Microbiota-derived Tryptophan Metabolites Provide a Novel Pathway for Regulation of Mucosal Barrier Function

The vast majority of microbial species within the human body contribute an important role in human health. Mucosal surfaces act as primary barrier against microbial toxins and invaders, while commensal bacteria work in a dynamic and intimate interaction with the epithelium to influence host cellular and immune responses. Indole, a tryptophan metabolite produced by anaerobic bacteria, is known for its intercellular signal activity and serves as an aryl hydrocarbon receptor (AHR) ligand. AHR is a ligand-dependent transcription factor activated by a variety of synthetic and biological molecules. The physiological function of AHR includes roles in immune regulation and mucosal barrier function. Further, IL-10 is an anti-inflammatory cytokine that inhibits production of numerous pro-inflammatory mediators in various cell types. This cytokine functions through binding to the IL-10 receptor alpha subunit (IL-10R1). An unbiased metabolomic profile of serum from healthy and colitic mice identified significant decreases in several microbial-derived indole derivatives (e.g. indoleacetate, indolepropionate, 5-OH-indoleacetate). Given that colitis is strongly associated with the induction of the epithelial IL-10R1, we hypothesized that indole-containing tryptophan metabolites regulate epithelial IL-10R1 expression. Human intestinal epithelial cells were exposed to indole derivatives and examined for expression of IL-10R1. These results revealed a prominent induction of IL-10R1 mRNA and protein expression following treatment with indole derivatives. Such results were explained, at least in part, by the activation of AHR. Indeed, IL-10R1 expression was diminished when indole derivatives were administered to AHR nuclear translocator (ARNT) knockdown cells. Likewise, the AHR inhibitor, CH-223191, significantly reduced IL-10R1 expression in cells exposed to indole derivatives. Together, these results provide strong evidence for a new role of tryptophan-indole derivatives in anti-inflammatory pathways mediated by IL-10 signaling in intestinal epithelia. The discovery of tryptophan metabolites as AHR ligands provides new insight to our understanding of the host-microbial axis during inflammatory processes. These studies also identify possible avenues for incorporating the indole-AHR pathway as novel therapies for mucosal disease.
Kinase Fusions are Therapeutic Targets in Distinct Subtypes of Melanoma

Cutaneous melanomas have one of the highest mutational burdens of all cancers, and over 50% of common malignant melanomas have an activating mutation in the oncogenic kinase BRAF. Although the majority of melanomas are related to sun-exposure, less common subtypes of melanoma such as acral lentiginous melanoma (ALM) and mucosal melanomas are not related to sun-exposure, have a lower mutational burden but increased chromosomal instability, and lack the common therapeutically targetable mutations in genes such as BRAF. Therefore, it is critical to identify the molecular mechanisms driving ALM and mucosal melanoma in order to develop and implement targeted therapies in these patients. Kinase fusions involving the genes BRAF, RAF1, ROS1, RET, ALK, and NTRK1 have been identified as oncogenic driving mechanisms in Spitzoid nevi and Spitzoid melanomas, but kinase fusions and response to therapy have not been investigated in ALM and mucosal melanoma. Using targeted RNA sequencing, we screened 22 samples from patients with ALM for fusions in ROS1, ALK, and NTRK1-3 kinase genes. We identified a ROS1 fusion in a patient with ALM, which contained exons 1-4 of the GOPC gene fused to the kinase domain exons of the ROS1 gene. This was an active patient that had failed multiple previous therapies, including immunotherapy. The patient was enrolled in a phase I basket trial of a ROS1/TRK/ALK inhibitor (entrectinib) and underwent a dramatic and durable response, with partial response of -55% (RECIST 1.1) persisting at 11 months. Although mucosal melanomas are rare, we have developed five patient-derived xenograft models from mucosal melanoma tumors and screened them by targeted RNA sequencing with an extensive gene fusion panel. We identified one mucosal melanoma sample which contained novel variants of the EML4-ALK fusion. This mucosal melanoma sample responds well to ALK inhibition in pre-clinical models, suggesting ALK fusions may be a therapeutic target in a subset of mucosal melanomas. Altogether, our data show oncogenic kinase fusions occur in ALM and mucosal melanomas and respond to targeted therapy. This highlights a new area of clinical screening and treatment for patients with these specific subtypes of melanoma.
The Influence of ENPP1 Expression in Mucosal Tissues

Previous work has established that extracellular adenosine signaling is protective in mucosal inflammation and promotes the resolution of ongoing inflammation. The sources of extracellular adenosine include enzymatic processing from nucleotides, such as ATP and AMP, which can be liberated from a variety of cell types, including leukocytes. Guided by a microarray screen of hypoxia-inducible genes in mucosal epithelial cells, we identified the strong induction of ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) in a time-dependent manner. ENPP1 is a type II transmembrane glycoprotein that can hydrolyze ATP and diadenosine triphosphate (Ap3A) to AMP and inorganic phosphate. ENPP1, present on the surface of chondrocytes, has been extensively studied for its role in tissue mineralization. Its potential role in the resolution of mucosal inflammation via AMP generation has yet to be determined. Presently, we have established that ENPP1 mRNA is expressed in T84 and Caco-2 intestinal epithelial cells, and this expression can be upregulated by conditions that drive epithelial metabolism (e.g., exposure to the short chain fatty acid butyrate). Likewise, studies in mouse intestinal epithelial cells lacking the transcription factor HIF-1α revealed significantly diminished expression of ENPP1. In parallel, it was revealed that activated human neutrophils are a source of Ap3A, previously thought to be made primarily by platelets. These studies indicate that Ap3A and ENPP1 at the mucosal surface may function to provide an additional source of adenosine, sub-serving its role in inflammatory resolution.
Presenters: Aryeh Fischer, MD
Contact info: aryeh.fischer@ucdenver.edu

Division: Rheumatology
Researcher Category: Faculty
Research Type: Clinical Research

Forced Vital Capacity Predicts Outcome in Scleroderma Associated Interstitial Lung Disease with Concomitant Pulmonary Hypertension

Background/Purpose: Interstitial lung disease (ILD) is the leading cause of death in scleroderma and is often accompanied by secondary pulmonary hypertension (Group 3 PH). The objectives of this study were to characterize the Group 3 PH population from “PHAROS” (Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma multicenter registry for the study of scleroderma-associated PH) compare them to those with Group 1 PH (pulmonary arterial hypertension), and identify specific variables with prognostic significance.

Methods: PHAROS is a prospective multi-center registry of scleroderma patients at high risk for, or with definite PH based on right heart catheterization (RHC) within six months of enrollment. In this study, we included those considered by PHAROS investigators to have Group 3 PH as defined by the presence of moderately-severe ILD (as determined by a forced vital capacity [FVC] < 65% predicted and/or significant ILD by chest computed tomography scan) along with RHC-confirmed mean pulmonary artery pressure of > 25 mmHg and pulmonary capillary wedge pressure < 15 mmHg and compared them to PHAROS subjects considered to have Group 1 PH (pulmonary arterial hypertension). Baseline demographics and clinical characteristics at the time of RHC were assessed, and univariate analyses (STATA, version 14.0) were performed to identify variables predictive of outcome.

Results: Sixty-three Group 3 PH patients were identified. Patients with Group 3 PH were more likely to be African-American, have diffuse skin involvement, anti-ScI70 positivity and more severe impairment on pulmonary function testing, but with better cardiac hemodynamics. With a median follow-up period of 913 days (interquartile range 383, 2158), 41% of the Group 3 PH cohort expired; with ILD progression as the most frequent cause of death (12 of 26, 46%). The 5-year survival was similarly poor for both groups: Group 1 PH 58% vs. Group 3 PH 61%. On univariate analysis of Group 3 PH patients, the only variable associated with survival time, was FVC (HR 0.967, 95% CI: 0.939-0.997; p=0.03): the lower the FVC, the higher the risk of death.

Conclusion: Moderate-severe ILD with concomitant PH is associated with a poor prognosis and the degree of physiologic restriction, as measured by FVC, is associated with worse survival time.
Perilipin-2 Modulates Lipid Absorption and Microbiome Responses in the Intestines of Mice

The intestinal microbiome has emerged as a critical factor linking diet to host physiology and metabolism. We have previously documented that deletion of the cytoplasmic lipid droplet protein Perilipin-2 (Plin2) in mice largely abrogates the long-term deleterious effects of a high-fat diet. We have also demonstrated associations between short-term, high-fat diet exposure, Plin2 genotype, and the diversity of the murine intestinal microbiome. In the current study, we examined how Plin2 modulates the response of gut microbiome composition and functional properties to both acute and chronic HF or low-fat diet exposures.

WT and Plin2-null mice raised on a standard chow diet were randomized to either a low-fat (10% fat calories) or high-fat (60% fat calories) diet. After four days, bulk RNA was extracted from colon content and subjected to metatranscriptomic and 16S rRNA gene sequencing. In parallel, mice were treated for 30-weeks of high-fat or low-fat diet and the gut microbiome profiled by 16S analysis.

16S rRNA sequencing demonstrated that following 4 days of high-fat diet, the gut microbiota of both WT and Plin2-null mice shifted towards an ostensibly obesigenic microbiota, characterized by increased Firmicutes and decreased Bacteroidetes. In contrast, by 30-weeks of high-fat diet, Plin2-null mice had re-normalized their gut microbiota to a non-obesigenic profile (i.e. lower Firmicutes/Bacteroidetes ratio), concomitant with lower weight gain, whereas WT mice still exhibited a Firmicutes-rich profile. RNA sequencing resulted in a median of 1.3x107 putative microbial mRNA reads per specimen. On both high-fat and low-fat diets, Plin2 deletion significantly modified the expression of multiple genes and pathways associated with fatty acid, amino acid, polysaccharide, and bile salt metabolic activities, which have been linked to regulation of intestinal function and obesigenic diet.

