An estrogen receptor α (ERα)-BMPR2-apelin axis mediates 17β-estradiol’s protective effects on right ventricular function in experimental pulmonary hypertension (PH)

Andrea Lee Frump, Margie Albrecht, Sandra Breuils-Bonnet, Bakhtiyor Yakubov, Mary Beth Brown, Steeve Provencher, Sebastien Bonnet and Tim Lahm

OBJECTIVES/SPECIFIC AIMS: Women with pulmonary arterial hypertension (PAH) exhibit superior right ventricular (RV) function and survival compared with men, and the female phenotype attributed to poorly understood cardiacprotective effects of 17β-estradiol (E2). We hypothesize that E2, through ERα, attenuates PH-induced RV dysfunction by upregulating the pro-contractile and pro-angiogenic peptide apelin. This ERα-mediated increase in apelin is mediated by the myocardial remodeling effector bone morphogenetic protein receptor 2 (BMPR2). METHOD/STUDY POPULATION: ERα, BMPR2, and apelin were measured by ELISA and western blot in RVs from patients with PAH-induced RV failure and in RV homogenates from male or female Sprague-Dawley rats with sugen/hypoxia (Sugen) or monocular over (MCT)-induced PH. MRI and cardiac catheter measurements were performed. RESULTS/ANTICIPATED RESULTS: E2 treatment increased BMPR2 and apelin expression in Sugen-RVs and human RVs. Treatment of Sugen-RVs with E2 increased RV survival and reduced cardiac hypertrophy. Apelin was measured in RVs from patients with PAH-induced RV failure and in RV homogenates from male or female Sprague-Dawley rats with Sugen/hypoxia (Sugen) or monocular over (MCT)-induced PH. MRI and cardiac catheter measurements were performed. RESULTS/ANTICIPATED RESULTS: E2 treatment increased BMPR2 and apelin expression in Sugen-RVs and human RVs. Treatment of Sugen-RVs with E2 increased RV survival and reduced cardiac hypertrophy. Apelin was increased in E2 treated hypertensive ERα knockout mice compared to E2 treated wild type mice. Downstream signalling was disrupted. DISCUSSION/SIGNIFICANCE OF IMPACT: E2 mediates an increase in BMPR2 and apelin expression, which may contribute to the beneficial effects of E2 in PH. This study will provide the foundation for future studies on the role of BMPR2-apelin axis in PH.

The effects of autoimmune inflammation on proliferation, differentiation, and androgen receptor signaling in adult prostate stem cells

Paula Cooper, Hsing-Hui Wang, Meaghan Broman, Hristos Kaimakiotis, Bennett Elzy, Scott Crist, Liang Cheng and Timothy Ratliff

OBJECTIVES/SPECIFIC AIMS: The primary goal of this project is to verify murine findings in the human setting. METHODS/STUDY POPULATION: The methods include primary cell isolation and culture, FACS, adoptive transfer, 3D-cell culture, histology, immunofluorescence, xenograft, and tissue recombination. The study population includes patients undergoing radical prostatectomy due to hyperplasia or adjacent bladder or prostate cancer. RESULTS/ANTICIPATED RESULTS: Having verified similar sensitivities to androgen receptor (AR) inhibitors between naive murine and human basal prostate stem cells, we anticipate that autoimmune inflammation in humans affects the response of basal prostate stem cells in a manner similar to the murine setting as well. This includes increased proliferation, differentiation, and response to AR inhibitors. DISCUSSION/SIGNIFICANCE OF IMPACT: The identification of survival mechanisms used by basal prostate stem cells in an androgen deprived environment may give insight to the process by which prostate cancer becomes androgen independent. The effect of inflammation on proliferation, survival, and AR signaling in these cells may also provide information relevant to cancer initiation and progression.

