CCTS Pilot Grant Awardees 2013

Debra Tonetti, Associate Professor, Pharmacy, Biopharmaceutical Sciences
Tibor Valyi-Nagi, Associate Professor, Medicine, Pathology
Lisa Tussing-Humphreys, Medicine, Section of Health Promotion Research, Assistant Professor
Jaehyung Cho, Medicine, Anesthesiology and Pharmacology
Xin Huang, Research Assistant Professor, Dentistry, Periodontics
Jamie Chriqui, Senior Research Scientist, Public Health, Institute for Health Research and Policy

PI: Debra Tonetti, Associate Professor, Pharmacy, Biopharmaceutical Sciences
Title: “Mechanism of Action of TTC-352 for the Treatment of Endocrine-Resistant Breast Cancer”
Co-I: Gregory Thatcher, Professor, Pharmacy, Medicinal Chemistry and Pharmacognosy

Lay Summary:
Breast cancer is the most common female malignancy, affecting 1 in 8 women. Up to 80% of breast cancers are estrogen receptor positive (ER+), a common type of breast cancer where estrogen fuels tumor growth. While the Selective Estrogen Receptor Modulator (SERM) tamoxifen (TAM), has been the gold standard for breast cancer therapy for over 30 years, 30-50% of patients either do not respond to TAM or they develop resistance to it. At the point of resistance to TAM, patient options are limited. Other potential drugs can include drugs that attempt to block ER function, yet often chemotherapy must be used in this setting which is associated with significant toxicities. Paradoxically, prior to the introduction of TAM, estrogen and diethylstilbestrol (DES) achieved similar success in the treatment of breast cancer, but also with unacceptable side effects. My laboratory has identified a biomarker, protein kinase C alpha (PKCα), to not only identify resistance to TAM, but also predict whether tumors will regress in response to estrogen. We also discovered that another SERM, raloxifene (RAL) is capable of initiating tumor regression, however upon discontinuation of treatment, tumor growth resumes. Based on the RAL molecular structure, we tested a series of compounds synthesized by Dr. Greg Thatcher and identified TTC-352. This compound, a novel Selective Estrogen Mimic (SEM) represents an alternate therapeutic strategy without the undesirable side effects of either TAM or estrogen. A patent has been filed and the Tonetti/Thatcher group was awarded a Proof of Concept Award from the Office of Technology Management to synthesize a prodrug, perform drug metabolism, pharmacokinetic (DMPK) and pharmacodynamic studies. However, studies on mechanism of action are urgently needed. In order to further develop TTC-352 for an Investigational New Drug (IND) application, and clinical trial, we will identify the mechanism of action. Preliminary data suggests that both estrogen and TTC-352 cause the ER to translocate out of the nucleus, likely blocking the ability to stimulate tumor growth. This study will allow us to verify the universality of ER translocation in two additional PKCα breast cancer models and determine whether the activity mediated by PKCα is required for estrogen and TTC-352 mediated ER translocation and tumor regression.

PI: Tibor Valyi-Nagi, Associate Professor, Medicine, Pathology
Title: “Peptide-Based Therapeutics Against HSV”
Co-I: Deepak Shukla, Associate Professor, Medicine, Ophthalmology and Visual Sciences

Lay Summary:
No cure against herpes simplex virus type-1 (HSV-1) exists and similarly, no vaccines or effective prophylactic treatments can prevent the virus from infecting humans. The virus very commonly infects humans and establishes lifelong infections. Recently, in collaboration with Dr. Deepak Shukla’s group, who will also be a co-investigator on this proposal, we have screened a peptide library and isolated two small peptides that block the virus from infecting cells. Our peptides, called G1 and G2, also stop the spread of the virus from cell-to-cell. We have shown this result using a mouse model of corneal infection. The current proposal will test our hypothesis that infected cells of the cornea can also be treated with our peptides and this treatment may be highly effective since the cells surface expression of the receptor protein to which our peptides bind go up upon HSV-1 infection. Two major specific Aims will be tested. The first Aim will design new ways to enhance protective and
therapeutic ability of our peptides, G1 and G2. New and more stable forms our peptides will be generated and then examined for their abilities (i) to prevent an infection (prophylactic mode) and also, (ii) to treat a preestablished infection (therapeutic mode). We will also determine whether a conjugate of our peptide with an existing drug called acyclovir can demonstrate stronger efficacy in reducing the infection. Multiple assays will be used to prove the efficacy. The second Aim will establish the prophylactic and therapeutic significance of our peptides using a mouse model of the infection. We will determine whether our peptides can prevent virus from establishing a life-long infection.