Our data demonstrate that Plin2 mediates acute and chronic effects of diet on the structure and function of intestinal microbiota. Resulting differences in the luminal concentrations of microbial metabolites, such as SCFAs and 2° bile salts, may modify the effects of high-fat diet on host metabolism through their pleiotropic effects on the host. Collectively, the data link Plin2-regulated lipid metabolism to changes in the functional properties of gut microbes implicated in diet-induced obesity.
The Impact of Increased Medicaid Payments for Primary Care Services on Access to Care for Medicaid Clients in Colorado

Section 1202 of the Health Care and Education Reconciliation Act amended the Affordable Care Act (ACA) requiring that Medicaid reimburse primary care providers (PCPs) at or above Medicare Part B rates in 2013 and 2014. The study objectives were to: (1) assess the extent to which the Section 1202 payment improved access to primary care for Medicaid client and increased the number of primary care providers accepting Medicaid (January 2013 – December 2014); and (2) estimate the impact of the Colorado extension (January – June 2015). We used Medicaid claims data provided by The Colorado Department of Health Care Policy & Financing to develop provider- and client-level measures of access to care for Medicaid clients. Interrupted time series and panel data models were used to assess if key program changes interrupted the time trends. The models were estimated using a generalized estimation equation method and included controls for seasonal variation, general time trends, and other factors that could influence the measures over time. The provider-level analysis includes all medical providers in Colorado that were authorized to receive Medicaid payments and rendered at least one qualifying service over the study period. A total of 17,939 providers are included in the analysis. Preliminary results from the provider-level analysis during the Section 1202 period suggested the higher payments were associated with an increase in the number of monthly Medicaid bump-eligible visits rendered by attested providers. On average, ever attested providers delivered an additional three bump-eligible visits per month after becoming attested, or approximately a 10% increase in the number of bump-eligible visits per month. This increased number of visits delivered by attested providers resulted in approximately 11,000 to 13,000 additional bump-eligible visits per month. Provider-level measures suggest that more PCPs are serving Medicaid patients as the number of Medicaid clients increases and that those providers serving Medicaid clients are increasing the number of bump-eligible visits over time. The provider-level models suggest that the Section 1202 enhanced payments increased both the number of providers rendering primary care services to Medicaid clients and the number of bump-eligible services rendered in a month by providers serving Medicaid clients.
Sample Methodology for Infant Fecal Microbiome Studies

The need to develop valid methods for sampling and analyzing infant fecal specimens for microbiome studies is increasingly important, especially for large population studies, and comparison studies of breastfed vs formula fed infants. Sampling methods need to be reproducible, stable, and accurate. A challenge in working with high biomass samples (such as stool) is that some bacterial DNA may derive from contamination - such as urine in diapers. Another notable and overlooked challenge is the different consistency between breast and formula-fed infant stool. Stool from exclusively formula-fed infants is generally homogeneous in nature whereas stool from young breastfed infants is more heterogeneous with notable liquid and pseudo-solid fractions. This makes collection of a representative sample difficult. We investigated the potential effects of often overlooked methodology factors such as sample collection and preparation and their implications for next-generation sequencing of infant fecal microbial communities by the 16s ribosomal ribonucleotide (rRNA) gene. Fecal samples were collected from a healthy exclusively breastfed infant via four different methods – whole diaper (standard), full bowel collection, liquid fraction only, and solid fraction only at different time points over a one week period. DNA was extracted from these samples and quantified for the 16s rRNA gene copies using qPCR. Amplicons were also generated from Illumina Miseq using the universal primers targeting the V3V4 region of the 16s rRNA gene. Microbiome of these fecal fractions yielded similar results, very few with variations in frequency of microbiome. We conclude that there is not a distinct difference between fecal samples and contamination introduced during sample collection and preparation.
Presenter: Nikolas Johs

Contact info: nikolas.johs@ucdenver.edu

Division: Infectious Diseases

Researcher Category: Student

Research Type: Clinical Research

The Social Ecological Model as a Framework for Understanding Physical Activity Barriers, Motivators, and Facilitators in HIV-infected Older Adults

Background: Antiretroviral treatment (ART) has greatly improved survival among those infected with HIV, and the proportion of adults over 50 years old with HIV is steadily increasing. Despite well controlled HIV disease, this cohort faces an increased burden of age-associated comorbidities and increased rate of disability and frailty. Benefits of regular physical activity and exercise on healthy aging in the general population are well established, however the unique barriers and facilitators to regular physical activity in the older, HIV-infected population are not well established.

Methods: Semi-structured focus group interviews were conducted among 2 target populations; HIV-infected persons aged 50-70 enrolled in the Exercise for Health Aging study and a group of HIV-infected volunteers aged 50 and older. Sessions were recorded and transcribed. Each transcript was coded individually by 2 team members, using a line-by-line emergent inductive approach. Codes were compared across transcripts, and a final set of codes was analyzed to identify major facilitators / barriers to exercise.

Results: A total of 51 participants participated in the study, 22 from the exercise intervention and 29 additional volunteers. The average age was 58 years and time since HIV diagnosis was 21 years. The majority of participants were white (68.6%), male (90.2%), and were on disability or unemployed (64.7%). The predominant themes that emerged from the data were consistent with the Social Ecological Model (SEM) framework. Participants identified the facilitators and barriers within each category of the SEM; intrapersonal (body image, sense of disability/disease), interpersonal (lack of social network, caregiver responsibilities, positive feedback), organizational (gym culture, age-friendly activities), community (HIV stigma, ageism, active community in Denver), and policy (no specific recommendations for exercise for those with HIV, lack of knowledge of insurance benefits).

Conclusions: The SEM model provides a meaningful way of conceptualizing the experiences of HIV-infected older adults with physical activity and exercise. Future interventions to increase physical activity in older HIV-infected adults or other older populations with health disparities should consider ways to incorporate SEM components to maximize physical activity uptake and maintenance.
Identifying Barriers to Kidney Transplant Evaluation

Background: Patients with end stage renal disease (ESRD) have a high risk of death. Kidney transplantation offers a significant survival advantage compared to chronic dialysis. However, only a minority of patients with ESRD receive a kidney transplant. Racial/ethnic minorities and those with lower socioeconomic status are less likely to be listed for a kidney transplant compared to whites. Our objective was to identify barriers to kidney transplant evaluation in our dialysis centers and determine if we have disparities in access to kidney transplant evaluations.

Methods: We conducted a survey of adult patients currently on hemodialysis. Participants were recruited from 4 dialysis units in the Denver Metro Area and completed an 11-item survey. The survey included demographic information such as age, race/ethnicity and gender as well as questions regarding time on dialysis, if a healthcare provider ever spoke to them about a kidney transplant and whether they have been evaluated for kidney transplant. Descriptive statistics were used to provide summaries of the responses.

Results: 140 patients completed the survey (response rate 68%). 119 patients (84%) reported having had a discussion of kidney transplantation with their doctor but only 51% reported having had an evaluation for a kidney transplant. The 68 patients (49%) who had not been evaluated were asked why. The most frequent responses contributing to why patients were not evaluated were that they weren’t sure how to proceed (48.5%), didn’t understand the benefits/process of transplant (43%) or were not referred by their provider (44%). Additionally, compared to whites, blacks and Hispanics reported less understanding of the benefits/process of transplant. Fewer blacks reported being evaluated for a transplant compared to whites and Hispanics and this trended towards statistical significance (p=0.059).

Conclusion: There were significant barriers to kidney transplantation evaluation in our dialysis units. Disparities in access to transplantation could be reduced through better provider communication and transplant education.
Role of Microbiota in Development of Autoimmune Arthritis

Background: Rheumatoid arthritis (RA) is an autoimmune disease with an unknown cause. Observations of dysbiosis and mucosal inflammation in patients with RA has raised interest in studying microbial-mucosal interactions as a potential trigger of RA. Using the murine collagen-induced arthritis (CIA) model, we hypothesized that microbiota and mucosal inflammation are required for the development of autoimmune arthritis.

Methods: 4-6 DBA1j mice were treated with or without broad-spectrum antibiotics in the drinking water 7 days preceding and throughout the induction of CIA, by immunization of bovine type II collagen (CII) in Complete Freund’s Adjuvant (CFA) on days 0 and 21. Fecal pellets and sera were collected every 7 days during the study for microbiome analysis and autoantibody development. Mice were euthanized on days 21 or 35 for tissue analyses for cytokines and autoantibodies by ELISA. Microbiome analyses were performed on fecal pellets by 16S sequencing of the bacterial rRNA.

Results: During the preclinical phase of CIA (without antibiotics), we find remarkable changes in the intestinal microbiome, specifically increased Clostridia and decreased Lactobacillus and Bacteroides. In parallel, intestinal permeability and cytokines IL-12 and IL-1β increased significantly (P value < 0.05). Furthermore, anti-CII autoantibodies were increased within the intestine, suggesting a developing mucosal immune response. In microbiota-depleted mice (antibiotic treated), CIA severity was reduced by 50% as assessed by clinical scores (P value < 0.01). Correspondingly, tissue cytokines and both serum and fecal anti-type II collagen antibody levels were reduced.

