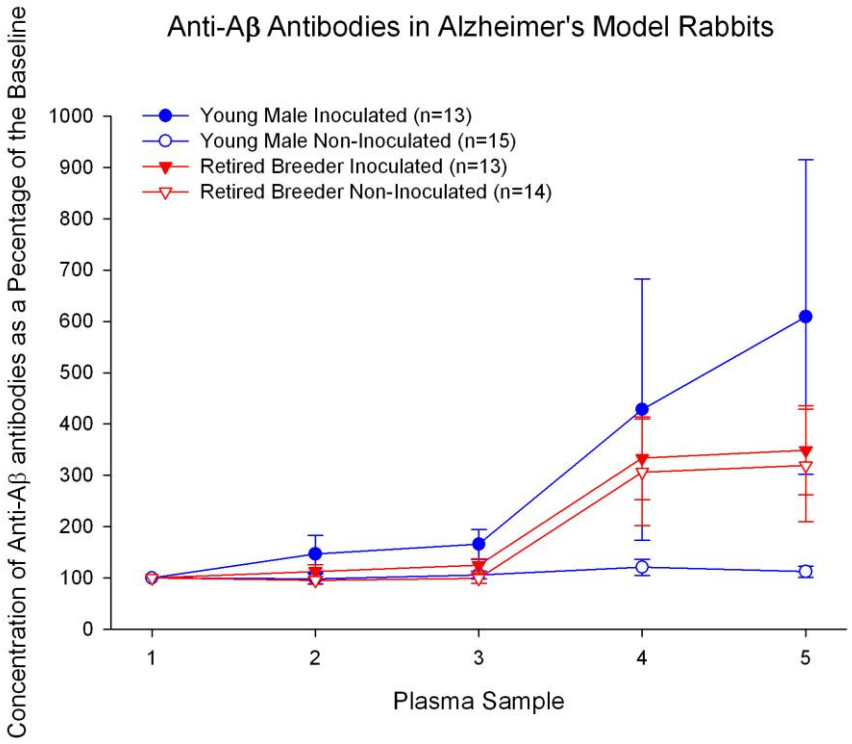

Figure 2. Concentration of plasma anti-Aβ antibodies as a percentage of baseline in young male and retired breeder female hyper-cholesterolemic rabbits that were inoculated or not inoculated five times.

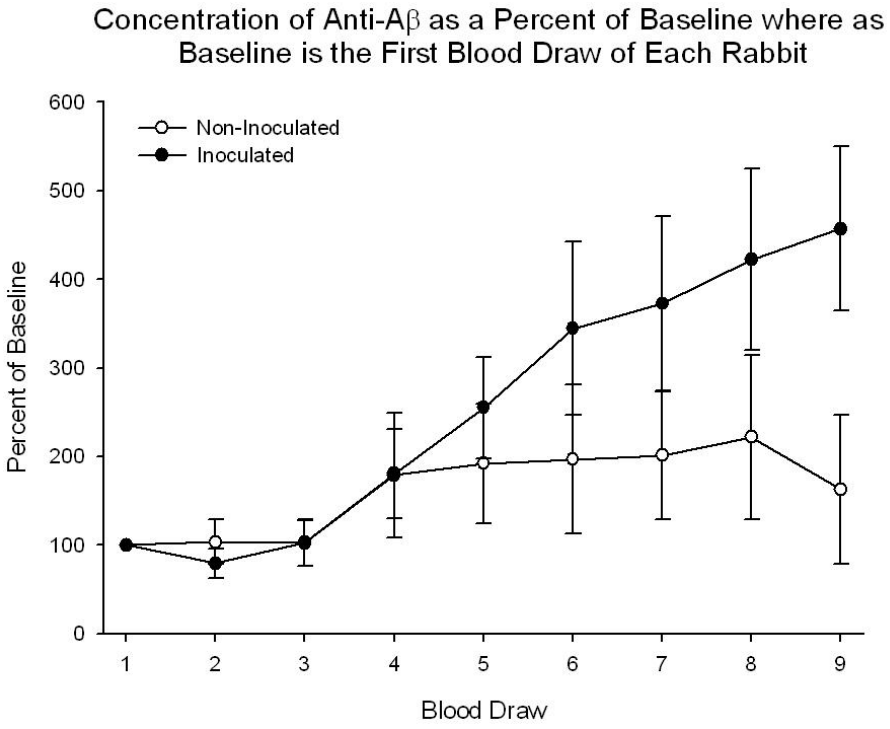

Figure 3. There was a statistically significant increase in anti-Aβ antibodies in rabbits inoculated biweekly with one CFA and 3 IFA administrations of $A\beta_{1-42}$ peptide coupled to KLH.

