were significantly altered. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results reveal novel genetic targets that underlie plasticity of fear-memory circuitry via their contribution of NMDAR-mediated fear consolidation and can inform future strategies for targeting fear related disorders like PTSD. CONFLICT OF INTEREST DESCRIPTION: Anantha Shekhar and Yvonne Lai are co-founders of Anagin, Inc., which is developing some of the related molecules for the treatment of PTSD.

4528

Sirtuin 3 activation as a potential renoprotective therapy in a mouse model of Alport syndrome

Bryce Jones[1], Komuraiah Myakala[2], Xiaoxin Wang[2], Andrew Libby[2], Shogo Takahashi[2], Kanchan Bhasin[2], Suman Ranjit[2], Avi Rosenberg[3], and Moshe Levi[2]
[1]Georgetown - Howard Universities; [2]Georgetown University; [3]Johns Hopkins University

OBJECTIVES/GOALS: Sirtuin 3 (Sirt3), a mitochondrial NAD^+-dependent deacetylase, is decreased in diverse models of kidney disease, and Sirt3 activation prevents disease progression in many of those models. We are investigating if pharmacological activation of Sirt3 ameliorates kidney disease in a mouse model of Alport syndrome. METHODS/STUDY POPULATION: Alport syndrome is a hereditary orphan disease arising from a defect in the collagen IV α3α4α5 heterotrimer, a component of the glomerular basement membrane. Male and female $Col4a3^{tm1Dec}$ knockout mice and wild type controls on the 129X1/SvJ background were harvested at 9–10 weeks of age. Serum and urine were collected prior to euthanasia; renal pathology was assessed by histology; and renal cortical mRNA and protein levels were assessed by qRT-PCR and western blot, respectively. Studies are ongoing using dietary administration of a Sirt3 activator, nicotinamide riboside (500 mg/kg/day), in Col4a3 transgenic mice on both the 129X1/SvJ and C57BL/6J backgrounds. RESULTS/ANTICIPATED RESULTS: $Col4a3^{-/-}$ mice have elevated BUN ($P < 0.0001$, both sexes), serum creatinine ($P < 0.001$, male; $P < 0.0001$, female), and urinary albumin-to-creatinine ratio ($P < 0.0001$, both sexes) compared to $Col4a3^{+/+}$ controls. On histology, $Col4a3^{-/-}$ mice have extensive renal fibrosis compared to $Col4a3^{+/+}$ controls. Sirt3 expression is decreased in the renal cortices of $Col4a3^{-/-}$ mice at the mRNA ($P < 0.0001$, male; trend, $P = 0.07$, female) and protein levels ($P < 0.05$, male; $P < 0.001$, female) compared to $Col4a3^{+/+}$ controls. All experiments had 5–9 mice per group. Results of the prevention study with nicotinamide riboside, a Sirt3 activator, are unknown at the time of abstract submission. DISCUSSION/SIGNIFICANCE OF IMPACT: $Col4a3^{-/-}$ mice have severe renal impairment and decreased renal cortical expression of Sirt3 at the mRNA and protein levels compared to $Col4a3^{+/+}$ controls. However, it is unknown at this time if pharmacologically activating Sirt3 prevents this renal decline.

4094

Structural Determinants of Immunogenicity for Peptide-Based Immunotherapy

Jason Devlin, University of Notre Dame[1], Jesus Alonso[2], Grant Keller[2], Sara Bobisse[3], Alexandre Harari[3], and Brian Baker[2]
[1]Indiana University School of Medicine; [2]University of Notre Dame; [3]UNIL CHUV, Ludwig Institute for Cancer Research, Switzerland