Conclusion: Taken together, these data suggest a model in which intestinal dysbiosis and mucosal immune responses drive the development of autoimmune arthritis. Future studies are aimed at elucidating the pathway by which microbiota and mucosal immune responses stimulate systemic autoantibody production that is necessary for the development of CIA.
The Short Chain Fatty Acid Butyrate Inhibits Pathobiont-Driven Gut CD4 T Cell Activation and HIV-1 Replication

A hallmark of progressive HIV-1 infection is the massive infection and depletion of CD4+ T cells in the intestinal mucosa, leading to microbial translocation and systemic immune activation. We previously reported that subjects with untreated chronic HIV-1 infection (HIV+) had an altered colonic mucosal microbiome which was linked to increased mucosal and systemic inflammation. Using bacterial 16S rRNA gene sequencing, HIV+ subjects had increased relative abundances (RA) of pro-inflammatory “pathobiont” bacteria (e.g. Prevotella) and decreased RA of bacterial families containing butyrate-producing bacteria (e.g. Roseburia). Butyrate is a short chain fatty acid (SCFA) involved in mucosal immune homeostasis and colonic epithelial barrier integrity. In order to address the hypothesis that a loss of butyrate might exacerbate Prevotella driven mucosal immune activation and enhance viral replication, we used an ex vivo intestinal cell model. Lamina propria (LP) mononuclear cells from normal human intestinal tissue (n=6-9) were infected with HIV-1BaL, exposed to P. stercorea, then cultured in the presence or absence of physiologic doses of butyrate (0-2mM). LP CD4 T cell activation, infection and death phenotype were determined by flow cytometry, while cytokine production was determined by ELISA. Our results indicate that physiologic levels of butyrate inhibit HIV-1 + P. stercorea specific LP CD4+ T cell activation, T helper cytokine production (IL-17, IFNγ and IL-22), HIV-1 infection and T cell apoptosis. The findings suggest that a loss of immunoregulatory SCFA resulting from gut dysbiosis could contribute to HIV-1 associated intestinal mucosal pathogenesis.
Elucidating the Role of Maternal Embryonic Leucine Zipper Kinase (MELK) in Adrenocortical Carcinoma

Adrenocortical carcinoma (ACC) is an aggressive cancer and although surgery is the first line therapy, most tumors are unresectable at presentation. Five year survival for ACC patients is less than 35%. Mitotane, an adrenolytic toxic insecticide, and EDP (etoposide, doxorubicin and cisplatin) are chemotherapeutic options, but have limited benefit. The recently formed UC Multi-Disciplinary Adrenal Tumor group provides an ideal platform for collaborative, translational research to advance the field. Using bioinformatic analyses of published databases we identified maternal embryonic leucine zipper kinase (MELK) as significantly overexpressed 5-25 fold in 40-50% of ACC samples compared to normal adrenal. Clinical data from The Cancer Genome Project (TCGA) suggests that increased MELK expression in ACC patients is correlated with shorter survival. MELK, a member of the AMPK/Snf1 family, is a highly conserved serine/threonine kinase overexpressed in some human cancers. Prior studies in breast, pancreatic and colon cancer showed that MELK is implicated in tumor growth and progression. To examine the role of MELK in ACC, a doxycycline inducible shRNA system was used to silence MELK in H295R ACC cancer cells. Loss of MELK (4-fold) was associated with a 3.5-fold decrease in tumorigenesis as measured by colony formation in soft agar (p=0.006). Next, we examined the role of MELK in cell survival. Since there is recent suggestion for the role of hypoxia in ACC we exposed control and shMELK H295R cells to a hypoxic, serum depleted microenvironment (1%O2). shMELK cells were associated with an increased rates of apoptosis (6-fold, 8-fold and 2.5-fold) as detected by caspase-3, PARP and TUNEL assays (p=0.001, p=0.00002, p=0.02 respectively). Incucyte assays demonstrated that MELK silencing was associated with decreased rates of proliferation (50%) in hypoxic conditions (p<0.01). Thus, silencing MELK in ACC human cells decreases tumorigenesis by increasing apoptosis and decreasing cell proliferation. MELK is an attractive novel target for future in vivo studies using a commercially available MELK inhibitor. Animal studies will provide evidence regarding anti-tumor activity of the MELK inhibitor which will form the basis for future studies on targeting MELK in patients with ACC.
Resident Bacteria Recruit IL-6 Producing Intraepithelial Lymphocytes in the Colon to Promote Barrier Integrity

Interactions between the microbiota and distal gut are important for the maintenance of a healthy intestinal barrier; dysbiosis of colon bacteria has emerged as a likely contributor to diseases that arise at the level of the mucosa. Intraepithelial lymphocytes (IELs) are positioned within the epithelial barrier, and in the small intestine, function to maintain epithelial homeostasis. We hypothesized that IELs of the colon modulate epithelial barrier function through the liberation of cytokines stimulated by interactions with resident bacteria. Our data demonstrate that candidate bacteria such as Alistipes species of the phylum Bacteroidetes recruit IELs to the colon where they produce IL-6 in a TCR-dependent fashion. IEL-derived IL-6 is functionally important in the maintenance of the epithelial barrier as IL-6/- mice were noted to have increased paracellular permeability and closer interaction with luminal bacteria which was reversed by transfer of IL-6+/+ IELs. IL-6 was found to signal in colonic epithelial cells and resulted in increased claudin1 and muc2 expression. Therefore, we conclude that the host microbiota provides a homeostatic role for epithelial barrier function through regulation of IEL derived IL-6.
Th2-Recruited Ly6C+ Monocytes Activate TGF-β via Thrombospondin-1 to Cause Pulmonary Vascular Disease Due to Schistosoma mansoni

Background: TGF-β signaling underlies many forms of vascular diseases, including Schistosoma and hypoxia-induced pulmonary hypertension (PH). TGF-β is largely regulated at the level of activation, but how TGF-β is activated in PH is unknown. We hypothesized that TGF-β activation by thrombospondin-1 (TSP-1) is required for the development of PH following Schistosoma exposure, a common cause of PH driven by Th2 inflammation.

Methods: We investigated TSP-1 loss of function in Schistosoma exposed mice, using pharmacological peptide inhibitors and bone marrow (BM) transplant of TSP-1/-, CCR2/- cells. TGF-β activity was measured using a PAI-1 promoter-luciferase reporter cell line. Results were correlated with hypoxic-PH bovine and scleroderma-PH human samples.

Results: Following Schistosoma exposure, TSP-1 expression in the lung increases, via Th2 inflammation-dependent recruitment of circulating Ly6C+ monocytes into the adventitial space. Cytokines directing recruitment of these monocytes are expressed by M2-activated tissue macrophages. TSP-1 inhibition and TSP-1 deficient BM protect against PH by blocking TGF-β activation. CCR2-deficient BM is also protective by preventing Ly6C+ monocyte recruitment. TSP-1 was increased in chronic hypoxia-exposed mice and its blockade also prevented PH in these animals. Further, TSP-1 was also increased in two bovine models of neonatal hypoxia-induced PH and chronic naturally occurring PH. In the plasma of patients with scleroderma, the TSP-1 plasma concentration significantly increased following PH development.

Conclusion: Blockade of Th2-dependent Ly6C+ monocyte-derived TSP-1 protects against TGF-β activation and pulmonary vascular disease due to Schistosoma. Targeting TSP-1-dependent pathologic activation of TGF-β could be a novel approach in inflammation-mediated, TGF-β-dependent vascular diseases.
Physicians and Pharmacists on Over-the-Counter Contraceptive Access for Women

Background: Recently, legislation has been proposed nationally to provide easier access to oral contraceptive pills. The proposed legislation entitled ‘Allowing Greater Access to Safe and Effective Contraception Act’ seeks to allow “routine-use contraceptives” to be sold over-the-counter (OTC). This legislation would increase women’s access to contraception; however, it could also increase out-of-pocket cost because the Affordable Care Act contraception provision only covers prescription contraception. Alternative legislation entitled ‘Affordability is Access Act’ requires health insurance to cover costs of oral contraception when it becomes OTC. California and Oregon have transitioned to allow access to oral contraception through pharmacist provision. Undeniably, political and healthcare leaders are investigating alternative methods of access to oral contraception. The goal of this study was to assess and compare the perceptions of Colorado physicians, pharmacists, and pharmacy technicians on OTC access to progestin-only oral contraception in order to identify specific concerns among health professionals to inform future policy and to guide effective provider education.

Methods: A physician-specific survey was created for faculty physicians in internal medicine, family medicine, and obstetrics and gynecology at the University of Colorado School of Medicine and emailed to faculty members. A pharmacist and pharmacy technician specific survey was created for pharmacists and pharmacy technicians practicing in Denver County outpatient pharmacies. Two student researchers visited 74 randomly selected pharmacies on the list of Denver pharmacies, and they attempted to obtain responses from one pharmacist and one pharmacy technician at each site. Survey responses were compared among respondent groups using chi-square and Fisher’s exact tests.

Results: Responses were collected from 56 physicians, 58 pharmacists, and 43 pharmacy technicians. There was a statistically significant relationship between profession (physician, pharmacist, and pharmacy technician) and how confident the survey participant felt about patients correctly using the progestin-only contraception without prior consultation (p-value=0.0318). Surveyed physicians, pharmacists, and pharmacy technicians “somewhat agree” that OTC progestin-only contraception will have a positive impact on women’s health (58.93%, 56.14%, and 41.64% respectively), and there was no statistically significant difference among groups (p-value=0.142). However, there was a significant difference on top concern of OTC access of progestin-only contraception (p-value <0.001). The biggest concern of physicians was efficacy of progestin-only contraception (35.71%), while pharmacists and pharmacy technicians both responded that patient self-assessment of contraindications was their top concern, (35.09% and 23.82% respectively).