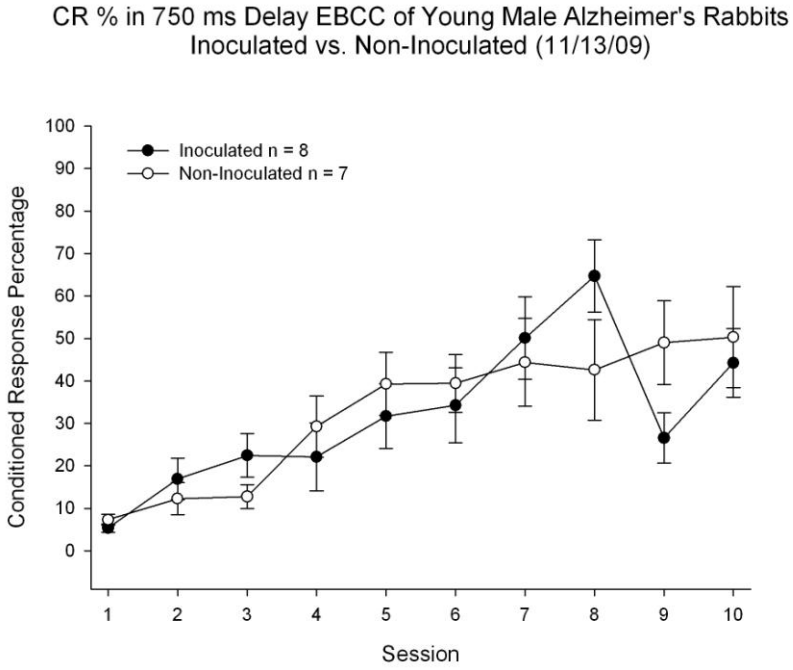

Figure 4. Eyeblink classical conditioning in the 750 ms delay eyeblink classical conditioning paradigm was tested in weeks 10-20 of the experiment, 9-10 weeks after diet initiation. Inoculations of $A\beta_{1-42}$ peptide did not ameliorate impaired eyeblink conditioning.

Research Project 20: Project Title and Purpose

Improving Biomedical Informatics Support at Temple Health Sciences Center - An increasing number of basic and clinical studies at Temple University require merging and analyzing a large volume of biomedical data collected from a variety of sources. Mastering appropriate informatics skills is one of recognized current challenges and is at the forefront of personalized medicine development. The purpose of the project is to provide informatics support within Temple Health Sciences Center (THSC) that will correct interoperability among existing clinical, molecular and other data resources and allow more efficient and potentially more accurate inference for the purpose of understanding disease states and pharmacotherapies. In particular, we propose *developing effective tools for biomedical data management, retrieval and data mining and providing informatics and analytical help as to efficiently support comprehensive translational research within THSC.*

Anticipated Duration of Project

1/30/2009 – 12/31/2011

Project Overview

Aim 1: Providing data management support for integration of genomic, molecular, and clinical information and creating framework for mining integrated biomedical data**.**

Implementation:
(a) *Developing and hosting biomedical websites:* Experience gained on developing and hosting DisProt and a dynamic website will be used towards developing and hosting biomedical websites to promote and facilitate research at other THSC laboratories.

(b) *Providing access to external biomedical data:* Data of interest to THSC investigators will be extracted from external genome, proteome, genotype, bioinformatics and other public biomedical data repositories.

(c) *Developing a data warehouse:* Given multiple databases our objective will be to develop a data warehouse that allows investigators to securely query de-identified data stored at these systems. Data from the clinical, administrative, molecular and other systems will be transformed and loaded into the repository.

(d) *Developing advanced analysis tools for constructed data warehouse*: Various data mining methods will be implemented to support knowledge discovery in the constructed data warehouse. State of the art methods will be implemented for association analysis, cluster analysis and anomaly detection.

Aim 2: Continuing expired bioinformatics support and introducing additional bioinformatics services at THSC aimed at expanding into clinical and translational medicine.

Implementation:
(a) *Providing access to bioinformatics and other analytical software:* Access and training will be provided to various bioinformatics software tools related to sequence database, analysis, and management, pathway analysis, protein structure prediction, analysis and visualization.

(b) *Analysis of protein structure*: We will analyze amino acid sequences to predict if a protein is fully ordered or it is likely to contain intrinsic disordered regions. For ordered proteins, we will predict their structure using tools developed elsewhere, while protein disorder will be characterized using our own methods.

