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An estrogen receptor α (ER α)-BMPR2-apelin axis mediates 17 β -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, a phenomenon attributed to poorly understood cardioprotective effects of 17 β -estradiol (E2). We hypothesize that E2, through ER α , attenuates PH-induced RV dysfunction by upregulating the pro-contractile and pro-angiogenic peptide apelin. This ER α -mediated increase in apelin is mediated by the myocardial remodeling effector bone morphogenetic protein receptor 2 (BMPR2). **METHODS/STUDY POPULATION:** ER α , BMPR2, and apelin were measured (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 (SuHx) or monocrotaline (MCT)-induced PH. H9c2 rat cardiomyoblasts and cardiac endothelial cells were stressed with TNF- α (10 ng/mL) or staurosporine (50 nM) \pm E2 (100 nM; 24 h). ER α -, BMPR2-, and apelin-dependence were evaluated by siRNA (5 pM). Downstream apelin target and pro-survival factor ERK1/2 expression was measured (western blot). $p < 0.05$ by ANOVA was considered significant. **RESULTS/ANTICIPATED RESULTS:** ER α correlated positively with BMPR2 and apelin expression in SuHx-RVs and human RVs. Treatment of SuHx-PH rats with E2 or ER α agonist increased RV BMPR2 and apelin, whereas RV apelin was decreased in E2-treated hypoxic ER α knockout mice ($p < 0.05$), but not in ER β knockout mice. In H9c2 cells, E2 or ER α agonist attenuated TNF- α - or staurosporine-induced decreases in BMPR2, apelin, and phospho-ERK1/2 ($p < 0.05$ for all endpoints). E2 protection was lost in presence of siRNA directed against ER α , BMPR2, or apelin ($p < 0.05$). ER α was necessary for E2-mediated increases in BMPR2, apelin, and ERK1/2, and BMPR2 was required for the E2-mediated increase in apelin ($p < 0.05$ for siRNA vs. scramble). ER α , BMPR2, and apelin protein was increased in decompensated human RVs but downstream phospho-ERK signaling was disrupted. **DISCUSSION/SIGNIFICANCE OF IMPACT:** E2, via ER α , increases BMPR2 and apelin in the failing RV and in stressed rat cardiomyoblasts. The E2-mediated increase in apelin is BMPR2-dependent and likely occurs through direct binding of ER α to the BMPR2 promoter. Harnessing this E2-ER α -BMPR2-apelin axis during RV compensation may lead to novel, RV-targeted therapies for PAH patients of either sex.

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Vaccine efficacy and immunogenicity of recombinant WAP and CAP-I 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*, is 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 *T. muris* stichosome proteins as vaccine candidates. **METHODS/STUDY POPULATION:** Through immunological screening of the *T. muris* stichosome cDNA library and *T. muris* ES macromolecules generated in culture, we identified, cloned, and expressed several vaccine candidates. In ranking these antigens, we selected the most promising recombinant proteins, WAP and CAP-I, and vaccinated AKR mice to evaluate the immunogenicity and efficacy of our candidate. In addition, we collected 240 serum samples in the *T. trichiura* endemic region of Honduras to measure the recognition of WAP and CAP-I in naturally infected human. **RESULTS/ANTICIPATED RESULTS:** We measured a statistically significant reduction in larval worm burden in WAP and ES vaccinated mice, but not CAP-I, by microscopy and real-time PCR of *T. muris* DNA. We found a significant relationship between antigen-specific IgG1:IgG2a ratio in the mouse serum and degree of protection. Mouse splenocytes, vaccine-sensitized draining lymph nodes, and mesenteric lymph nodes were antigen-stimulated and their secreted Th1, Th2, and Th17 cytokines were measured by Luminex[®]. Stimulated mouse lymphoid cells were further analysed for T helper, T cytotoxic, and central/effector memory T cells by Flow

Cytometry. Human IgG1, IgG2, and IgE antibody titers against WAP and CAP-I were measured by ELISA. **DISCUSSION/SIGNIFICANCE OF IMPACT:** In our study, we describe the T cell dependent mechanism of humoral immunity of 2 promising ES-derived vaccines recombinant proteins, WAP and CAP-I. 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.

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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 Kaimakliotis, Bennett Elzey, 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.

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Temperature regulating wheelchair cushion for prevention of pressure ulcers

Metin 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/ANTICIPATED 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.

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Dietary fat stimulates growth of pancreatic cancer growth through the cholecystokinin receptor

Sandeep Nadella, Jill Smith, Julian Burks, Abdulhameed Al-Sabban, Juan Wang, Robin Tucker and Gloria Inyang
Georgetown - Howard Universities, Washington, DC, USA

OBJECTIVES/SPECIFIC AIMS: Epidemiologic studies have found that the incidence of pancreatic cancer is greatest in countries that consume diets high

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 through the CCK receptor that is over-expressed on 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) in genetically engineered mice lacking the CCK peptide (CCK-KO). After injection of 2×10^6 Panc02 cells subcutaneously, mice were fed either a high-fat diet or a control diet for 37–42 days. Tumor volumes and weights were measured and histology performed. **RESULTS/ANTICIPATED RESULTS:** Dietary fat significantly increased the size of pancreatic cancer xenografts and this effect was reversed by CCK receptor blockade. Receptor antagonist therapy also significantly reduced 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.

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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, acute 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 ($> 0.3 \times 10^3$ /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 ($p = 0.05$), C16DH ($p = 0.05$), C18:1DH ($p = 0.002$), C20 ($p = 0.05$), Sphingosine ($p = 0.05$), and SIP ($p = 0.04$). In EBC, asthma was associated with higher levels of C18:1DH ($p = 0.05$), C20 ($p = 0.05$), C22 ($p = 0.05$), Sphinganine ($p = 0.05$), Sphingosine ($p = 0.04$), and SIP ($p = 0.06$). When data were stratified for 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 ($p = 0.006$), C16DH ($p = 0.006$), C18:1DH ($p = 0.06$), 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 SIP ($p = 0.02$). Notably, only C18:1DH 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, C18:1DH, was elevated in asthmatics regardless of allergic status. Notably, this sphingolipid was recently identified to be associated with exercise induced wheezing (EIW) and asthma persistence overtime, in a large case-control study of children with and without asthma (Perzanowski et al., in press). EIW has been identified as a specific phenotype of asthma, and can be present with or without allergy/atopy. 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

Perzanowski M, et al. Distinct serum sphingolipid profiles among school-age children with exercise-induced wheeze and asthma persistence. *American Journal of Respiratory and Critical Care Medicine* 2017 (in press).

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

Kevin O'Brien, Michele Basso and James Schmedeler

OBJECTIVES/SPECIFIC AIMS: Incomplete spinal cord injury (iSCI) is a life-long 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 iSCI 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 iSCI 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 iSCI, 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 iSCI. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The goal is to determine if downhill training can improve eccentric motor control and extend recovery beyond established plateaus. OpenSim modeling of the experimental data will help quantify changes in eccentric control of individual muscles to clarify where specific gains are made.

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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 NF κ B 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 NF κ B 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 NF κ B 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; NF κ B target genes, including IFNG and IL6; and the shared NF κ B and Wnt pathway co-activator CREBBP, despite unchanged transcript levels for β -catenin (CTNBN1). 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-p65 protein levels. **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, suggesting that a receptor level interaction may be occurring. Interestingly, the required co-activator CBP, shared between NF κ B and Wnt pathways, has decreased transcription following steroid treatment, which may provide a mechanism for limited Wnt