Conclusions: Our study has shown that there are differing views among healthcare professionals on OTC provision of progestin-only contraception. This study builds upon previous literature including the work of Daniel Grossman et al. that indicates that most women are able to self-assess for contraindications using simple checklists. Given recently proposed legislation, it is crucial to seek the perspectives of primary stakeholders, including physicians and pharmacists. Legislators should address the concerns of healthcare professionals before moving forward with OTC provision. While provider/physician prescribing is a barrier to contraceptive access and adherence, moving oral contraception to OTC status may create unforeseen barriers including removing insurance copays and increasing direct costs to women. Affordable prices, assured insurance reimbursement, and clear packaging may alleviate some concerns.
Intracellular Purine Metabolism in Intestinal Epithelial Cell Barrier Formation, Maintenance, and Healing

Deterioration of the intestinal epithelial cell (IEC) barrier plays a fundamental role in the pathogenesis of inflammatory bowel disease (IBD). A metabolomic analysis of colon tissue revealed broad depletion of purine metabolites in a murine model of colitis, metabolites well known in IEC signaling, barrier formation and maintenance, and energy homeostasis. In contrast, examination of the purine metabolites in T84 cells during the early stages of barrier formation show elevated purine levels relative to cells with a fully formed barrier, indicating a likely need for the metabolites in barrier restitution and wound healing. Previous work has shown that supplementing IECs with butyrate and creatine substantially increases barrier function. With this knowledge we explored the effect of the supplementations on purine metabolism to identify possible biomarkers of, and therapeutic targets for IBD. Presented herein are the progress and results of our research of this important group of metabolites.
PTEN Protects against Angiotensin II-induced Pathological Cardiovascular Fibrosis and Remodeling

Pathological cardiovascular remodeling, in particular fibrotic changes that reduce vascular compliance and contribute to cardiac complications, develop in hypertensive heart disease and are major contributors to heart failure. Inflammation and immunity play significant roles in pathological cardiovascular remodeling and fibrosis. Therapeutic interventions that reduce recruitment and/or activation of these cells will likely be beneficial in reducing fibrosis-mediated vascular stiffening and cardiac dysfunction. Our published data demonstrate that loss of the lipid and protein phosphatase PTEN contributes to significant cardiovascular fibrosis and accumulation of inflammatory cells. Here we determined the role of PTEN overexpression on AngII-induced cardiovascular fibrosis and remodeling. Using a mouse model with systemic elevation of PTEN expression (sPTEN), sPTEN and wildtype (WT) control mice were subjected to subcutaneous AngII infusion by osmotic pumps for 28 days. Compared to WT mice, AngII-induced aortic and cardiac fibrosis was significantly blunted in sPTEN mice as shown by picrosirius red and masson's trichrome staining. Movat’s pentachrome stain showed reduced medial mucin deposition in sPTEN compared to WT mice when treated with AngII. Interestingly, PTEN elevation did not prevent AngII-mediated cardiomyocyte hypertrophy, suggesting that PTEN protects against fibrosis, but maintains cardiac contractility. To examine the infiltration of immune cells, immunohistochemistry was performed to identify T cells and macrophages. AngII-mediated adventitial accumulation of T cells and macrophages was blunted in sPTEN mice. Collectively, these data support a role for PTEN as an anti-fibrotic and anti-inflammatory target.
Semaphorin 7a is a Driver of Lymphovascular Metastasis

Postpartum breast cancers, defined as breast cancers diagnosed within 5-10 years of last childbirth, are nearly twice as likely to become metastatic; this devastating diagnosis affects thousands of young women annually. In pre-clinical models of postpartum breast cancer we have shown that tumor growth, invasion, lymph vessel density (LVD), lymphovascular invasion (LVI), and lymph node (LN) and lung metastasis are increased when breast tumor cells are implanted into postpartum mouse mammary glands compared to those in nulliparous mice. In addition, we revealed increased LVD and LN metastasis in postpartum patients (Lyons, TR et al., Nat Med 2011 and JCI 2014). We identified Sem7a protein as increased in postpartum breast cancer patient samples and in nulliparous patient tumors that recurred. In addition, increased SEMA7A mRNA expression is associated with decreased overall and distant metastasis free survival, as well as with increased LVI in patient datasets (Black, S...Lyons, TR, Oncogene 2016). Using silencing and overexpression approaches we observe that growth, invasion, LVD, LVI, as well as LN and lung metastasis are dependent on Sem7a. In addition, we observe that Sem7a is important for adhering to lymphatic endothelial mono-layers and survival in non-adherent conditions. Cumulatively, our results suggest that Sem7a supports multiple steps in the metastatic cascade. As such, we propose that Sem7a is a driver of metastasis for postpartum patients and a risk factor for metastasis in non-postpartum patients and therefore should be explored as a biomarker and therapeutic target.
Presenter: Jason Mansoori, MD

Contact info: jason.mansoori@ucdenver.edu

Division: Pulmonary Sciences and Critical Care Medicine

Researcher Category: Fellow/Postdoc

Research Type: Outcomes Research

Variable Fluid Resuscitation Practices Associated with Poor Risk-Adjusted Performance and Potential Harm in Critically Ill Septic Patients

Background: Practice variability, including in the intensive care unit, is pervasive in the way tests are ordered and medical or surgical treatments are prescribed (Wennberg et al, BMJ 2011). However, there is little systematic quantitation of the variability in usual care of critically ill septic patients. Unwarranted variability in clinical decision-making raises the potential for harmful practice and is a considerable threat to high-value care.

Objective: To determine the effect on hospital mortality of severity-adjusted treatment variability in fluid resuscitation of critically ill septic patients.

Methods: We conducted a retrospective outcomes analysis using the Premier Inc. database of day-one fluid resuscitation practices in critically ill, mechanically ventilated adult patients discharged with ICD-9-CM codes compatible with sepsis and septic shock. Models for predicted day-one fluid resuscitation, propensity to receive five or more liters of fluid, and predicted hospital mortality were developed and applied, comparing observed to predicted day-one fluid resuscitation and hospital mortality.

Results: 32,319 patients from 400 hospitals met criteria for analysis. There was a wide range of prescribed fluid resuscitation volumes (median volume 3,050mL, IQR 1,975-5,000mL) and a significant association between increasing amounts of day-one fluid resuscitation and worsened observed hospital mortality compared with predicted mortality. Our predicted day-one fluid resuscitation model identified eight fluid reductive factors and five fluid additive factors. Compared to the expected-resuscitation group, risk-adjusted observed hospital mortality was statistically significantly worse in under-resuscitated (52.1% vs. 49.2%, p = 0.037) and over-resuscitated (57.5% vs. 49.2%, p < 0.001) patients without any fluid reductive factors. In patients with one or more fluid reductive factors, risk-adjusted hospital mortality was statistically significantly worse only in over-resuscitated patients (47.1% vs 41.5%, p < 0.001).

Conclusions: These data demonstrate a high degree of severity-adjusted treatment variability and an association with harm amongst critically ill, mechanically ventilated septic patients. A significant performance gap was observed between patients with and without one or more fluid reductive factors.
**The Introduction of Direct Oral Anticoagulants Is Associated With Improved Overall Oral Anticoagulation Rates in Atrial Fibrillation: Insights from the NCDR PINNACLE Registry**

**Background:** While oral anticoagulation (OAC) reduces the rate of stroke and thromboembolism for patients with atrial fibrillation (AF), previous work has demonstrated OAC with warfarin is underutilized for AF. Direct oral anticoagulants (DOACs) provide effective alternatives to warfarin and thus may improve overall OAC rates. However, the association of DOAC use on overall OAC rates in AF is unknown.

**Methods:** Within the NCDR PINNACLE registry, we identified 3,164,236 outpatient encounters with 655,000 non-valvular AF patients with CHADS-VASc scores $>1$ occurring between April 1, 2008 and September 30, 2014. We analyzed temporal trends of overall OAC, overall DOAC, and individual DOAC (dabigatran, rivaroxaban, and apixaban) rates. Multivariable logistic regression was performed to identify factors associated with OAC and DOAC use, including CHADS-VASc score, age, weight, CAD, PAD, dyslipidemia, stroke/TIA, heart failure, gender, hypertension, and diabetes mellitus. To assess for practice level variation in OAC and DOAC use, we then used a two-level hierarchical logistic regression model including practice specific random effect.

**Results:** Overall OAC rates increased from 52.4 to 60.7% during the study period among eligible AF patients ($p$ for trend $<0.01$). Warfarin use decreased from 52.4% to 34.8% ($p$ for trend $<0.01$) and DOAC use increased from 0% to 25.8% ($p$ for trend $<0.01$). In the final quarter of the study period, rivaroxaban was the most frequently prescribed DOAC (12.1%), followed by dabigatran (6.6%), and apixaban (5.7%). In a subset analysis, most patients who received a DOAC had previously received warfarin. There was significant practice level variation in rates of use of OAC (range 11-78.8%, median odds ratio [MOR] 1.52, 95% confidence interval [CI] 1.45-1.57) and of DOACs (range 0-40.4%, MOR 3.58, 95% CI 3.05-4.13). Increasing CHADS-VASc score was associated with higher rates of use of overall OAC but lower rates of use of DOACs.