(c) *High throughput data analysis:* Steps to be supported for a typical gene expression study include (i) Image processing; (ii) Identification of differentially expressed genes; (iii) Control of false discovery rate; and (iv) Pathway analysis.

(d) *Fusion of clinical, molecular and environmental data*.

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None

Expected Research Outcomes and Benefits

The project will immediately result in a much stronger informatics component of the Keystone Biomedical Informatics Resource and so will benefit a Clinical and Translational Science Awards grant proposal currently in preparation by a large group of investigators from Temple Health Sciences Center jointly with collaborators at Fox Chase Cancer Center and Geisinger Health System.

Other expected outcomes in the next 2-year period will include informatics support for ongoing and planned projects at Temple Health Sciences Center that have bioinformatics tasks defined within their specific aims.

Summary of Research Completed

In this period we have continued some collaborations with basic medical, clinical and translational projects within Temple Health Sciences Center and established several new

collaborations.

Privacy-preserving Decision Making in Clinical Settings

Prevention of identity information leak and preservation of privacy are important security concerns in clinical data transactions. An end-to-end framework using a graph-based approach that addresses both these concerns is proposed in our study. Our framework is motivated by the emergence of grid-based and standards-based (eg: HL7) distributed systems that are graph structures at an abstract level and the flexibility of graphs in capturing structural information. The lifecycle of a clinical query transaction in the context of evidence-based medicine is modeled and a two stage distributed knowledge-mining tool is developed to support a decision-making query in a clinical setting over multiple sites. Experimental validation using data distributed among four sites provides the initial feasibility evidence of the proposed two stage method that involves 1) generating a global view of the distributed graph based on a query and 2) distribute statistics gathering for decision making without actual data exchange. The results of this project are published at Mathew, G., Obradovic, Z. "A Privacy-preserving Framework for Distributed Clinical, *Proc. IEEE International Conference on Computational Advances in Bio and Medical Sciences,* January 2011, Orlando, Florida.

Analysis of Regulatory Elements in Maternal mRNA Sequences

As a result of collaboration with Dr. K. Latham's group, a paper submitted in 2010 has since been published (Potireddy S, Midic U, Liang CG, Obradovic Z, Latham KE, "Positive and negative cis-regulatory elements directing postfertilization maternal mRNA translational control in mouse embryos," *Am J Physiol Cell Physiol*. 2010 Oct;299(4):C818-27).

We continued cooperation on a related project with another PhD student from Dr. Latham's group. We extended the methodology previously used to search for regulatory linear motifs, to cluster them into more complex motifs, which have greater coverage (sensitivity) than the individual motifs. This study is still ongoing, and it was part of one doctoral thesis in Dr. Latham's group.

Our collaboration with Dr. Latham's laboratory in this period also resulted in another published paper (Lee YS, Vandevoort CA, Gaughan JP, Midic U, Obradovic Z, Latham KE, "Extensive effects of in vitro oocyte maturation on rhesus monkey cumulus cell transcriptome," *Am J Physiol Endocrinol Metab*. 2011 Apr 12.). We designed an automated procedure which, given a set of protein sequences from rhesus monkey (the focus of the study), searched for homologous sequences in a large number of other genomes, to produce empirical evidence for conservation of those genes across species.

Recently, we started collaborating on a new project, which includes analysis of microRNA expression analysis. MicroRNAs are short RNA molecules that bind to targets in messenger RNAs (mRNAs), which results in translational repression or gene silencing. MicroRNA studies can effectively complement gene expression and SNP studies, because they can explain important phenomena that cannot be detected by other types of studies. This project will give us valuable hands-on experience with this kind of data.

Temporal Analysis of Social Networks of Alcohol Abuse

The analysis of social networks often assumes time invariant scenario while in practice actor attributes and links in such networks often evolve over time and are inextricably dependent on each other. We developed a new method to predict actor attributes and links in temporal networks. Our approach takes into account the attributes corresponding to the participating actors together with topological and structural changes of the network over time. This is achieved by building two conditional predictors to jointly infer links and actor attributes. The proposed prediction method was significantly more accurate than alternatives when applied to prediction of alcohol consumption in group of teenagers from the same school observed over time. A paper on this study is currently in press (Ouzienko, V., Guo, Y. and Obradovic, Z. "A Decoupled Exponential Random Graph Model for Prediction of Structure and Attributes in Temporal Social Networks," *Statistical Analysis and Data Mining*).

