Harold Amos Medical Faculty Development Program

Name: Tamia Harris-Tryon, MD, PhD

Institution: UT Southwestern Medical Center

Mentor(s): Lora Hooper, PhD

College: Haverford College

Medical/Dental/Nursing PhD School: Johns Hopkins School of Medicine

Residency: Johns Hopkins Hospital

Fellowship: UT Southwestern Medical Center

Title of Project: The Function of Small Proline Rich Proteins in Cutaneous Host Defense

Human skin functions as a physical barrier, preventing the entry of foreign pathogens while also accommodating a myriad of commensal microorganisms. A key contributor to the skin landscape is the sebaceous gland. Mice devoid of sebocytes are prone to skin infection, yet our understanding of how sebocytes function in host defense is incomplete. Here we show that the small proline-rich proteins, SPRR1 and SPRR2 are bactericidal in skin. SPRR1B and SPPR2A were induced in human sebocytes by exposure to the bacterial cell wall component lipopolysaccharide (LPS). Further, LPS injected into mouse skin triggered the expression of the mouse SPRR orthologous genes, *Sprr1a* and *Sprr2a*, through stimulation of MYD88. Both mouse and human SPRR proteins displayed potent bactericidal activity against MRSA (methicillin-resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa* and skin commensals. Thus, *Sprr1a-/-;Sprr2a-/-* mice are more susceptible to MRSA and *Pseudomonas aeruginosa* skin infection. Lastly, mechanistic studies demonstrate that SPRR proteins exert their bactericidal activity through binding and disruption of the bacterial membrane. Taken together, these findings provide insight into the regulation and antimicrobial function of SPRR proteins in skin and how the skin defends the host against systemic infection.

Harold Amos Medical Faculty Development Program

Name: Maria Isabel Carlo

Institution: Memorial Sloan Kettering Cancer Center

Mentor(s): Kenneth Offit, MD

College: Harvard College

Medical/Dental/Nursing PhD School: Harvard Medical School

Residency: Brigham and Women’s Hospital

Fellowship: Memorial Sloan Kettering Cancer Center

Title of Project: Identification of Novel Germline Variants Associated with Increased Risk of Renal Cell Carcinoma

Background: Renal cell carcinoma (RCC) is a highly heritable, and has a poor prognosis when identified in advanced stages. Most known RCC genetic syndromes are associated with distinct pathologic RCC subtypes, such as Fumarate Hydratase (FH)-deficient RCC seen in patients with germline variants in the FH gene. Despite several known genetic syndromes, the majority of familial RCC remains unexplained. To date, a key barrier to the discovery of RCC-susceptibility genes has been the lack of large cohorts of RCC patients with available genomically-profiled tumors.

Methods: To nominate and validate novel RCC-susceptibility genes, we assembled a cohort of over 1240 RCC patients who have undergone tumor and germline targeted exome sequencing for over 380 cancer-associated genes. We excluded patients with known germline variants predisposing to cancer. To enrich for patients at higher risk of hereditary syndromes, we focused on patients with more than one primary RCC or RCC and second malignancies. We analyzed the germline for predicted loss-of-function variants in cancer-associated genes. For candidate genes, we analyzed somatic data to look for loss of heterozygosity (LOH). For select patients with candidate variants, we recruited family members for co-segregation analysis.

Results: In a subgroup of RCC patients enriched for multiple primary malignancies, we identified germline and somatic predicted loss-of-function variants in the gene *KEAP1*, a known tumor suppressor gene and negative regulator of NRF2, the key activator of the antioxidant response pathway. RCCs with *KEAP1* biallelic loss had papillary or unclassified histology. In a proband with RCC and the germline *KEAP1* p.Gln217\* variant, we sequenced 4 first degree relatives, and identified that the variant co-segregated with individuals with cancer. Furthermore, we identified LOH or a second somatic *KEAP1* mutation in all 3 tumors available from the proband and family. In a pan-cancer cohort, we identified 1021 tumors with somatic *KEAP1* mutations, of which 79% had LOH in the second allele.