**Conclusions:** In a large U.S. outpatient registry, the introduction of DOACs into clinical practice was associated with higher rates of overall OAC use in AF patients at high risk for stroke. However, OAC and DOAC use differed by CHADS-VASc score. Significant practice level variation exists in rates of use of DOACs for AF.
Deletion of c-FLIP in CD11b+ Macrophages Protects against Bleomycin-induced Lung Fibrosis

The biologic mechanisms that underlie the development of lung fibrosis are incompletely understood. There is evidence that adult-derived CD11b+ macrophages recruited to the lung following injury (recMø) may influence this process. In the LPS model of non-fibrotic lung injury, recMø undergo caspase-8 dependent apoptosis that contributes to the resolution of injury. We show that in the bleomycin model of fibrotic lung injury, recMø persist and fail to undergo apoptosis. We hypothesized that restoring recMø apoptosis would prevent the development of fibrosis. To target recMø apoptosis during fibrosis, we established c-FLIPΔrecMø mice where inducible deletion of the anti-apoptotic protein c-FLIP, which specifically inhibits caspase-8 dependent apoptosis, was targeted to recMø using the hCD68-rtTA system. Deletion of c-FLIP in recMø immediately after injury attenuated recMø accumulation following bleomycin by sensitizing recMø to caspase-8 dependent cell death. Inducible deletion of c-FLIP in recMø immediately after injury or during the onset of fibrosis protected against the development of fibrosis, as shown by decreased collagen deposition and improved lung compliance. recMø drive the progression of lung fibrosis; c-FLIP and recMø may provide a therapeutic target for the treatment of fibrosis.
The Role of Alarmins in Chronic Beryllium Disease: Oxidative Stress, Necrosis and Dendritic Cell Activation

Chronic beryllium disease (CBD) is a granulomatous lung disease that occurs in individuals who have been sensitized to beryllium (Be). Sensitization of Be-specific CD4+ T cells requires dendritic cells (DCs) to present both antigenic and costimulatory signals to naïve CD4+ T cells. Previous work showed that Be exposure promoted migration of pulmonary DCs expressing elevated levels of CD80 and CD86 to the draining lymph nodes (LNs). This effect was MyD88-dependent, however the receptors involved were not determined. In this study, we hypothesized that the innate immune system is triggered by the release of DNA and IL-1alpha. These alarmins are constitutively present in the nucleus and are released upon necrotic cell death. We have shown that following pulmonary exposure to Be, alveolar macrophages were reduced in number coincident with increased cell death in the BAL. This precluded the release of IL-1alpha and DNA into the alveolar space. Here we show that alveolar macrophages stimulated with Be(OH)2 and BeSO4 in vitro released DNA and IL-1alpha. This effect was dependent upon phagocytosis and required reactive oxygen species (ROS). Be-exposed mice that had been depleted of alveolar macrophages had reduced levels of IL-1alpha and DNA compared to mice with normal numbers of alveolar macrophages. Both IL-1R and TLR9 are MyD88-dependent receptors and mice injected with ligands for either receptor (IL-1 or DNA) upregulate costimulatory molecules on DCs. However Be-induced upregulation of costimulatory molecules was similar in Be-exposed WT, IL-1R and TLR9KO mice. Thus, we hypothesized that both pathways must be to be disrupted in the same mice to see an effect. To do this, we treated TLRKO mice with an IL-1alpha blocking antibody, exposed them to Be in the lung, and followed DC costimulatory molecule expression in the LNs. Compared to WT mice, upregulation of CD80 and CD86 on DCs was impaired in the TLR9KO mice treated with anti-IL1alpha. Thus, DNA and IL-1alpha play redundant roles in Be-induced activation of DCs. Taken together, these data suggest that Be taken into phagosomes can initiate the release of ROS leading to necrosis and release of IL-1alpha and DNA. These alarmins can both induce upregulation of CD80 and CD86 on DCs. These effects of Be on DCs provide potent sensitization of Be-specific CD4+ T cells in CBD.
The Role of Bone Marrow-derived cPLA2α in Experimental Renal Fibrosis

Background: Progressive histologic renal fibrosis heralds the development of chronic kidney disease (CKD), a growing worldwide health epidemic. Renal fibrosis is characterized by poorly defined signaling events between resident renal epithelial, endothelial, and stromal cells with recruited inflammatory cells. The group IVA cytosolic phospholipase A2 (PLA2G4A, or cPLA2α) enzyme is the rate-limiting enzyme in eicosanoid production and responsible for a host of biologic effects in multiple tissues, including the kidney. Published data has implicated many distinct eicosanoid products in the progression of CKD in unrelated animal models; though the complexity of the involved pathways indicates that eicosanoids produced by different cell types may act to either promote or inhibit injury. Current data support a pivotal role for cPLA2α, derived specifically from circulating inflammatory cells, in directing the “eicosanoid storm” which accompanies tissue injury, infection, autoimmunity, and organ fibrosis. We sought to establish the role of bone marrow-derived cPLA2α in an animal model of renal fibrosis in order to evolve a more sophisticated understanding of eicosanoid expression from specific cell types in the diseased renal microenvironment.

Methods: Wild type (WT) C57BL/6 mice were lethally irradiated and transplanted with bone marrow obtained from either WT mice or cPLA2α-deficient (Pla2g4a-/-, or KO) mice. Animals were allowed to fully engraft and were then subjected to unilateral ureteral obstruction (UUO). After UUO, uninjured contralateral (control) kidneys and injured obstructed (UUO) kidneys were collected for flow cytometry, RNA, protein, and histologic analysis.

Results: Compared with WT-engrafted controls, KO-engrafted mice had attenuation of renal fibrosis at 14 days after UUO as assessed by picrosirius red (PSR), collagen 3, and alpha smooth muscle actin (αSMA) staining. KO-engrafted animals had decreased message of pro-fibrotic αSMA and fibronectin; and decreased pro-inflammatory chemokines such as fractalkine, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor alpha (TNFα). Additionally, 3 days after UUO KO-engrafted animals had less infiltration of CD45+ leukocytes and pro-inflammatory CD11b positive-Ly6c positive cells than WT-engrafted controls.

Conclusion: These data highlight a critical role for cPLA2α expression, derived specifically from invading inflammatory cells, in both inflammatory cell recruitment and renal fibrosis progression after ureteral obstruction in mice.
Presenter: Alia Moore, MD

Contact info: alia.moore@ucdenver.edu

Division: General Internal Medicine

Researcher Category: Fellow/Postdoc

Research Type:

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**HPV Vaccination in Correctional Care: Attitudes, Knowledge and Barriers among Female Inmates**

Background: Women in correctional care settings are four times more likely to develop cervical cancer than their non-incarcerated peers. The Human Papillomavirus (HPV) has been implicated in up to 70% of cervical cancer diagnoses, and justice-involved women are at increased risk for HPV infection due to limited healthcare access and a high frequency of risky sexual behaviors. The HPV vaccine protects against both genital warts and carcinogenic strains of HPV, and is an important healthcare measure that could reduce the cervical cancer illness burden within this population. However, while justice-involved women would likely benefit from the HPV vaccine, it is important to identify the factors that may influence their decision to seek or accept it.

Methods: This study will assess attitudes toward, knowledge of, barriers to, and reported rates of HPV vaccination among English-speaking female inmates aged 18-26. Participants will be recruited via opportunistic sampling. The close-ended survey instrument will be administered face-to-face, and includes questions adapted from existing, validated health behavior surveys as well as original questions based upon the Health Belief Model. The study is currently in progress, and is being conducted in cooperation with the Colorado Department of Corrections.

Results: Based on prior surveys of HPV vaccination in similar populations, study participants are anticipated to have high rates of HPV vaccine acceptability in the setting of significant barriers to vaccination, including incarceration, cost, lack of insurance and healthcare access, mistrust of the medical community, and incomplete knowledge of the benefits of the vaccine.

Conclusions: Gathering information on reported vaccination rates as well as knowledge and acceptability of, and barriers to receiving the HPV vaccine will provide crucial information that will guide future educational and therapeutic interventions for this population. It may also inform policy decisions within correctional facilities aimed at improving female preventive care and vaccine delivery.
Loss of PTEN Correlates with Reduced αSMA Expression in Dedifferentiated Smooth Muscle Cells of Human Coronary Arteries Affected by Atherosclerosis or Continuous Flow Left Ventricular Assist Devices

Phosphatase and tensin homolog (PTEN) is a negative regulator of vascular smooth muscle cell (SMC) proliferation and injury-induced vascular remodeling. Recently, we have shown nuclear PTEN interacts with serum response factor (SRF) to regulate SRF-dependent transcription and control SMC differentiation. In this translational study, we examined the relationship between PTEN expression and SMC differentiation in human coronary arteries and the effects of atherosclerosis and continuous flow LVAD. PTEN and SMC α-actin (αSMA) were detected by immunofluorescence confocal microscopy in 55 human coronary arteries from 27 individuals with heart failure undergoing cardiac transplantation and from 5 non-transplanted hearts. Areas of PTEN and αSMA abundance and expression levels of PTEN and αSMA in single cells were quantified with Matlab and compared among intimal and medial SMCs. Levels of PTEN and αSMA were significantly reduced in intimal SMCs compared to medial SMCs in arteries with non-atherosclerotic hyperplasia (NAH), atherosclerotic hyperplasia (AH) or complex plaque (CP). αSMA+ cells in CP vessels had significantly less PTEN compared to NAH vessels (P=0.017). Analysis at the single cell level showed similar findings. NAH arteries from patients with an LVAD showed marked vascular remodeling, thicker media, and reduced PTEN and αSMA in medial SMCs compared to matched vessels from patients without LVAD. This was correlated with increased medial collagen deposition and vessel stiffening in vessels from LVAD compared to non-LVAD patients. In summary, PTEN correlates with αSMA expression in human coronary arteries and the extent of SMC dedifferentiation increases in vessels with atherosclerosis or LVAD exposure.
**This is My Gym**

Introduction: Jodi Holtrop, PhD and Andrea Nederveld, MD received a partnership development grant from CCTSI in cycle 7. The goal of this grant project was twofold; to bring together a group of individuals in Mesa County, CO to strategize about possible community responses to pediatric obesity and also to engage providers in Western Colorado around participation in Practice-Based Research Networks (PBRNs).

Project Activities: In the fall of 2015, the first meeting of the Mesa County Pediatric Obesity Task Force was held. A diverse group of people was recruited for this project. Early on, consensus was reached that the task force wanted to concentrate on obesity prevention for all children in our community, particularly children from lower socioeconomic status. The task for met several times over six months, using Bootcamp Translation techniques. The primary outcome was the conviction that families need to be physically active through play and exploration of their built and natural environments, recognizing that there are many such opportunities available in Mesa County. We created a slogan “This is my Gym” that we would like develop into a media campaign showing kids and families engaged in physical activity in various locations around Mesa County. We chose to focus our future activities on providing support and encouragement for families to participate in free or low-cost activities in their own geographic locations and to find ways to engage their friends and neighbors as well. In addition, Drs. Holtrop and Nederveld met with several providers regarding practice-based research network participation.