Analysis of the Plasma Proteome in Advanced Chronic Obstructive Pulmonary Disorder

In collaboration with Dr. Salim Merali and Dr. Steven Kelsen we are working on analysis of the plasma proteome in advanced Chronic Obstructive Pulmonary Disorder (COPD) patients. In this study a test group of 15 patients with advanced COPD is compared to a control group of 15 ex-smokers with less severe COPD, and four candidate biomarkers of advanced COPD were identified. Our task was to estimate the predictive power of these candidate biomarkers, as well as their combination, for which we used logistic regression as the predictive model and the ROC (receiver operating characteristic) and AUC (area under curve) to measure the predictors' performance.

We also had to deal with the issues stemming from the small size of the dataset. We developed bootstrapping methodology that produced 95% confidence interval for AUC and 95% confidence bands for ROC. The experiments have shown that using three of four biomarker candidates together gives the best prediction results, while the fourth candidate biomarker was shown to be unreliable for prediction of COPD. The whole study (with our contribution) is described in the paper "Analysis of the Plasma Proteome in Advanced COPD: Identification of Candidate Biomarkers" (in review).

Analysis of Drug Abuse Synergism

In collaboration with Dr. Ronald Tallarida we analyzed literature related to drug abuse synergism. PubMed (http://www.pubmed.org/) is a database of abstracts and papers on biomedical and related topics, containing over 20 million records. Initially, we started this as a text mining project, with plans to develop a classifier that would help search for papers in PubMed that treat drug synergism with a very specific methodology. After analysis of PubMed corpus, we decided that the text mining approach is not viable. The results of the analysis also gave an important insight into the state of research in the drug synergism field, and are the focus of the paper "Searching for Synergism Among Combinations of Drugs of Abuse" that is currently in the submission process.

Analysis of Intrinsic Disorder in Human Genome

Studies of intrinsically disordered proteins that lack a stable tertiary structure but still have important biological functions critically rely on computational methods that predict this property based on sequence information. Although a number of fairly successful models for prediction of

protein disorder were developed over the last decade, the quality of their predictions is limited by available cases of confirmed disorders. To more reliably estimate protein disorder from protein sequences, we have recently developed an iterative algorithm that integrates predictions of multiple disorder models without relying on any protein sequences with confirmed disorder annotation. The iterative method alternately provides the maximum a posterior (MAP) estimation of disorder prediction and the maximum-likelihood (ML) estimation of quality of multiple disorder predictors. Experiments on data used at three most recent Critical Assessment of Structure Prediction evaluations (CASP7, CASP8, and CASP9) have shown the effectiveness of the proposed algorithm. The proposed algorithm can potentially be used to predict protein disorder and provide helpful suggestions on choosing the suitable disorder predictors for unknown protein sequences. The results of this study are currently in press (Zhang P., Obradovic, Z. "Unsupervised Integration of Multiple Protein Disorder Predictors: The Method and Evaluation on CASP7, CASP8 and CASP9 Data," *Proteomic Science Journal*).

This year we have also studied supervised learning from multiple annotators which is an increasingly important problem in machine leaning and data mining. We have developed a probabilistic approach to this problem when annotators are not only unreliable, but also have varying performance depending on the data. The proposed approach uses a Gaussian mixture model (GMM) and Bayesian information criterion (BIC) to find the fittest model to approximate the distribution of the instances. Then the maximum a posterior (MAP) estimation of the hidden true labels and the maximum-likelihood (ML) estimation of quality of multiple annotators are provided alternately. Experiments on CASP9 protein disorder prediction tasks show performance improvement of the proposed approach as compared to the majority voting baseline and a previous data-independent approach. Moreover, the approach also provides more accurate estimates of individual annotators performance for each Gaussian component, thus paving the way for understanding the behaviors of each annotator. The results of this study are currently in press (Zhang P., Obradovic, Z. "Learning from Inconsistent and Unreliable Annotators by a Gaussian Mixture Model and Bayesian Information Criterion," *Proc. European Conference on Machine Leaning and Principles of Knowledge Discovery and Data Mining*, Sept. 2011).

One interesting and unexplained result of the analysis of intrinsic disorder in human genome (Midic, U., Oldfield, C.J., Dunker, A.K., Obradovic, Z., Uversky, V.N. "Unfoldomics of Human Genetic Diseases: Examples of Ordered and Intrinsically Disordered Members of the Human Diseasome, " *Protein and Peptide Letters*, vol. 16, no. 12, 2009, pp. 1533-1547) was the significant difference of predicted disorder content in experimentally confirmed human protein sequences, and protein sequences obtained with automated annotation (i.e. prediction) procedures. One possible explanation of this is that the sequences in the second group contain many incorrectly predicted parts that may have higher predicted disorder content, which may in turn increase the overall predicted disorder content in that group. To test this hypothesis we (Midic, U. and Obradovic, Z.) developed a classifier that aims at distinguishing between real protein sequences and incorrectly predicted parts of protein sequences (these were constructed from mRNAs of confirmed protein sequences, by translating non-coding parts of mRNAs and by intentionally translating coding parts in a wrong reading frame). The preliminary results are positive, i.e. the predictor can successfully distinguish between two types of sequences. When applied on the two groups of sequences from the human genome study, there were significant

differences between the predictions obtained for the two groups. This partially confirms the proposed explanation, but further research is needed.