OBJECTIVES/GOALS: Neoantigen vaccine immunotherapies have shown promise in clinical trials, but identifying which peptides to include in a vaccine remains a challenge. We aim to establish that molecular structural features can help predict which neoantigens to target to achieve tumor regression. METHODS/STUDY POPULATION: Proteins were prepared by recombinant expression in *E. coli* followed by *in vitro* refolding. Correctly folded proteins were purified by chromatography. Affinities of protein-protein interactions were measured by surface plasmon resonance (SPR) and thermal stabilities of proteins were determined by differential scanning fluorimetry. All experiments were performed at least in triplicate. Protein crystals were obtained by hanging drop vapor diffusion. The protein crystal structures were solved by molecular replacement and underwent several rounds of automated refinement. Molecular dynamics simulations were performed using the AMBER molecular dynamics package. RESULTS/ANTICIPATED RESULTS: A T cell receptor (TCR) expressed by tumor-infiltrating T cells exhibited a 20-fold stronger binding affinity to the neoantigen peptide compared to the self-peptide. X-ray crystal structures of the peptides with the major histocompatibility complex (MHC) protein demonstrated that a non-mutated residue in the peptide samples different positions with the mutation. The difference in conformations of the non-mutated residue was supported by molecular dynamics simulations. Crystal structures of the TCR engaging both peptide/MHCs suggested that the conformation favored by the mutant peptide was crucial for TCR binding. The TCR bound the neoantigen/MHC with faster binding kinetics. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that the mutation impacts the conformation of another residue in the peptide, and this alteration allows for more favorable T cell receptor binding to the neoantigen. This highlights the potential of non-mutated residues in contributing to neoantigen recognition.

4322

Structure-guided design of the TIL1383I T cell receptor

Jesus Alonso[1], Nishant Singh, Jason Devlin[1], Lauren Davancaze, and Brian Baker
[1]University of Notre Dame

OBJECTIVES/GOALS: Our goal is to employ a structure-guided design approach to engineering a safer and more effective variant of the TIL1383I T cell receptor (TCR) currently under study in clinical trials for malignant melanoma METHODS/STUDY POPULATION: Using our unpublished structure of TIL1383I we are in process of designing a panel of TCR variants with the goal of identifying candidates that improve "focus" towards the tyrosinase antigen presented on the MHC class I molecule HLA-A2. RESULTS/ANTICIPATED RESULTS: Structural analysis of TIL1383I revealed key residues, particularly beta-chain residues E97, G101, L102, responsible for engaging the tyrosinase peptide bound to HLA-A2. The crystal structure of TIL1383I in complex with tyrosinase-HLA-A2 also highlighted its uncharacteristic binding geometry and we therefore hypothesize that this binding orientation is associated with the observed CD8 co-receptor independence of TIL1383I. Indeed, functional analysis with TIL1383I-transduced CD8-positive and CD8-negative T cells, transduced T cells expressing a truncated CD8 lacking the intracellular LCK signaling domain, and tyrosinase peptide variants presented by HLA-A2 mutants outline this co-receptor independence. Combined with our interrogation of tyrosinase peptide cross-reactivity via a peptide positional scanning library approach, structure-guided design resulted in the identification of TIL1383I variants with improved binding affinities to the tyrosinase

https://doi.org/10.1017/cts.2020.93 Published online by Cambridge University Press

peptide as well as an understanding of structural characteristics that may contribute to TIL1383I's co-receptor independence.

4222

Synthesis and application of cyanuric chloride lipids for peptide presentation

David Nardo[1], and Vincent J. Venditto[2]
[1]University of Kentucky Center for Clinical and Translational Science; [2]University of Kentucky