Conclusions: We show that the tumor suppressor *KEAP1* is a candidate RCC susceptibility gene, and that biallelic *KEAP1* loss may be a driver of tumorigenesis in RCC. RCC with *KEAP1* loss has a distinct histologic phenotype. In RCC and pan-cancer, loss of heterozygosity in the second allele appears to be a common mechanism for loss of function in tumors with *KEAP1* variants. Future work includes functional characterization of the KEAP1/NRF2 pathway in tumors with *KEAP1* loss.

Harold Amos Medical Faculty Development Program

Name: Jason Watts

Institution: National Institute of Environmental Health Sciences

Mentor(s): Vivian Cheung

College: University of Pennsylvania

Medical/Dental/Nursing PhD School: University of Pennsylvania

Residency: Internal Medicine, Duke University

Fellowship: Nephrology, University of Michigan

Title of Project: RNA Polymerase Pausing Regulates Renal Gene Expression

**Background:**  This project studies how RNA polymerase pausing is regulated and its effect on gene expression in the kidney. Synthesis of RNA is a discontinuous process where RNA polymerase undergoes punctuated pauses during RNA chain elongation. We found that RNA Polymerase II (RNAPII) pauses in a highly regulated manner. At over 1,000 genes, RNAPII paused at the same nucleotide locations across individuals and human cell types. This finding enabled us to identify elements in the nucleic acid sequence that contribute to the precise regulation of RNA polymerase pausing. Here, using mitochondrial transcription as a model system, we examine the role of nucleic acid structure in the regulation of RNA polymerase pausing, gene expression, and cellular function.

**Results:** The mitochondrial genome encodes proteins that are required for energy production in eukaryotic cells and these essential genes are transcribed by the mitochondrial RNA polymerase (mtRNAP). To characterize mtRNAP pausing, we used a transcription run-on technique (PRO-seq), which maps active RNA polymerase at high resolution. In primary fibroblast cells from different individuals, we found that as the polymerase transcribes the mitochondrial genome it pauses over 400 times at consistent locations. These brief stops occur most often after mtRNAP has transcribed through guanine (G)-rich regions. Using computational and experimental approaches, we found that the G-rich sequences can form guanine (G)-quadruplexes, which are secondary structures that are stabilized by non-canonical base pairing between guanine residues. We treated fibroblasts with a drug (RHPS4) that stabilizes G-quadruplexes and we found that transcription by mtRNAP was reduced due to more frequent pausing. This resulted in significantly lower expression of mitochondrial-encoded genes and consequently decreased ATP generation. Renal proximal tubule cells primarily act to reclaim solutes from the glomerular filtrate, and they require ATP from mitochondria for transporter function. We treated proximal tubule cells with RHPS4 to stabilize G-quadruplexes and found significantly reduced transporter function due to loss of mitochondrial ATP production.

**Conclusion:** We find that mtRNAP pausing is mediated by guanine-rich sequences which form guanine-quadruplex secondary structures. G-quadruplex mediated mtRNAP pausing regulates mitochondrial gene expression, mitochondrial ATP production, and the function of renal proximal tubule cells. In future studies, we will examine how proximal tubule cells regulate the abundance of mitochondrial G-quadruplexes to tune transcriptional output to meet the energy demands in the cells.

Harold Amos Medical Faculty Development Program

Name: Hasina Outtz Reed, M.D. Ph.D.

Institution: Weill Cornelle Medicine

Mentor(s): Augustine Choi, M.D.

College: Princeton University

Medical/Dental/Nursing PhD School: Columbia University

Residency: University of Pennsylvania

Fellowship: University of Pennsylvania

Title of Project: “Lymphatic vascular dysfunction in emphysema: uncovering the missing link”

Rationale: The lymphatic vasculature is critical for lung function, but whether there are defects in lymphatic function in lung disease is understudies. In mice, lymphatic dysfunction alone is sufficient to cause lung injury with many of the hallmarks of human emphysema. Whether there are changes in lymphatic function in cigarette smoke (CS)-induced emphysema is unknown.

Objective: We investigated whether lung lymphatic function is altered in the pathogenesis of CS-induced emphysema.

Methods: Lung lymphatics in patients with emphysema were analyzed using immunohistochemistry and compared to lung lymphatics in control smokers. Using a mouse model of CS-induced emphysema, we analyzed lung lymphatics using immunohistochemistry, drainage and cell trafficking assays, and confocal microscopy. Thoracic lymph from CS-exposed mice was harvested for proteomic analysis and compared to lymph from control mice. The effect of CS on lymphatic endothelial cell permeability was assessed using *in vitro* transport assays.