PI: Lisa Tussing-Humphreys, Medicine, Section of Health Promotion Research, Assistant Professor
Title: “Obesity, Colonic Iron Metabolism, and Colorectal Cancer Risk”
Co-I: Xavier Llor, Medicine, Section of Digestive Diseases and Nutrition; Carol Braunschweig, Applied Health Sciences, Kinesiology and Nutrition; Giamila Fantuzzi, Applied Health Sciences, Kinesiology and Nutrition

Lay Summary:

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the United States (US) and third most common cancer in adults. Obesity is a significant risk factor for developing CRC however, the mechanism in which obesity impacts this risk is unknown. Dietary factors may influence changes in the colon that promote the cancer process. This is possible because undigested foodstuffs come in direct contact with the colon through the fecal stream. Iron is one such dietary factor associated with CRC. Recent studies demonstrate that obesity is associated with iron deficiency and reduced dietary iron absorption. The change in iron status and absorption observed in obesity is a result of an increase in the body’s main iron regulator, hepcidin. Obesity is associated with body inflammation which causes hepcidin to increase. Hepcidin controls iron by regulating the iron exporter, ferroportin-1. Ferroportin-1 is located on tissues that store or transfer iron including the small intestine. When hepcidin is increased, expression of ferroportin-1 is reduced causing iron to be trapped in the tissue. In obesity, increases in hepcidin lead to decreased expression of ferroportin-1 in the small intestine and reduced dietary iron absorption. As a result, obese individuals have higher amounts of iron in their fecal stream. Studies have shown that higher levels of iron in the fecal stream can lead to greater iron uptake by the colon. When the colon accumulates iron, adverse changes can occur including, inflammation, DNA damage, and increased cell growth. These processes are instrumental to the development of intestinal cancers. We believe that the changes in iron regulation and dietary iron absorption observed in obesity leads to alteration in the colon that elevate an obese individual’s risk of developing CRC. To investigate this, we will conduct a study comparing blood and healthy colon tissue obtained from 30 obese and 30 lean participants enrolled in the University of Illinois at Chicago-led Chicago Colon Cancer Consortium. We will accomplish the following aims: 1) determine the influence of obesity on body iron status, hepcidin, and inflammation; 2) determine the influence of obesity on healthy colon tissue expression of iron transport proteins, including ferroportin-1, and hepcidin; 3) determine the influence of obesity on the amount of iron in healthy colon tissue; and 4) determine the influence of obesity of healthy colon tissue expression of markers of CRC-risk linked to elevated iron exposure including inflammation, DNA damage, and cell growth. Conducting this study will help us to determine if obesity-related changes in iron regulation may be a link to understanding obesity-driven CRC. This study will also provide us the opportunity to obtain the preliminary data necessary to design and conduct a prospective clinical trial examining the effect of dietary iron exposure on CRC-risk in obesity. Ultimately, this work could influence current US federal dietary iron intake recommendations and clinical iron supplementation practices for obese individuals which would have a tremendous public health impact.

PI: Jaehyung Cho, Medicine, Anesthesiology and Pharmacology
Title: “Molecular Mechanisms Mediating Vascular Occlusion in Thrombo-Inflammatory Disease”
Co-I: Xiaoping Du, Medicine, Pharmacology; Masuko Ushio-Fukai, Medicine, Pharmacology

Lay Summary:
The long-term objective of the proposed studies is to elucidate the regulatory mechanisms of heterotypic platelet-leukocyte interactions in thrombo-inflammatory disease, thereby identifying a novel therapeutic target for treatment of the pathological consequences.