In this period we also studied the relationship between protein sequence alignment and intrinsic disorder in proteins. Initially we proposed to extend the amino acid alphabet with 20 more symbols (20 amino acids in intrinsically disordered regions), develop a 40x40 matrix of substitution scores and to modify alignment methods to use the extended alphabet and the expanded matrix. However, evaluation on a reference dataset has shown that the proposed procedures have much worse performance than standard alignment methods used with standard matrices (such as BLOSUM62). The reason for this was the loss of specificity when pairs of unrelated disordered sequences were aligned. We decided to back-track and apply similar methodology to obtain expanded scoring matrices from BLOCKS, i.e. the same dataset that BLOSUM matrices were derived from. In addition to the 20x20 matrix and 40x40 matrix, we constructed a 40x20 matrix which is applied in an unsymmetrical way: one sequence is expressed in the extended alphabet (i.e. disorder is taken into consideration) and the other is expressed in the original alphabet (disorder is ignored). This approach is particularly fitting for alignment of query sequences to large sequence databases, where a disorder predictor can be easily applied to the query sequence, but its application to all sequences in the database can be prohibitively time-consuming. Evaluation of the three matrices has shown that, while the 40x40 matrix again suffers from low specificity, the 40x20 matrix performs slightly better than the 20x20 matrix. The preliminary results on this study were reported in the Preliminary II examination of Ph.D. student Uros Midic, and a paper on this study is currently in the submission process.

Research Project 21: Project Title and Purpose

Neuroimaging of Dextroamphetamine Effects in Aphasia - Aphasia is a neurological disorder characterized by a loss of the ability to understand or produce speech that occurs when language areas of the brain are damaged. The main treatment for the disorder has traditionally been speech and language therapy. However, recent studies have suggested that the outcome of this therapy can be significantly enhanced with the use of a particular medication called dextroamphetamine. The purpose of this project is to use an advanced brain imaging technique (fMRI) to examine the influence of this medication on brain activity while individuals with aphasia are engaged in tasks requiring speech processing. This type of assessment will facilitate an understanding of the nature of the positive treatment effects that have been described and may eventually allow us to predict which patients are likely to benefit from this form of adjuvant treatment.

Anticipated Duration of Project

9/9/2009 – 12/31/2011

Project Overview

Aphasia is an acquired communication disorder in which a person's ability to produce and/or comprehend language is impaired due to damage to neural systems that mediate the processing of speech. Aphasia affects more than 1 million Americans, with 80,000 new cases each year

from stroke alone. Speech/language therapy is the traditional form of treatment for the disorder. However, recent neuropharmacologic studies have suggested that the outcome of speech/language therapy in patients with aphasia can be enhanced by adjuvant treatment with low doses of the medication dextroamphetamine (D-AMPH). The nature of this effect and its neurophysiological correlates remain unspecified. This project therefore proposes to use functional magnetic resonance imaging (fMRI) to evaluate the effects of D-AMPH in stroke patients with aphasia. This technique examines changes in brain activity that occur when participants are engaged in cognitive tasks. We aim to test the hypothesis that a low dose of D-AMPH used in previous neuropharmacologic studies of aphasia results in increased brain activation in the left hemisphere during processing of speech. In order to test this, we will develop a protocol that is suitable for patients with aphasia to perform in the scanner environment. We will then examine the effects of D-AMPH and placebo on cerebral activation indexed by fMRI and on behavioral responses recorded during performance of tasks requiring speech-processing. We propose to evaluate whether fMRI is sensitive to changes in cognitive processing in individual patients that result from administration of low doses of D-AMPH.