Response was very favorable and several expressed interest. A larger informational meeting was held in April, 2016 and from that meeting, it was decided to pursue development of a Western Slope specific PBRN.

Future Directions: Two funded grant proposals resulted from our work, the first by the Colorado Health Foundation under the “Activating Places and Spaces Together” funding opportunity. This project will create activity committees in Title I elementary schools with the purpose of increasing family activity in the existing built environment surrounding the schools. The second was submitted to CCTSI as a Community Engagement Pilot Program and will support pilot testing and evaluation of a summer activity program for Mesa County children and families. A steering committee was formed to being planning the PBRN. This committee is planning to meet monthly during 2016 and 2017, and has begun to consider possible starting projects and funding.
Suppressing Autophagy Leads to Increased Apoptosis in Pkd1 Knockout Models

Background: Increased proliferation and apoptosis play a role in cyst growth. The link between autophagy and apoptosis in PKD is not known. Accumulation of p62 during autophagy is crucial for activation of caspase-8 and subsequent activation of caspase-3, the major mediator of apoptosis.

Methods: 90 and 150 day old mice with a kidney specific tamoxifen-inducible Pkd1 knockout were treated with bafilomycin (Baf), a lysosomal inhibitor, to measure autophagic flux. Human Pkd1/- (WT 9-12) RCTE cells with a homozygous mutated Pkd1 were treated with lysosomal inhibitor chloroquine (C) to measure autophagic flux. LC3-II (autophagic flux), cleaved caspase-3 (CC-3), cleaved caspase-8 (CC-8), p62 (autophagy/apoptosis crosstalk) were measured by immunoblot. Annexin-V staining (apoptosis) was measured by flow cytometry.

Results: The decrease in LC3-II with Baf (decreased autophagic flux) and increased p62 in Pkd1/- kidneys was associated with increased CC-3 and CC-8. C resulted in an increase in LC3-II in control RCTE (+/+ ) cells but not in Pkd1/- cells suggesting decreased autophagic flux. Decreased autophagic flux and increased p62 in human Pkd1/- cells was associated with increased apoptosis (annexin V staining), increased CC-8 and increased CC-3.

Conclusions: The lack of effect of the lysosomal inhibitors to increase LC3-II in Pkd1/- kidneys and in Pkd1/- cells suggests a defect in autophagy resulting from a block of autophagosome-lysosome fusion and degradation. Suppressed autophagy is associated with increased apoptosis and apoptosis/autophagy crosstalk. Autophagy inhibition with Baf or chloroquine leads to increased apoptosis in Pkd1/- models.
Mechanisms of Decreased Acute Kidney Injury (AKI) and Decreased Tumor Growth by Mitogen-activated Extracellular Signal-regulated Kinase (MEK) Inhibition

Background: The pathogenesis of cisplatin (Cis) AKI involves the MEK/ERK pathway. The MEK inhibitor U0126 blocks ERK activation. The effect of U0126 on AKI and tumor growth was determined in a 4-week model of cisplatin AKI in tumor bearing mice.

Methods: Mice were injected with lung cancer cells. 10 days later, Cis (10 mg/kg/wk) and U0126 (5 mg/kg 2X/wk) were given for 4 weeks. RIP3, a marker of necroptosis, cleaved casp-3 (CC-3), a marker of apoptosis, PD-L1, an immune system suppressor, p-JNK and p-ERK (MAPK signaling) were measured by immunoblot. MAPK Signaling RT² Profiler™ PCR Array was used to profile genes in the kidney.

Results: U0126 resulted in a significant decrease in BUN, SCr in mice with or without cancer. In kidney, U0126 protected against AKI despite increasing RIP3. ERK regulated cell cycle genes specifically cyclins, cyclin-dependent kinases (Cdk) and Cdk inhibitors were up to 78-fold increased by Cis and reduced by U0126. U0126 decreased tumor weight, potentiated the effect of cisplatin, increased CC-3, decreased p-ERK and p-JNK, and had no effect on PDL1 in tumors.

Conclusions: The effect of U0126 to decrease AKI and cancer growth is independent of the presence of cancer. In kidney, U0126 increased RIP3 and the large increase in gene expression of cell cycle proteins regulated by ERK was decreased by U0126. Cell cycle proteins regulated by ERK signaling in AKI and cancer merits further study.
Clinical Phenotype of Patients with Interstitial Pneumonia with Autoimmune Features: An Inception Cohort from an Autoimmune Lung Center

Background: Some patients considered to have idiopathic interstitial lung disease (ILD) possess certain clinical features that suggest an underlying autoimmune process; however, they do not meet established criteria for any connective tissue disease (CTD). Differing criteria and terms have been used to describe these patients. With an aim to achieve consensus around how to label, define, and study these cohorts the consensus-derived classification of “interstitial pneumonia with autoimmune features” (IPAF) (Table 1) was recently published. The objective of this study was to describe the clinical phenotype of a single center inception cohort consisting of patients that fulfill classification criteria for IPAF.

Methods: All patients received clinical care from a rheumatologist (AF) experienced in ILD between June 2015 and March 2016. All were confirmed to have ILD by thoracic high resolution computed tomography and/or surgical lung biopsy. Based on a combination of clinical, serologic, radiologic or pathologic features, each patient was considered by AF to have an autoimmune basis for their ILD. None had a defined CTD or alternative etiology to account for the presence of ILD (e.g., infection, drug, environmental or occupational exposure). Clinical data at the time of initial evaluation at our center were extracted from the comprehensive electronic medical record and used to determine whether each patient fulfilled classification criteria for IPAF.

Results: The cohort consisted of 7 patients. Median age was 62 years (47-67) and the cohort was female predominant (n=5, 71%) and ethnically diverse (4 were White, 1 each were Black, Latino, and Asian-American). None were active smokers but 3 had a prior history of cigarette smoking. The median percent predicted forced vital capacity was 63 (41-92) and median percent predicted diffusing capacity for carbon monoxide was 37 (14-60). Two of the 7 did not have a feature from the clinical IPAF domain, but all 7 had at least one feature in both the serologic and morphologic IPAF domains (Table 2). All were initiated on immunosuppression with a combination of corticosteroids and a steroid-sparing agent: 4 with azathioprine and 3 with mycophenolate mofetil.

Conclusion: In this single center study we describe the clinical, serologic, and thoracic morphologic features of an inception cohort of patients fulfilling classification criteria for IPAF. The prospective study of this and other IPAF cohorts will better inform our understanding of this novel group of ILD patients.
Characterization and High-Throughput Drug Screening of a Cardiomyopathy Using Cardiomyocytes from Patient-Derived Induced Pluripotent Stem Cells

Danon disease is an untreatable and severe X-linked form of cardiomyopathy that leads to profound cardiac hypertrophy, causing death or a need for cardiac transplantation in males by the third decade. Danon disease is known to be caused by loss of the lysosome-associated membrane protein-2 (LAMP-2) protein, which is a membrane glycoprotein likely involved in lysosome-autophagosome fusion and vesicle trafficking during autophagy. However, it is unknown how LAMP-2 protein deficiency leads to the development of Danon disease. To better characterize the molecular and cellular pathogenic mechanisms involved and enable high-throughput screening (HTS) of compound libraries, human induced pluripotent stem cells (iPSCs) were generated from patients with Danon disease and differentiated into cardiomyocytes. RNA transcription levels from the Danon iPSC-derived cardiomyocytes (iPSC-CMs) were analyzed using RNA-Sequencing (RNA-Seq) and expression of key genes confirmed via quantitative real-time PCR (qRT-PCR). The data show significant upregulation of genes involved in cellular respiration and sarcomere expression in Danon iPSC-CMs compared to unaffected control iPSC-CMs. The upregulation of cellular respiration transcripts in our data align with published data suggesting mitochondrial function is affected in these cells, including increased levels of mitochondrial oxidative stress. Our RNA-Seq findings of potentially impacted molecular pathways in the Danon iPSC-CM model are being used to identify biomarkers for conducting HTS drug discovery. Following development and validation of a primary HTS assay, a pilot HTS using a library of 355 kinase inhibitors and then a HTS drug discovery using a custom designed diversity library of 20,000 compounds will be performed and hits confirmed using secondary and tertiary assays. These confirmed hits may offer novel potential therapeutic treatments for individuals with this currently devastating and untreatable cardiomyopathy.
Influence of Altered Intestinal Microbiome on Intraepithelial Lymphocyte Trafficking

Intraepithelial lymphocytes (IELs) are a unique population of antigen-experienced T cells that are distributed between intestinal epithelial cells and function to protect the epithelium from microbial invasion and maintain homeostasis. Intestinal microbiome studies have shown an alteration of individual species of bacteria in individuals with inflammatory bowel disease and ankylosing spondylitis compared to healthy controls. However, the mechanistic relationship between the microbiota and immune cells, and the subsequent pathogenic targeting of the spine has not been defined. Ongoing studies have revealed that bacterial dysbiosis in patients with IBD and mouse models alters the function of IELs. We hypothesize that resident bacteria educate IELs in the intestine, which will then traffic systemically and cause arthritis under dysbiotic conditions. To model dysbiosis in mice, broad-spectrum antibiotics (ampicillin, neomycin, metronidazole, and vancomycin) are administered in drinking water and recolonization of microbiota is achieved through cohousing with unmanipulated littermates. KikGR mice contain a transgene for a photoconvertible green-to-red fluorescent protein, and endoscopy-guided violet light allows for photoconversion of distal colonic IELs in vivo. TNFΔARE/+ X KikGr mice contain a mutation in the AU-rich element of the TNF-α gene resulting in increased mRNA stability and systemically elevated TNF-α. As a result, these mice develop ileitis and arthritis beginning around 8 weeks of age. Trafficking of photoconverted lymphocytes is determined by flow cytometry of tissues. One week following photoconversion of the distal colon lymphocytes in KikGR mice, labeled intestinal lymphocytes were detected systemically in tissues including the spleen, liver, lungs, and Achilles entheses; while few lymphocytes remained in the colon. Treatment of mice with antibiotics following photoconversion resulted in substantially reduced trafficking to distal tissues as well as a depletion of the colonic population. After recolonization, labeled colonic lymphocytes substantially increased in the colon while few remained in distal tissues. Analysis of TNFΔARE/+ mice demonstrates significant dysbiosis occurs as mice age and develop disease compared to TNF+/+ littermates. Our goal is to identify triggers for systemic IEL trafficking from the intestine during health and IBD-related ankylosing spondylitis and define the role of trafficked IELs in the target tissue.
Development of *Ex Vivo* Drug Sensitivity Profiling to Guide Therapeutic Decisions in Multiple Myeloma