Temperature regulating wheelchair cushion for prevention of pressure ulcers

Meitn Yavuz, Ali Ersen and Linda Adams

OBJECTIVES/SPECIFIC AIMS: According to the US census, there are 3.3 million Americans who have to use wheelchairs in order to maintain their mobility. About 50% of these patients develop a pressure ulcer at some point during their life time. Three major factors contribute to pressure ulceration: pressure, tissue temperature, and maceration due to sweating. The objective of this study is to develop a temperature regulating wheelchair cushion in order to address elevated tissue temperatures and related sweating. METHODS/STUDY POPULATION: We instrumented a wheelchair with cooling elements, a water filled cushion and a pump. The pump moves the water through the cooling elements where water temperature drops down to 15°C. The water then moves to the cushion where it cools the tissue and then back to the cooling elements. RESULTS: We recruited 1 healthy subject to sit on the instrumented wheelchair and then obtained thermographs of the cushion surface using an infrared thermal camera. After 1 minute of sitting on the cushion the minimum temperature was recorded as 27°C. After 10 minutes the temperature dropped to 23.3°C. DISCUSSION/SIGNIFICANCE OF IMPACT: In this ongoing proof-of-concept study we are investigating if circulating chilled water inside a wheelchair cushion is a feasible method to regulate tissue temperatures at the 25–28°C range. This range has been shown to delay ulceration under loading conditions that simulate sitting on a wheelchair. Initial results indicate that this may be an effective ulcer prevention method.

Dietary fat stimulates growth of pancreatic cancer through the cholecystokinin receptor

Sandee Nadella, Jill Smith, Julian Burks, Abdulhameed Al-Sabban, Juan Wang, Robin Tucker and Gloria Iyang

OBJECTIVES/SPECIFIC AIMS: Epidemiologic studies have found that the incidence of pancreatic cancer is greatest in countries that consume diets high in dietary fat. The mechanism by which dietary fat stimulates growth of pancreatic cancer through the cholecystokinin receptor is unknown. We hypothesize that dietary fat stimulates growth of pancreatic cancer through the cholecystokinin receptor. METHODS/STUDY POPULATION: We exposed pancreatic cancer cells to dietary fat and measured cell proliferation using an automated cell proliferation system. RESULTS/ANTICIPATED RESULTS: We found that dietary fat stimulates growth of pancreatic cancer cells through the cholecystokinin receptor. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings provide new insights into the role of dietary fat in the development of pancreatic cancer and have implications for developing novel therapeutics to prevent pancreatic cancer.

Vaccine efficacy and immunogenicity of recombinant WAP and CAP-1 proteins in AKR mice

Neima Briggs, Leroy Versteeg, Bin Zhan, Rojelio Mejia, Brian Keegan, Coreen Beaumier, Jagannadha Sastry, Maria Elena Bottazzi and Peter Hotez

Baylor College of Medicine, Houston, TX, USA

OBJECTIVES/SPECIFIC AIMS: Trichuris trichiura, a leading cause of chronic colitis worldwide, resulting in growth stunting, anemia, and cognitive deficits, predominately in children. Our long-term goal is to develop a vaccine against T. trichiura. Both T. trichiura and the closely related Trichuris muris release excretory/secretory (ES) macromolecules from the stichosome organ, which facilitates intracellular invasion into the cecum. We exploited the high degree of genetic sequence homology between T. trichiura and T. muris to evaluate the immunogenicity and efficacy of our vaccine candidates. In this study, we describe the T cell dependent mechanism of humoral immunity of 2 promising ES-derived vaccines recombinant proteins, WAP and CAP-1. We evaluated the immune response, indicating a driving a Th2-induced humoral response necessary for protection. We further predict protection and allergenicity of WAP in humans using serum from a cohort in an T. trichiura endemic region.
in fat. The gastrointestinal peptide cholecystokinin (CCK) is released from the duodenum in response to dietary fat. CCK has also been shown to stimulate growth of pancreatic cancer cells. The aim of this investigation was to determine if dietary fat promotes growth of pancreatic cancer through the actions of CCK at its receptor. METHODS/STUDY POPULATION: The effects of dietary fat on growth of murine Panc02 pancreatic cancer xenografts were studied in 3 different systems with immune competent mice: (1) pharmacologic blockade with a CCK receptor antagonist, (2) genetic knockout of the CCK receptor by CRISPR, and (3) systems with immune competent mice: (1) pharmacologic blockade with a CCK receptor antagonist, (2) genetic knockout of the CCK receptor and (3) tumor-associated fibrosis and increased the influx of CD8+ lymphocytes in the micro-environment. Panc02 cancer cells lacking CCK receptors failed to respond exogenous administration of CCK in vitro and to dietary fat in vivo. Dietary fat did not stimulate Panc02 tumor growth in CCK-KO mice. DISCUSSION/SIGNIFICANCE OF IMPACT: The mechanism by which dietary fat stimulates growth of pancreatic cancer is by CCK and this effect is independent of obesity. This is a significant finding because of the potential beneficial effects of medications which can block the effects of CCK in populations at risk for pancreatic cancer consuming a high-fat diet.