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Expected Research Outcomes and Benefits

Aphasia is a disorder of the ability to produce and/or comprehend language that occurs when language areas of the brain are damaged. The disorder affects more than 1 million Americans, with 80,000 new cases each year from stroke alone. It is associated with profound personal, economic and social implications and aphasia is consistently cited as one of the primary determinants of diminished quality of life in stroke survivors. Speech/language therapy is the primary treatment and can result in significant improvements over time. Medications have traditionally had little to no effect on the outcome of this disorder. However, recent studies have suggested that low doses of dextroamphetamine (D-AMPH), a medication typically used to treat attention deficits, can significantly improve outcome of speech/language therapy. It has been suggested that this combination of behavioral/medical treatment enhances the brain's ability to reorganize functions related to language, thus enhancing long-term outcome. However, treatment is not beneficial to all patients and the nature of the effect is not well understood. A better understanding of the mechanisms involved in the treatment effect would allow a more rational approach to prescribing this form of treatment. This project proposes to utilize an advanced brain imaging technique, functional magnetic resonance imaging (fMRI), to evaluate the

influence of low dose D-AMPH on brain activation during speech processing in patients with aphasia. This controlled assessment of D-AMPH's effects will help elucidate its influence on brain function in patients with aphasia and provide a better understanding of the nature of the positive treatment effects that have been described when it is used in combination with speech/language therapy. The use of fMRI may potentially be developed as a highly efficient approach to examine medication effects and in predicting which patients are likely to benefit from this form of adjuvant treatment. If successful, this approach could potentially accelerate progress in clinical trials and research in aphasia.

Summary of Research Completed

In the past year, we have continued in our recruitment efforts to enroll more patients in the study, have collected data on new patients, and have been analyzing acquired data. We have also had to spend a significant amount of time dealing with a number of technical and administrative problems that emerged in the course of the year that resulted in some loss of data. These problems relate to accessing data from the 3 Tesla Siemens MRI magnet at Temple University Hospital, which is used to obtain our scans. This magnet is used for both clinical and research purposes. When installed, no special provisions were made to provide researchers on main campus access to data collected using the magnet. Like clinical scans, research scan data is uploaded from the scanner computer to a Picture Archiving and Communication System (PACS) server in the hospital. Data on the PACS server is directly accessible by clinicians within the hospital system. However, researchers do not have direct access to information stored on the PACS since this server is primarily for hospital use by physicians and staff who have received clearance to access patients' private medical data. In order to obtain research scan data, then, researchers must request that the data be burned on DVDs by the medical records office at Temple University Hospital. Approximately 1,400 files are generated for each session for each research patient in this study. The burned DVD's are flawed in almost 40-50% of cases. Due to the data format (DICOM), researchers may only discover the errors when attempts are made to convert the files to formats used by fMRI data analysis software back in the lab. When it is discovered that a burned DVD is flawed, we then have to return to the hospital and ask the file room staff to burn the DVD again. One of our patient's data had been incompletely burned to DVD on more than one occasion. On investigating the nature of the recurrent problem, we eventually discovered that the data from the completed scan had not been pushed in its entirety from the Siemens scanner computer to the PACS server. Due to the cumbersome process in place in accessing research data, this error was not detected until it was too late to retrieve the original data from the fMRI computer itself. The scanner computer keeps scan data for only a limited time, and is not backed up anywhere besides the PACS server. The original data had been erased from the scanner computer and was unrecoverable.

Given that the patients for our study are difficult to recruit and require considerable time and effort to get them through the protocol (of which the fMRI data is only a part), a loss of data represents a substantial setback. As a result, we (along with other researchers) approached the Department of Radiology requesting that the protocol in place for researchers to access to their fMRI data be changed in order to avert further incidents. After meetings with the Institutional Review Board, and the Associate Dean for Research and consultations with the Legal Department at Temple University Hospital, it was eventually agreed that a single portal be made

available to researchers on main campus through a centralized server to be managed by Information Technology personnel of Temple University. This server was to be functional in January or February of this year. We now understand that it is undergoing testing and may be available by the end of the summer. This will mark a significant improvement in data management and access for this project and for other projects as well.

Having developed a protocol which appeared to lateralize and localize language functions in the brains of healthy young college aged adults (see previous progress report), we have analyzed data on five patients of 10 patients screened for the protocol. For each of these patients, one scan represents the patient's brain activation during performance of a language comprehension task while on dextroamphetamine while the other session represents activation while the patient is on a placebo. We are currently blinded as to which session is active drug and which is placebo. Each session's functional scans were preprocessed, visually inspected and manually adjusted to optimize the coregistration with that session's high-resolution anatomical T1 scan. To ensure accurate comparisons across sessions, the first session's high-resolution anatomical scan was manually adjusted in all six dimensions using a yoked interface to match the spatial orientation of session two's high-resolution anatomical scan. The adjustments made to the first session high-resolution anatomical scan were then applied to the functional datasets so that they were coregistered to second week's anatomical scan. This process allowed scans to be contrasted at the single subject level (i.e. the same subject on and off drug).

