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

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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 × 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 ($p = 0.05$), C16DH ($p = 0.05$), C18:1DH ($p = 0.002$), C20 ($p = 0.05$), Sphingosine ($p = 0.05$), and S1P ($p = 0.04$). In EBC, asthma was associated with higher levels of C18:1DH ($p = 0.05$), C20 ($p = 0.05$), C22 ($p = 0.05$), Sphinganine ($p = 0.05$), Sphingosine ($p = 0.04$), and S1P ($p = 0.06$). 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 S1P ($p = 0.02$). 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 Schmiedeler

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

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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-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 NFκB and Wnt pathways, has decreased transcription following steroid treatment, which may provide a mechanism for limited Wnt

activation following steroid therapy. Although steroids play a significant role in regulating the amount of inflammatory damage that occurs during IBD treatment, our data suggest that they may be limiting pathways required for effective healing as well.

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Successful hand function recovery after stroke

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OBJECTIVES/SPECIFIC AIMS: Upper-extremity (UE) impairment affects 88% of stroke survivors due to dysfunctional shoulder-hand coordination. Patients may be able to grasp with the arm at rest, but unable to grasp in a functional context (eg, from a high shelf) because shoulder use elicits involuntary hand muscle activity. Further, much rehabilitation research is directed at unsuccessful stroke recovery (patients with persistent UE impairment) but very little towards patients who show successful clinical recovery (such as those with mild UE impairment) even though these patients have attained the desired rehabilitation outcome. We examined the neurophysiological trajectory of successful compared to unsuccessful post-stroke recovery in the context of functional UE movements to clearly identify what factors are necessary for successful recovery of functional UE movements after stroke. METHODS/STUDY POPULATION: We studied 3 populations: (1) mildly-impaired patients, early (at <17 d, 30 d, 90 d, and 180 d) after stroke as a model of successful post-stroke recovery, (2) moderately-impaired, chronic patients (>6-months post stroke) with persistent hand function impairment, as a model of incomplete post-stroke recovery (unsuccessful recovery), and (3) Healthy age-range matched controls. We used transcranial magnetic stimulation (TMS) in all 3 groups at the given time points to measure corticomotor excitability (motor evoked potentials, recruitment curve), corticomotor inhibition (short-interval intracortical inhibition, long-interval intracortical inhibition), and intracortical facilitation of hand muscles with the shoulder positioned in different degrees of flexion or abduction (these shoulder positions are known to elicit involuntary, undesired hand muscle activation, which leads to UE dysfunction and impairment in individuals with stroke). RESULTS/ANTICIPATED RESULTS: Data collection are in process and will be presented. Preliminary data from controls shows that corticomotor excitability of selected hand muscles is affected by changes in shoulder position. Preliminary findings in controls are consistent with clinical findings in stroke that certain shoulder positions elicit involuntary and undesired hand muscle activation, leading to UE dysfunction and disability. Findings from the stroke groups will be presented. DISCUSSION/SIGNIFICANCE OF IMPACT: We hypothesize that this centrally-facilitated coupling between shoulder and hand muscles is disrupted after stroke, which may play a central role in the inability of patients to perform functional UE movements. By comparing the TMS metrics in mildly-impaired Versus moderately-impaired chronic patients, we will be able to identify the longitudinal change in neurophysiology underlying shoulder-hand coordination that is associated with successful or unsuccessful clinical recovery of UE function after stroke. Thus, these findings will help us distinguish between the neurophysiology underlying successful from unsuccessful UE recovery leading to more mechanism-based interventions for UE dysfunction post stroke in the future.

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Enumeration of circulating tumor cells for monitoring cancer treatment response