Interaction of platelets and neutrophils on the activated endothelium mediates vaso-occlusion under thrombo-inflammatory conditions. Following vascular injury, activated endothelial cells express various adhesion molecules, thereby supporting neutrophil and/or platelet adhesion. Platelet P-selectin interacts with P-selectin glycoprotein ligand-1 on neutrophils, which initiates heterotypic neutrophil-platelet interactions. Subsequently, the surface expression of αMβ2 integrin increases on activated neutrophils by granular secretion, and binding of activated αMβ2 integrin to platelet glycoprotein Iba induces firm interaction between two cell types. Despite our extensive understanding of receptors and counter receptors for heterotypic neutrophil-platelet aggregations, the regulatory mechanisms of heterotypic cell-cell aggregation remain unknown. Using in vivo intravital microscopy analysis with Akt knockout (KO) mice and chimeric mice generated by reciprocal bone marrow transplantation between WT and Akt KO mice, we have demonstrated that blood cell Akt2, but not Akt1 and Akt3, regulates neutrophil adhesion to the activated endothelium and neutrophil-platelet interactions during TNF-α-induced vascular inflammation. Using in vitro systems in which platelets and/or neutrophils were treated with an Akt2 specific inhibitor (Akti XII), or both cells were isolated from WT and Akt KO mice, we observed that neutrophil Akt2 is a key regulator for the heterotypic platelet-neutrophil aggregation. Consistently, Akt2 KO neutrophils and neutrophils pretreated with Akti XII exhibited reduced adhesive function of αMβ2 integrin during cell activation. Therefore, we will test the hypothesis that Akt2 plays a critical role in regulating function of neutrophil αMβ2 integrin which is a crucial receptor for the interaction with platelets, thereby orchestrating neutrophil-platelet aggregations on the activated endothelium in thrombo-inflammatory diseases.

Aim 1: We will determine the molecular mechanism by which Akt2 regulates αMβ2 integrin function. Biochemical and cell biological experiments using pharmacologic inhibitors and KO mice will be performed to study membrane translocation, conformational change, adhesiveness, and inside-out signaling of αMβ2 integrin. Since Akt2 also regulates NADPH oxidase (NOX2) activity, we will study whether reactive oxygen species (ROS) regulates αMβ2 integrin function and platelet-neutrophil interactions using NOX2 KO mice and ROS scavenger-treated blood cells and mice in vitro and in vivo.

Aim 2: We will examine the pathophysiological role of Akt2 in platelet-neutrophil aggregations during vasooclusion in vascular inflammation and sickle cell (SC) disease as models of thrombo-inflammatory disease. Using real-time intravital microscopy, we will test Akti XII in TNF-α-inflamed WT mice and in SC mice. Further, we will use SC mice deficient in blood cell Akt2 using shRNA introduced in hematopoietic stem cells. Using pharmacologic and genetic approaches, the inhibitory effect of the Akt2 inhibitor and gene deletion on the death rate under thrombo-inflammatory conditions will be examined in mice.

Our study will provide important evidence that Akt2 could be a novel therapeutic target for prevention and treatment of vaso-occlusion in thrombo-inflammatory diseases.

PI: Xin Huang, Research Assistant Professor, Dentistry, Periodontics
Title: “Virtual and High-Throughput Screening for Anti-Hemophilia Drugs that Target the Protein Z-Dependent Protease Inhibitor (ZPI)-Protein Z Interaction”
Co-I: Steve Olson, Professor Emeritus, Dentistry, Periodontics; Michael E. Johnson, Professor & Director Emeritus, Dentistry, Periodontics

Lay Summary:

Virtual and high-throughput screening for anti-hemophilia drugs that target the protein Z-dependent protease inhibitor (ZPI) - protein Z interaction

Hemophilia is a bleeding disease caused by genetic defects in procoagulant blood clotting factors that impair normal blood clotting. The current treatment for hemophilia is to replace the missing coagulation factor. Replacement therapy with plasma-derived or recombinant coagulation factors have significantly improved the fate of hemophilia patients. But several problems persist. Not only are replacement therapies highly expensive, but they also activate immune responses from patients, leading to the occurrence of alloantibodies that renders