After thresholding each session's activation maps with a p value of .001 or less, several regions of interest, including classical language areas, were visually inspected for activation within and across hemispheres. Functional scans were overlaid on top of week 1's high-resolution anatomical scan to determine the location of activations. To more precisely characterize the differences between active drug and placebo, for each task, week 1's functional scan was subtracted on a voxelwise basis from week 2's scan.

In order to compare brains of different shapes and sizes, brain scans from normal subjects are typically warped to a standardized space such as the Montreal Neurological Institute's standard MRI template often referred to as "Colin 27" to make comparisons across multiple subjects. Warping algorithms find anatomical landmarks in each scan to try to precisely fit each brain region to match the same brain region in the standard template image. However, the participants in this study have cerebral lesions which may obscure or displace some of the anatomical landmarks necessary to accurately warp to a standard space. Moreover, the damage can potentially induce significant anatomical distortions which the warping algorithms may misinterpret. As a consequence of these issues, there is no single approach that has been established when working with lesioned brains. This is a salient problem in the neuroimaging community and literature continues to be published discussing how best to approach normalization with focal lesions. One method has been to use cost function masking, while another approach has been to use unified segmentation.

In order to evaluate whether the standard warping algorithms can be successful in our population, we examined both normalized and non-normalized data. Visual inspection of warped and unwarped versions of the same scans revealed that the process retained the participants' lesions and cerebral asymmetries reasonably well. We applied a threshold of $p<.001$

for each activation map and performed a region of interest (ROI) analysis of classical language regions available from the Talairach-Tournoux Atlas. In addition, because it has been shown that individuals with cerebral lesions may not activate in classical language areas, we also surveyed a number of non-classical ROIs which may also represent a drug effect. Regions of interest included cingulate gyrus, fusiform gyrus, inferior, middle, superior, and transverse temporal gyrus, insula, inferior and middle frontal gyrus, angular gyrus, supramarginal gyrus, inferior and parietal lobules, and parahippocampal gyrus.

Because the drug schedules are still blinded, it is unclear in which week a drug effect would be expected. Nonetheless, one salient finding is that for each subject, there is one week which has significantly more activation in either the left hemisphere language areas or homologous areas of the right hemisphere than the comparison week. For most, this represented a twofold increase from one week to another.

A pattern of increased activation in language areas of the left hemisphere has been described in normal controls in response to somewhat higher doses of dextroamphetamine. As we have not yet been unblinded as to which week patients received active medication or placebo, we cannot say at this time that the session differences in perisylvian activation we observed are indeed related to drug effects in our patients with aphasia. However, this seems a possible explanation of the session differences. This was not attributable to an order effect, since there was no systematic relationship between the week of the scan and the pattern of greater activation.

Contrary to what has been observed in normal controls, changes we observed were not necessarily limited to increases of activation in classical left hemisphere language areas. In three of our participants, the increased activation involved increases in right perisylvian areas suggesting that language dominance has reverted to the right hemisphere post-stroke. Two patients show left hemisphere dominance for language in one session and an increase in left hemisphere activation on the second session. This effect is most evident in inferior and middle temporal gyrus, inferior and middle frontal gyrus, and left fusiform gyrus. The effect was present but less robust in cingulate and parahippocampal gyrus.

An example from one patient is illustrated in Figure 1 below. In this illustration, the left hemisphere of the brain is on the right side of the axial and coronal images. The upper panel represents activation obtained during week 2 while the lower panel represents activations obtained in week 1. As can be appreciated, this patient demonstrated greater activation in left hemisphere on both occasions. During week 2, activation increased substantially, particularly in inferior frontal areas, superior temporal gyrus, supramarginal gyrus and middle temporal gyrus. This is not simply an order effect since other patients show a similar difference between weeks, but the direction of the difference varies.