Allergic asthma is associated with elevated sphingolipid levels in children

Jennie G. Ono, Benjamin I. Kim, Tilla S. Worgall and Stefan Worgall

OBJECTIVES/SPECIFIC AIMS: To determine if altered sphingolipid metabolism and composition are associated with childhood-onset asthma. METHODS/STUDY POPULATION: Sphingolipid profiles and composition were analyzed in a pilot cohort of pediatric with asthma (n = 22), and in nonasthmatic controls (n = 17). The cohort includes males and females, ages 5–17 years with no prior history of asthma or wheezing, and those who have been previously diagnosed with asthma by a pediatric pulmonologist. Subjects who have a history of prematurity, chronic lung disease, respiratory infection, malignancy, autoimmune disorders, immunodeficiency, or sickle cell anemia were excluded. Asthma and nonasthma phenotypes were determined through clinical history, standardized asthma symptom checklists, medical record review and spirometry. Masses of sphingolipids were quantified by mass spectrometry (HPLC-MS/MS) in serum and exhaled breath condensates (EBC). Allergy status was determined through clinical questionnaire, blood IgE (>150 IU/mL) and blood eosinophils (>0.3 × 103/mcl). RESULTS/ANTICIPATED RESULTS: Multiple species of sphingolipids and ceramides were found to be higher in the serum and EBC of asthmatics compared with controls in the overall cohort. In serum, these species include C16 (p = 0.05), C16:2D (p = 0.05), C18:1D (p = 0.002), C20 (p = 0.05), Sphingosine (p = 0.05), and S1P (p = 0.04). In EBC, asthma was associated with higher levels of C18:1D (p = 0.05), C20 (p = 0.05), C22 (p = 0.05), Sphinganine (p = 0.05), Sphingosine (p = 0.05), and S1P (p = 0.04). When data were stratified by allergic status, the increases in serum sphingolipids were largely associated with total IgE levels greater than 150 IU/mL. Sphingolipids which were increased in allergic asthma (n = 13) compared with allergic controls (n = 5) included C16 (p = 0.006), C16:2D (p = 0.006), C18:1D (p = 0.06), C20 (p = 0.048), C22 (p = 0.02), C24 (p = 0.02), C24:1 (p = 0.02), Sphinganine (p = 0.02), Sphingosine (p = 0.01), and S1P (p = 0.02). Notably, only C18:1D remained increased in asthmatics regardless of allergic status, in both low and high total IgE subjects. DISCUSSION/SIGNIFICANCE OF IMPACT: Data from this pilot cohort suggest that sphingolipids are altered in asthmatic compared with nonasthmatic children, particularly in association with a history of allergy and elevated blood IgE. This trend was also demonstrated in exhaled breath condensate, suggesting that sphingolipids are altered both in serum and airway fluid. Only 1 species of sphingolipid measured, C18:1D, was elevated in asthmatics regardless of allergic status. Notably, this sphingolipid was recently identified to be associated with exercise induced wheezing (EW) and asthma persistence overtime, in a large case-control study of children with and without asthma (Perzanowski et al, in press). EW has been identified as a specific phenotype of asthma, and can be present with or without asthma/astag. Taken together, these data suggest that altered sphingolipids may contribute towards the underlying pathophysiology of asthma, the understanding of which can lead to improved characterization of asthma phenotypes.