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OBJECTIVES/SPECIFIC AIMS: The goal of this research is to use circulating tumor cells (CTC) enumeration and characterization to monitor anticancer treatment response. Emerging evidence strongly suggests the implications that epithelial-to-mesenchymal transition may have in cancer metastasis. Consequently, we hope to elucidate the significance of mesenchymal and stem-like CTCs in the peripheral blood of metastatic pancreatic cancer patients by analyzing the prevalence and frequency trends of CD133 + CTCs, as they relate to clinical events. We also hope to determine if there is a correlation between EpCAM + CTCs and CD133 + CTCs numbers with tumor size, disease stage, and patient clinical outcome. METHODS/STUDY POPULATION: Blood samples of patients with metastatic pancreatic cancer (stage IV) were obtained from the University of Florida Health Cancer Center after informed consent through an IRB-approved protocol. CTC capture, characterization, and enumeration was performed on the blood of these cancer patients during their anticancer treatment. Patients had blood drawn for this purpose at time points aligned with clinical phlebotomy (every 2 weeks). CTC capture was performed by introducing treated patient blood samples into antibody-functionalized microdevices. The PDMS devices were functionalized by immobilizing either anti-EpCAM or anti-CD133, through an avidin-biotin complex. After capture, cells were fixated and permeabilized with 4% paraformaldehyde and 0.2% Triton X-100, respectively. Three-color immuno-cytochemistry (anti-cytokeratin-FITC, anti-CD45-PE, and DAPI) was performed to identify CTCs from nonspecifically captured blood cells. To be counted as a CTC, based on the FDA-approved technical definition, a cell with the appropriate cell size and morphology must be nucleated (DAPI+), express cytokeratin (CK+), and lack the leukocytic CD45 marker (CD45−). RESULTS/ANTICIPATED RESULTS: We tested the clinical utility of the device for monitoring the response of patients with advanced pancreatic cancer to a chemotherapy treatment consisting of anticancer drugs including 5-fluorouracil, leucovorin, oxaliplatin, and dasatinib. We have detected EpCAM + CTCs in 47/47 (100%) and CD133 + CTCs in 41/47 (87.2%) of blood samples, coming from a cohort of 16 patients. We studied the correlation between the CTC numbers and the clinical result of patients in the study. We found that the size and changes in the size of the primary tumor (confirmed by CT scans) correlated with the frequency and increase/decrease trends in the number of CTCs detected. We expect to find some relationship between the number of detected CD133 + CTCs, or rather stem-like CTCs, and the clinical outcome of patients (eg, disease progression leading to withdrawal from study). DISCUSSION/SIGNIFICANCE OF IMPACT: Enumeration of patient CTCs and stem-like CTCs at different stages of a patient's treatment may correlate with disease stage and prognosis, and prove useful in monitoring early recurrence, patient-specific treatment response, and newly acquired resistances; all of which would aid in providing guidance for the next step in clinical intervention. This type of liquid biopsy technology has great potential to make an impact in the future of personalized medicine and point-of-care diagnostics, as well as become a sturdy tool for translational research.

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Defining critical features of the immune microenvironment in melanoma using multiplex immunohistochemistry and spatial analysis

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OBJECTIVES/SPECIFIC AIMS: Precise biomarkers are urgently needed to characterize the tumor immune microenvironment in primary melanoma tumors both for prognostication and to predict the benefit of immuno-therapeutic intervention. The goal of this work is to define spatial relationships between CD8 + T cells, CD68 + macrophages and Sox10 + melanoma cells in order to define features correlating with prolonged survival METHODS/STUDY POPULATION: Five micrometer slides from either the primary biopsy or subsequent wide local excision procedure were stained using Opal multiplex IHC for DAPI, CD3 (LN10, Leica), CD8 (4B11, Leica), CD68 (KP1, Biogenex), SOX10 (BC34, Biocare), HLA-DR (LN-3, Abcam), and Ki67 (MIB1, Abcam). Cell phenotypes within representative fields preselected by a trained dermato-pathologist and were visualized using the Mantra quantitative pathology workstation (PerkinElmer), and analysis of spatial distribution of CD3+ CD8+ cells analyzed using inForm® image analysis software (PerkinElmer), and Spotfire software (TIBCO). In order to test whether mIHC can better characterize the tumor immune microenvironment, we screened databases at the Herbert Irving Cancer Center (HICC) at Columbia University for stage II/III melanoma patients diagnosed between 2000 and 2012, with available FFPE of primary melanoma tissue and documented clinical follow-up. We identified a preliminary population of 57 patients to begin our analysis. Clinical follow-up was available on 35 patients of whom 21 patients were alive with no evidence of recurrence or died with no evidence of recurrence and 14 had died of melanoma. Twenty-four patients had more than 24 months of survival information available but no detailed clinical information to determine cause of death. RESULTS/ANTICIPATED RESULTS: First, we evaluated whether density of immune cells in tumor and stroma predicted prognosis in 35 patients with disease specific survival information. We find that high number of CD3 + CD8 + cells in tumor correlates with Disease Specific Survival (DSS) ($p = 0.0323$*) and CD3 + CD8 + cells in stroma may also correlate with DSS ($p = 0.0671$). This is consistent with what is known in the literature regarding tumor infiltrating lymphocytes (TILs). We also found that CD68 + cells in stroma predict poor prognosis (0.0259*). This is consist with the proposed