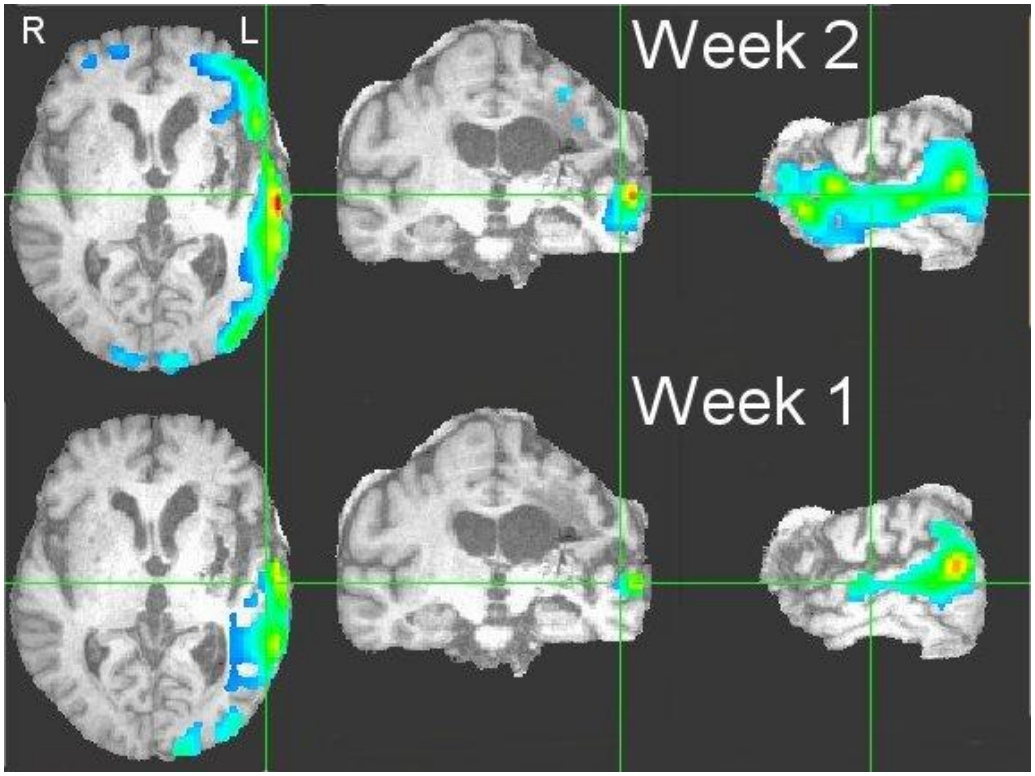

Figure 1: From left to right-- coronal, axial and sagittal views of activations elicited by the sentence comprehension task in a participant with aphasia. The upper row of images was obtained during week 2, while the lower row are images taken during week 1. The patient demonstrates asymmetric left hemisphere activation. There is greater perisylvian activation in the left hemisphere during week 2. This is evident on both week 1 and week 2 scans. However, note the marked increase of activation in the region of superior and middle temporal gyrus in the left hemisphere during week 2 (upper row).

Research Project 22: Project Title and Purpose

Geographic Information System (GIS) and Visualization for Health Disparities Research Core Facility Infrastructure - Temple University's Department of Geography and Urban Studies is undergoing a significant expansion to invigorate its research environment and to improve the education of the next generation of GIS scientists. To advance biomedical research, there is an urgent need for a new core facility that has the capacity to conduct state-of-the-art GIS and visualization research on health disparities, environmental health, and the study of the human impacts and health effects of natural disasters and technological hazards. These improvements will involve the reconstruction of an outdated 1200 square foot lab. Currently, this space does not have necessary information and communication technologies (ICT) infrastructure to support the use of new equipment or new telecommunications tools for transmitting data and analytical outputs across a growing network of collaborators on health, environment, and disaster/hazards research. The planned infrastructure project includes both the ICT and space renovations to accommodate new equipment that will be used for GIS, visualization and telecommunications efforts in health disparities research.

Anticipated Duration of Project

7/1/2011 – 12/31/2011

Project Overview

The objective of the proposed infrastructure project is renovation of space and installation of equipment in the Department of Geography and Urban Studies at Temple University. Renovation involves removal of old, outdated equipment, demolition of existing interior walls, removing outdated telecommunications systems, constructing and finishing new interior walls, installing new, additional electrical power capacity, internet drops, telecommunications cables, ventilation, and built-in storage and work spaces. The project includes purchase and installation of telecommunication devices, networked GIS stations, and associated high end printing capacity, including a 3D printer and workstation set up.

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Expected Research Outcomes and Benefits

This project will provide new laboratory space and associated infrastructure improvements, including renovation of electrical, ventilation, internet connectivity, and telecommunications connectivity needed for the installation of state-of-the-art equipment to enhance the ability of Temple University research investigators to conduct health geographic analyses using geographic information systems and spatial analytical technologies. This research will include the examination of spatial variations in health disparities, diffusion of diseases and chronic health conditions; geographic analyses of the distribution of health services; advanced modeling of environmental health hazards; modeling of disaster and technological hazards and technical support for the implementation of telehealth studies in rural areas in Pennsylvania.

Summary of Research Completed

This project is scheduled to begin on 7/1/2011.