replacement therapy ineffective. An alternative strategy to correct the hemostatic imbalance in hemophilia would be to reduce natural anticoagulant factors that limit blood clotting except when it is needed at sites of injury. However, this method is seldom explored. The protein Z-dependent protease inhibitor (ZPI) is a newly identified anticoagulant protein that circulates in blood in a tight complex with its cofactor, protein Z (PZ). The importance of the ZPI-PZ complex as an anticoagulant regulator of blood clotting is indicated from the increased risk of abnormal blood clotting, i.e., thrombosis, in individuals with deficiencies in ZPI or PZ and from animal studies in which knockout of the ZPI or PZ genes increases the susceptibility to thrombosis especially when combined with other procoagulant risk factors. Our preliminary studies confirm that the ZPI-PZ complex controls the amount of the procoagulant factor, thrombin, formed upon activation of blood coagulation in plasma and that disruption of the complex by a PZ antibody significantly enhances thrombin formation both in normal and hemophilic plasmas. We therefore have hypothesized that blocking the ZPI-PZ complex could restore hemostasis in hemophilia patients and potentially provide a safer and more cost-effective therapy that is not associated with the risk of immune system activation. Our laboratory has determined the X-ray structure of the ZPI-PZ complex and demonstrated by mutagenesis studies that two ZPI residues are hotspots that mediate the interaction. One of these residues in particular binds to a hydrophobic pocket in PZ that drugability analysis shows is an ideal candidate for a small molecule drug. We have designed a simple and sensitive fluorescence assay that employs a fluorescent-labeled ZPI to report PZ binding. We therefore propose to use this assay to screen small molecule libraries for compounds that can disrupt the ZPI-PZ complex and potentially function as anti-hemophilic drugs. Computer-based virtual screening will be done to allow a vast array of compound libraries to be surveyed for hits and this will be complemented with high-throughput screening of compound libraries. Hits will be validated by functional assays in which the ability to disrupt the ZPI-PZ complex is evaluated from a reduced rate of inhibition of the target, blood coagulation factor Xa, and from an enhanced production of thrombin in normal and hemophilic plasmas. A small molecule drug that is able to correct the hemostatic defect of hemophilia would have major advantages over current protein replacement therapy regarding patient immune responses, safety, and cost. If achieved, this study will not only advance understanding of ZPI/PZ anticoagulant function and mechanisms, but potentially will provide an improved therapy for hemophilia.

PL: Jamie Chriqui, Senior Research Scientist, Public Health, Institute for Health Research and Policy

Title: “Developing a Baseline Understanding of Unhealthy Snack and Beverage Availability in Chicago Public Schools (CPS)”

Lay Summary:

This collaborative pilot study between the UIC Institute for Health Research and Policy and the Chicago Public Schools (CPS) Office of Student Health and Wellness aims to compile critical baseline data on the availability of junk foods, sugar-sweetened beverages and high-fat milks sold through vending machines and cafeteria lines (i.e., competitive foods and beverages, CF&B) in Chicago Public Schools (CPS) prior to the implementation of CPS’ newly adopted Healthy Snack and Beverage Policy. The data will be compiled through on-site audits of vending machines and à la carte lines in the cafeteria of a representative sample of up to 90 non-chartered, non-alternative CPS schools that have not already achieved Healthier US School Certification status from the U.S. Department of Agriculture (USDA). The on-site audit tools will be adapted from checklists originally used for the USDA’s School Nutrition Dietary Assessment Survey-IV (SNDA-IV) and will assess the presence of a variety of foods and beverages sold through vending machines and à la carte lines as well as the pricing and placement of items in vending machines. Emphasis will be placed on high schools since data from CPS indicate that CF&B are commonly available at that level. The CPS policy is scheduled to be implemented gradually beginning in school year 2013-14. Thus, two years’ of data collection are proposed that will allow for at least one (and, possibly, two) baseline years (and, for some schools, one year of post-implementation data). The data from this study will be combined with baseline data already compiled by CPS including student body mass index (BMI), student dietary behaviors, and school-level academic yearly progress as well as to be compiled focus group data on student, parent and school administrator perceptions, for inclusion in a larger R01 grant application planned for submission to the National Institutes of Health in the Fall of 2013. The larger study will provide critical data for CPS and its stakeholders as well as the public health and education communities. Importantly, the results of the R01 will provide insights into challenges/barriers associated with implementing strong CF&B standards for the USDA which will soon issue a proposed (and eventually final) rule to regulate CF&B’s nationwide. This study and the planned R01 study build off of the existing literature in this area and prior
research conducted by the PI that illustrates that strong policies can effectuate change in the school food environment, student consumption, and BMI outcomes. This study is directly responds to the 2013 CCTS Pilot Grant Funding Opportunity as it focuses on improving the health of the population through the conduct of policy relevant research.