Measurements and Main Results: Analysis of human lung tissue revealed significant lung lymphatic thrombosis in patients with emphysema compared to control smokers, and that this increased with disease severity. In a mouse model, CS exposure led to lung lymphatic thrombosis, decreased lymphatic drainage, and impaired leukocyte trafficking. Analysis of thoracic lymph confirmed enrichment of inflammatory and coagulation pathways in the lymphatics of CS-exposed mice compared to control mice. *In vitro* assays demonstrated a direct effect of CS on lymphatic endothelial cell integrity.

Conclusions: CS exposure causes lung lymphatic dysfunction with thrombosis, impaired leukocyte trafficking, and changes in the composition of thoracic lymph. In patients with emphysema, lymphatic thrombosis is seen in severe disease.

Harold Amos Medical Faculty Development Program

Name: Ahmara G. Ross, MD PhD

Institution: University of Pennsylvania, Scheie Eye Institute

Mentor(s): Kenneth Shindler, MD, PhD and Jean Bennett, MD, PHD

College: Bryn Mawr College

Medical/Dental/Nursing PhD School: Jefferson Medical School/ Thomas Jefferson University

Residency: University of Pittsburgh

Fellowship: University of Pennsylvania, Scheie Eye Institute Neuro-ophthalmology (2016-2017) and Glaucoma (2017 to 2018)

Title of Project: Harnessing the potential of SIRT1 to treat acute and chronic glaucoma with gene therapy

Pharmacologic activation or genetic over-expression of the SIRT1 signaling pathway continues to show promise by preventing retinal ganglion cell (RGC) loss and axonopathy in both acute models of optic nerve damage. Evidence suggests a mechanism of action that involves upregulation of genes in responsible for increased oxygen consumption and neutralization of oxidative stress. Previous manuscripts demonstrate a role for gene therapy in improving visual and structural outcomes in a subacute model of optic neuropathy, however results were limited by transduction specificity and efficiency. We hypothesize that AAV-mediated overexpression of SIRT1 in RGCs specifically can reduce RGC loss, thereby preserving visual function. The RGC specific neuroprotective potential of RGC-selective SIRT1 gene therapy in an acute optic nerve crush (ONC) model showed promise, so we followed experimentation using a chronic magnetic microbead model of optic nerve damage. Briefly, cohorts of C57Bl/6J mice received intravitreal injections of therapeutic or control AAVs using a ganglion cell promoter, 8 weeks later, the IOP was chronically elevated was induced using a magnetic microbead model to induce optic neuropathy. Retina and optic nerves were harvested to investigate RGC survival by immunolabeling and optic nerve axon were stained and counted. AAVSNCG.eGFP and AAV-SNCG.SIRT1 vector showed a 42% and 39% efficiency, compared with a 25% (p <0.05) efficiency previously published (AAVCMV.eGFP). The magnetic microbead model demonstrated chronically elevated IOP with MB, 24±5 mmHg compared with BSS injected animals 9.8±1.2 mmHg (p<0.003) for 8 weeks. This elevated IOP corresponded to a significant reduction of visual function by optic kinetic responses (OKR; 0.160±0.098 cyc/degree) compared with BSS control injected normotensive animals 0.412±0.078 cyc/degree). This loss of visual function also manifested as a time-dependent degree in retinal ganglion cells at six weeks (BSS:1685.75±708; MB: 1019±212; p<0.05) and eight weeks (MB: 804±724; p<0.003). This loss of visual function overtime, but most prominently by 8 weeks as measured by OKR (MB, AAV-eGFP: 0.206±0.018; MB, AAV-SIRT1: 0.309±0.038; p<0.03), RGC preservation (MB, AAV-eGFP: 1026±432; MB, AAV-SIRT1: 1214±398; p<0.03), and axonal preservation (MB, AAV-eGFP: 4642±1121; MB, AAV-SIRT1: 4510±1217; p>0.03). Over-expression of SIRT1 through AAV-mediated gene transduction suggests a RGC selective component of neuroprotection which is effectively sustained using a chronic model of optic neuropathy context making it a strong and first of its kind, therapeutic candidate for use in acute, sub-acute, and chronic optic nerve diseases.