Multiple myeloma (MM) is incurable, but for most patients it can be managed until treatment resistance develops. When resistant to IMiDs (immunomodulatory drugs) and proteasome inhibitors (PIs), the prognosis becomes poor. Patients have a debilitating course in which they are treated with sequential lines of combination therapy, comprised of at least 15 agents in 6 classes (IMiDs, PIs, alkylator chemotherapies, steroids, monoclonal antibodies and histone deacetylase inhibitors). This wealth of treatment options has led to confusion among treating hematologist/oncologists in choosing the next regimen in their patients’ care and in many cases a delay in the identification of an effective drug regimen resulting in increased morbidity and mortality. We have developed a novel flow cytometry-based assay of bone marrow aspirates to reproducibly measure the sensitivity of patient MM cells to these drugs. This assay could serve to address the lack of biomarkers that are predictive of treatment response. Our data shows initial progress towards defining parameters for resistance and the development of a format for high-throughput clinical testing. We have been assessing assay predictiveness retrospectively for use in three separate clinical scenarios: (1) at diagnosis to identify drug refractoriness, (2) at first relapse to identify early development of resistance, and especially (3) after multiple relapses to identify the agents that retain activity. As predicted, samples have displayed increasing drug resistance *ex vivo* as the disease course progresses. In patients with multi-relapsed disease, we have identified drugs that retain activity, which would provide treating physicians with a useful decision making information. The results support design of a prospective clinical trial to validate the clinical utility of this assay. If predictive of response, we foresee this assay could be widely adapted and dramatically improve outcomes for patients with MM and avoid wasted time, money and debilitating complications on ineffective drugs.
Druggable Genome and Proteome Landscape in Breast Cancer Xenografts

Extensive analysis of cancer proteomes is now possible due to recent advances in mass spectrometry (MS). Here, we employed quantitative proteomics to profile protein expression across 24 breast cancer patient-derived xenograft (PDX) models including 22 representing diverse breast cancer subtypes and 2 EBV-positive B-cell proliferative lesions arising in the NOD/SCID/ mouse strain. Integrated proteogenomic analysis showed positive correlation between expression measurements from transcriptomic and proteomic analyses; further, PAM50-based gene expression subtypes were largely re-capitulated using non-stromal protein markers. However, several protein/phosphoprotein events such as overexpression of AKT proteins and ARAF, BRAF, HSP90AB1 phosphosites were not readily explainable by genomic analysis, suggesting that druggable translational and/or post-translational regulatory events may be uniquely diagnosed by MS. Proteogenomic analysis also validated a number of predicted genomic targets and identified pathway crosstalks from phosphoprotein-specific antibodies reacting with multiple receptor tyrosine kinases. Drug treatment experiments targeting HER2 and various components of the PI3K pathway (e.g., PIK3CA and mTOR) supported proteogenomic response predictions in 7 xenograft models. In conclusion, proteomic investigation increases potential therapeutic options, can exhibit higher specificity than antibody-based phosphoprotein approaches and highlights the potential of PDX drug response evaluation in the context of detailed MS-based pathway analysis.
Protein-structure-guided Discovery of Functional Mutations across 19 Cancer Types

Local concentrations of mutations are well known in human cancers. However, their three-dimensional spatial relationships in the encoded protein have yet to be systematically explored. We developed a computational tool, HotSpot3D, to identify such spatial hotspots (clusters) and to interpret the potential function of variants within them. We applied HotSpot3D to >4,400 TCGA tumors across 19 cancer types, discovering >6,000 intra- and intermolecular clusters, some of which showed tumor and/or tissue specificity. In addition, we identified 369 rare mutations in genes including TP53, PTEN, VHL, EGFR, and FBXW7 and 99 medium-recurrence mutations in genes such as RUNX1, MTOR, CA3, PI3, and PTPN11, all mapping within clusters having potential functional implications. As a proof of concept, we validated our predictions in EGFR using high-throughput phosphorylation data and cell-line-based experimental evaluation. Finally, mutation–drug cluster and network analysis predicted over 800 promising candidates for druggable mutations, raising new possibilities for designing personalized treatments for patients carrying specific mutations.
Proteogenomic Integration Broadens Treatment Targets in Human Cancer

Although large-scale, next-generation sequencing (NGS) studies of cancers hold promise for enabling precision oncology, challenges remain in integrating NGS with clinically validated biomarkers. To overcome such challenges, we constructed a Database of Evidence for Precision Oncology ("DEPO") by aggregating information relating druggability to genomic, transcriptomic, and proteomic biomarkers. Using a pan-cancer cohort of 6,570 tumors, we found that only 9% could be matched to drugs according to their cancer type. By repurposing drugs across 22 cancer types using shared mutational biomarkers of druggability, we discovered that an additional 22% of tumors are druggable. Using a proximity-based clustering tool, we uncovered putative druggable mutations in close spatial proximity to known druggable mutations in 7% of tumors. We conducted outlier analysis of mRNA and protein expression data, which inferred potential druggability in 39% of tumors. Our analyses also revealed co-occurring druggable proteogenomic alterations in 32% of tumors with complete mutational, mRNA expression, and protein expression data, indicating a role for individualized combinational therapy. Finally, we identified variations in druggable biomarkers across ethnicities. Our results suggest that an integrated analysis platform utilizing a comprehensive evidence-based database can expand the percentage of druggable tumors from 9% to 60% and aid ongoing efforts to bring precision oncology to patients.
The Damaging Effects of Electronic Cigarettes on Lung Structure

Introduction: In recent years, electronic cigarettes (e-cigs) have gained popularity, initially as an aid to smoking cessation. As a result of marketing and flavors e-cig use has now grown amongst never-smokers and young adults. Due to lack of regulation of e-cigs by the FDA, little is known about their chemical composition and effects on pulmonary structure and function. Studies investigating the effect of e-cigs on lung parenchyma in human or animal models are lacking. In this study we compared the effects of exposure of e-cigs to tobacco smoke (TS) on lungs in rats to determine whether they are a safer alternative to cigarettes.

Methods: Rats were divided into three groups and exposed to: room air (RA-control); Blu® e-cigarettes; and Kentucky 2R4F reference cigarettes (TS) for four hours per day, five days per week, over four weeks. Daily nicotine exposure levels were estimated to be 48 mg, and 51 mg for the e-cigs and TS groups, respectively. Lung tissues were subjected to histopathological evaluation and western blot analysis. Alveolar wall density was measured by tissue surface area and calculated as a percentage using ImageJ software. Data were analyzed using one-way ANOVA and Tukey’s multiple comparisons test.

Results: Exposure to e-cigarette and tobacco cigarette smoke resulted in a significant decrease in alveolar density when compared to room air controls (p<0.05). Mean units ± standard deviation of alveolar density for room air control, e-cigarette exposure group, and cigarette group were 8.473 ± 2.237, 4.418 ± 0.652, and 3.885 ± 1.213, respectively. Western blot analysis revealed that caspase-3 was activated by both TS and e-cigarette compared to RA-control, which may explain the decrease in alveolar density. Activation of p38 and Erk together with caspase implicates a role for MAPK in its activation.

Conclusions: Our results show that exposure to e-cigarettes has the same damaging effects as tobacco cigarette smoke in a rat model. This research serves as the basis for further study that may refute the suggestion that e-cigarettes are a healthy alternative to cigarettes. The potential detriment to health supports stricter regulation of e-cigarettes including transparency regarding ingredients.
**Presenters:** Jacqueline Turner  
**Contact info:** jacqueline.turner@ucdenver.edu  
**Division:** Medical Oncology  
**Researcher Category:** Student  
**Research Type:** Basic Science

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**Investigating the Role of Different 5’ Partners in BRAF Gene Fusions in Melanoma**

Melanoma development and progression is often driven by activation of the MAP kinase pathway though aberrant activation of the BRAF kinase. BRAF is activated by mutations, amplifications, alternative splicing, or recently discovered gene fusions. BRAF fusions involving different 5’ gene partners have been described in cutaneous melanomas and Spitz nevi, however, oncogenic potential and treatment response between BRAF fusions with different 5’ gene partners has not been assessed. We used fluorescence in situ hybridization to analyze a cohort of 59 melanoma patient tumors and identified two different BRAF gene fusions, AGK-BRAF and ARMC10-BRAF. The ARMC10 5’ gene partner has not been described in melanoma and treatment response of ARMC10-BRAF has not been reported in any cancer type. We generated patient-derived xenograft (PDX) models for the two BRAF fusions and treated them with MAP kinase pathway inhibitors specific to MEK1/2 and ERK1/2. Both PDX models responded to the inhibitors, however, the ARMC10-BRAF fusion was more sensitive and showed stronger tumor regression. To further understand differences between BRAF fusions we used computational modeling to predict the protein structures of wild type BRAF, mutated BRAFV600E, and six BRAF gene fusions with 5’ partners including, AGK, ARMC10, KIAA1549, TRIM24, PPFIBP2, and ZKSCAN1. We found large variations in the MEK1/2 kinase binding domain between different 5’ partners, and we are currently overexpressing these BRAF fusions in a generic background to perform additional experiments and determine the tumorigenic potential and treatment response between these different 5’ partners. Patients harboring gene fusions should only be treated if the fusion demonstrates actionable driver potential. Understanding differential responses in BRAF gene fusions will improve clinical treatment of patients with BRAF gene fusions.
Maintenance of Serum Ionized Calcium during Exercise Attenuates the Exercise-Related Increase in Bone Resorption