Reference

Targeted eccentric motor control to improve locomotion after incomplete spinal cord injury

Kevin O’Brien, Michele Basso and James Schmiedeler

OBJECTIVES/SPECIFIC AIMS: Incomplete spinal cord injury (SCI) is a lifelong disability that typically results in a profound loss of locomotion capability. Current rehabilitation methods rarely restore full community ambulation, which in turn limits quality of life. Most individuals with SCI exhibit persistent deficits in eccentric muscle control and reach recovery plateaus below the levels necessary for independent community ambulation. Eccentric motor control is essential during the weight acceptance phase of gait, which is emphasized during downhill walking. METHODS/STUDY POPULATION: The overground locomotion of subjects with chronic SCI was analyzed both prior to and following a 12-week downhill body-weight-supported treadmill training regimen and compared to that of matched healthy controls in terms of kinematics, kinetics, and EMG activation. RESULTS/ANTICIPATED RESULTS: We expect to find significant differences between the controls and subjects with SCI, with deficits in eccentric motor control accounting for some of these differences. In addition, we expect the downhill training to yield significant improvement in eccentric muscle control that translates into improvements in functional, overground walking for the subjects with SCI. DISCUSSION/ SIGNIFICANCE OF IMPACT: The goal is to determine if downhill training can improve eccentric muscle control in chronic SCI and establish plateaus. OpenSim modeling of the experimental data will help quantify changes in eccentric control of individual muscles to clarify where specific gains are made.

Steroid therapy limits stem cell activation required to enact mucosal healing in inflammatory bowel disease

Evan Brady Lynch, Tatiana Goretsky, Emily Bradford, Tianyan Gao and Terrence Barrett

OBJECTIVES/SPECIFIC AIMS: Intestinal stem cells (ISC) primarily act in the repair of ulcerated epithelium, and their proliferative capacity relies on Wnt/β-catenin signaling. However, the role of GCs on basal epithelial cell signaling has not been fully characterized. The objective of this study was to interrogate a mechanism by which steroids may limit ISC activation. GCs inhibit NFκB signaling, which has been shown to play a role in nuclear β-catenin activation in epithelial cells. We hypothesized that GCs limit Wnt/β-catenin signaling required for ISC activation and epithelial restitution by inhibiting NFκB activation in epithelial cells. METHODS/STUDY POPULATION: To examine the effects of GCs on intestinal epithelial cells, we treated a nontransformed human colonic epithelial cell line (NCM460) with dexamethasone and observed the effects on NFκB and Wnt/β-catenin signaling events. We isolated mouse epithelial cells from the distal colon for stem cell culture as 3D “organoids.” We obtained pure epithelial cell preparations from mucosal biopsies isolated from patients treated at GI clinics at the University of Kentucky Chandler Hospital and VA Medical Center, Lexington. Steroid treated patients with equivalent levels of inflammation, but no mucosal ulceration were used as controls. RESULTS/ANTICIPATED RESULTS: In steroid-treated NCM460 cells, we saw an increase in steroid-responsive genes GILZ and SGK1. We saw a significant decrease in transcripts for Wnt target genes, including Axin2 and cmyc; NFκB target genes, including IFNG and IL6; and the shared NFκB and Wnt pathway co-activator CREBBP, despite unchanged transcript levels for β-catenin (CTNNB1). This data was corroborated in 3D stem cell cultures from cells isolated from mouse colon tissue, which had significant decreases in transcripts for stem cell markers Lgr5 and Ascl2, proliferative markers Ki67 and PCNA, and Wnt target Axin2. NCM460s transfected with a lentivirus carrying a TCF/LEF luciferase construct showed a 2.5-fold decrease in TNF-stimulated luciferase activity with dexamethasone treatment. Interestingly, this effect can be rescued by glucocorticoid receptor (GR) blockade with RU-486. Intestinal epithelial cells from patient biopsies showed significant decreases in colitis-induced Axin2, p-LRP6 (a positive marker of Wnt Signaling) and nuclear β-catenin, which correlated with decreased p-NFκB and p-EP300. DISCUSSION/ SIGNIFICANCE OF IMPACT: Together, these data suggest that steroid therapy inhibits Wnt/β-catenin signaling at multiple levels and effects stem cell proliferation in pure stem cell cultures. Decreases in TCF/LEF transcriptional activation (nuclear β-catenin’s DNA binding target) can be reversed with steroid receptor blockade with RU-486. While that a receptor level interaction may be occurring, Interestingly, the required co-activator CBP, shared between NFκB and Wnt pathways, has decreased transcription following steroid treatment, which may provide a mechanism for limited Wnt