Exercise is recommended to build or maintain bone mass, but there is evidence that vigorous or prolonged exercise is associated with bone loss under certain conditions. It is our contention that disruptions in calcium homeostasis during exercise lead to increases in parathyroid hormone (PTH) and bone resorption. PURPOSE: To determine if preventing the decline in serum ionized calcium (iCa) during exercise attenuates the increases in PTH and a marker of bone resorption (carboxy-terminal collagen crosslinks (CTX)). METHODS: Healthy, cycling-trained men (n=11, aged 18-45 y) underwent two identical 1-hour cycling bouts at ~75% VO2peak under conditions of calcium gluconate versus half-normal saline infusion. Blood was sampled every 5 minutes to adjust the calcium infusion rate, with a goal to maintain serum iCa at ~0.2 mg/dL above baseline. The same infusion protocol was replicated under the saline condition. Blood samples to assess PTH, CTX, bone formation (procollagen type 1 amino procollagen (P1NP)), and total calcium (tCa) were taken every 15 minutes during exercise and hourly for 4 hours post-exercise. RESULTS: Serum iCa was successfully maintained above baseline during Ca infusion and was significantly different from the saline condition during exercise (all time points p<0.01). Compared to saline, the Ca infusion markedly reduced the increase in serum PTH during exercise (p=0.004) and the suppression of PTH persisted throughout the 4-hour recovery period (AUC p<0.001). Similarly, the increase in CTX during exercise was suppressed with Ca infusion (p=0.003) and remained below the saline condition through recovery (AUC p<0.001). tCa was also a significantly higher during exercise (p<0.001) with Ca infusion, and remained elevated during recovery (AUC p=0.01). There were no differences between conditions for P1NP during (p=.47) or after exercise (p=.10). CONCLUSIONS: The increase in bone resorption was attenuated when the exercise-related decline in serum iCa was prevented, suggesting a calcium-dependent relationship. There was no effect of Ca infusion on P1NP, but the duration of post-exercise sampling may have been too short to capture any changes. The results are limited to young, trained, men during cycling exercise. Future research should investigate sex- and age-differences and other exercise modalities.
Presenter: Patrick Wood, MD

Contact info: patrick.wood@ucdenver.edu

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Researcher Category: Fellow/Postdoc

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Introduction: Perturbations in glucose homeostasis for patients with rheumatoid arthritis (RA) who initiate tumor-necrosis factor inhibitors (TNFis) are described in case reports and series. These events include reduced blood glucose, reduction of insulin requirements and resolution of diabetic disease with TNFi initiation. Hypoglycemic effects have also been reported in various non-biologic disease modifying anti-rheumatic drugs (DMARDs). The mechanism of these effects is unclear, although obesity-related cytokine pathways have been proposed. We evaluated the scope of these effects using a large patient registry.

Methods: Data from the Veterans Affairs Corporate Data Warehouse, Decision Support System National Pharmacy Extract, and Pharmacy Benefits Management were obtained for patients enrolled in the Veterans Affairs Rheumatoid Arthritis (VARA) registry. Blood glucose and hemoglobin A1c values were extracted for patients who initiated treatment with TNFi, prednisone, or non-biologic DMARDs during follow-up. Proximal values from 6 months prior to drug initiation were compared to values obtained between 8 weeks to 6 months post-initiation using t-tests and univariate regression analysis.

Results: Post-treatment A1c and blood gluoses were significantly increased from pre-treatment levels among those who started prednisone according to the t-test (103 vs 98.67 mg/dL, p < .01). Post-initiation A1c values were significantly lower in those started on sulfasalazine compared to pre-drug (5.3 vs 5.6%, p=.02). With linear univariate regression, drug initiation was associated with lower blood glucose values among those started on hydroxychloroquine (p=.01). No statistically significant differences in blood glucose or A1c were noted in patients started on any TNFi, methotrexate or leflunomide. Multivariate regression analysis is pending, which will attempt to account for comorbidity and other confounding variables.

Conclusion: Statistically significant decreases in A1c and blood glucose values were found surrounding the initiation of sulfasalazine and hydroxychloroquine. These data demonstrate support for potentially important glycemic effects that correspond to previously reported anecdotal hypoglycemic events, although complete multivariate analysis on these data are pending. Corresponding increases in glucose concentrations among prednisone users suggest face validity and adequate sensitivity to detect clinical differences. No statistically significant change was noted surrounding the initiation of TNFis, although complete multivariate data analysis is pending.
Vascular Endothelial Function Associated with Serum Calcium among CKD Patients

This study examined how vascular endothelial function is associated with serum calcium. Endothelial dysfunction or deleterious alterations of endothelial function, which can be measured as endothelial dependent dilation (EDD), is a key early signal in the development of atherosclerosis. It is also involved in plaque progression and the occurrence of atherosclerotic complications. Calcium plays an important role in energy production and storage, intracellular transport, and signal transduction. Furthermore, some evidence of association between elevated serum calcium and cardiovascular morbidity and mortality has been found among patients with chronic kidney disease (CKD) and post-menopausal women. In this study we investigated the association between serum calcium and vascular endothelial function among CKD patients. Using data from a randomized controlled trial that was designed to examine the effect of vitamin D (cholecalciferol, treatment for 6 months) on EDD as measured by brachial artery flow-mediated dilation (FMD) among CKD patients, in which serum calcium was measured at baseline and then monthly for six months after randomization and FMD was measured at baseline and end of study, we first identified four trajectory groups of serum calcium by using group-based trajectory modeling (GBTM), and then linked the group membership to FMD by using general linear models (GLMs). It was found that FMD at end of study was associated with trajectory group membership after adjustment for baseline FMD and demographic variables (i.e. race, sex, and age).
Microbial-derived Butyrate Regulates Interleukin-10 Receptor and Claudin 2 Expression to Enhance Intestinal Epithelial Barrier Formation

The interactions between the enteric microbiota and distal gut play important roles in regulating human health. Short chain fatty acids (i.e. butyrate) produced by anaerobic microbes represent a major energy source for the host colonic epithelium. A decreased concentration of luminal butyrate has been strongly associated with colonic disease, including inflammatory bowel disease (IBD). Recent work from our lab has revealed that butyrate enhances intestinal trans-epithelial electrical resistance (TEER) and barrier through stabilization of the hypoxia-inducible factor (HIF-1α). Interestingly, the anti-inflammatory interleukin-10 receptor (IL10RA) expressed on the apical side of the intestinal epithelium, is also important for barrier formation. These findings have led us to hypothesize that butyrate is augmenting intestinal barrier through the IL10R pathway.

Using human intestinal epithelial cells (IECs), we have discovered that butyrate induces IL10RA expression and enhances intestinal barrier formation. Lentiviral knockdown of the IL10R in IECs led to decreased TEER and barrier formation. Conversely, overexpression of IL10R led to enhanced epithelial resistance and barrier formation. The addition of Trichostatin A, a histone deacetylase (HDAC) inhibitor, to IECs increased IL10RA expression suggesting that butyrate is mediating the receptor through its role as an HDAC inhibitor. Further, Stat3 inhibition resulted in decreased IL-10RA promoter activity suggesting that Stat3 activation is also important for IL-10RA expression.

Studies in germ free mice revealed decreased IL10RA in the colonic epithelium supporting our hypothesis that microbial-derived butyrate is important for IL10RA expression. We are currently investing how butyrate and interleukin-10 receptor over-expression increase intestinal epithelial barrier by examining the expression of cell junction proteins. Preliminary results suggest that butyrate and IL10RA downregulate the expression of Claudin 2, a tight-junction protein that forms a paracellular channel for small cations and water. Interestingly, Claudin 2 is upregulated in a number of intestinal diseases, including IBD. Our current results indicate that microbial-derived butyrate participates in a diverse set of functions in the gut: regulation of the interleukin-10 cytokine receptor, barrier formation; it could even be a potential therapeutic for mucosal protection.
Comparing Human ES Cells and Patient iPS Cells Derived Dopamine Neuron Transplantation in a Rat Model of Parkinson’s Disease

Dopamine neuron transplantation into the brain could be a promising strategy for treating Parkinson’s disease, which is caused by the progressive degeneration of midbrain dopamine neuron. In our clinical experience, we have found that transplanted fetal dopamine neurons can survive for the life of the Parkinson patient and lead to significant motor function improvement. In this study, we have compared dopamine neurons generated from human embryonic stem (ES) cells and Parkinson patient derived induced pluripotent stem (iPS) cell lines for their function in a rat model of Parkinson’s disease. Dopamine neurons differentiated from human ES cells were treated with or without rat striatal neuron conditioning medium. Three Parkinson patient iPS cell lines were differentiated to dopamine neurons in similar method. At differentiation Day 25, dopamine neurons were dissociated and 0.5 million of total cells were transplanted into 6-hydroxydopamine lesioned nude rat striatum. Transplant growth was followed with monthly tests of methamphetamine-induced circling. Six months after transplant, all groups showed significant reduction in methamphetamine circling compared to sham operated controls. Animals were sacrificed and dopamine neuron survival were assessed with immunohistochemical staining for tyrosine hydroxylase. Results showed dopamine neuron survival was similar between ES cells and iPS cell derived dopamine neuron transplant groups. Since the overall dopamine neuron survival was still low, it only led to partially reduction of circling. We conclude that optimizing both in vitro differentiation and transplant methods are important for transplantation of stem cell-derived dopamine neurons into patients with Parkinson’s disease.