OBJECTIVES/GOALS: My long-term career objective is to become an established independent researcher focused on understanding and modulating immune responses to biologics in order to enhance their efficacy and understand the underlying mechanisms by which these interact with the immune system. METHODS/STUDY POPULATION: In this study we will evaluate the utility of cyanuric chloride based synthetic lipids in the presentation of peptide epitopes of the gene delivery vector, adeno-associated virus (AAV). The lipopeptide conjugates will be administered to mice via liposomal formulations to assess their ability to induce immune responses by ELISA as compared to mice treated with the AAV. The three-dimensional conformation of the peptides will be evaluated using nuclear magnetic resonance to determine their similarity with the natural conformation that these peptides adapt on the viral surface. Additionally, to assess the translatability of this conjugation strategy, we will test the ability of our lipopeptides to bind to serum antibodies from human subjects. RESULTS/ANTICIPATED RESULTS: We anticipate that peptide presentation using cyanuric chloride lipids will achieve a robust response in mice following immunization. Immunizations with our lipids should induce the production of antibodies targeting AAV, albeit a milder response that the virus itself, given the complexity of viral epitopes. Nuclear magnetic resonance will inform us on how to improve the synthetic lipids to optimize peptide presentation by altering the characteristics of the lipid anchors. Finally, by using human serum to test for the ability of our lipopeptides to bind to antibodies in serum from patients positive for AAV antibodies, we can become informed on whether our strategy has utility in human studies or whether our method is limited to animal models of human disease. DISCUSSION/SIGNIFICANCE OF IMPACT: The current work seeks to develop a strategy to present peptides from a well characterized biologic, AAV, on a liposome surface. Our ultimate purpose is to employ liposomal formulations as decoys that target AAV-specific lymphocytes to improve the *in vivo* efficacy of AAV.

4296

Targeting ERG Through Toll-Like Receptor 4 in Prostate Cancer

Ben Greulich[1], Josh Plotnik, and Peter Hollenhorst
[1]Indiana University School of Medicine

OBJECTIVES/GOALS: The objective of this research was to learn how the oncogenic transcription factor, ERG, is regulated in prostate cancer. If we could learn how ERG is regulated and which genes are important for its oncogenic phenotype in prostate cells, we could design new therapeutic strategies against ERG, which has proven to be difficult to target. METHODS/STUDY POPULATION: We conducted an shRNA screen in prostate cells to determine candidate genes and pathways that are important for ERG function. To validate the findings of the screen, we performed a variety of cell-based functional assays, including trans-well migration, wound healing, and clonogenic survival assays. To further investigate the mechanism between ERG and the genes revealed by the screen, we performed biochemical and molecular biology experiments such as Western blotting and qRT-PCR for protein and mRNA expression, co-immunoprecipitation assays to determine protein-protein interactions, and chromatin immunoprecipitation (ChIP-qPCR) to determine transcription factor binding to DNA sites. RESULTS/ANTICIPATED RESULTS: The screen revealed that genes involved in the toll-like receptor 4 (TLR4) pathway are important for ERG-mediated migration. We tested the effect of a TLR4 inhibitor on ERG function and observed decreased migration and clonogenic survival exclusively in ERG-positive cells. Expression of pMEK and pERG was reduced when TLR4 was inhibited, which suggests a mechanism in which TLR4 upregulates pMEK, leading to the phosphorylation and activation of ERG. This is supported by functional assays in which cells expressing a phosphomimetic ERG are resistant to the TLR4 inhibitor. We demonstrated that ERG drives the transcription of *TLR4* and its endogenous ligands *HSPA8* and *BGN*. Therefore, ERG can sensitize the cell to TLR4 activation by increasing the number of receptors as well as providing the ligands needed for stimulation. DISCUSSION/SIGNIFICANCE OF IMPACT: This research provides a new therapeutic pathway for treating ERG-positive patients through TLR4 inhibition. This can be beneficial because many patients become resistant to the standard therapy, leaving very few treatment options. TLR4-based therapies could provide an alternative for patients who have developed resistance.

4263

The cardioprotective effects of ramipril during the course of experimental hypercholesterolemia in rabbits

Zvezdana Zivorad Kojic[1]
[1]University of Belgrade, School of Medicine

OBJECTIVES/GOALS: High cholesterol is among the major causes of cardiometabolic complications. People with high cholesterol have about twice the risk of heart disease as people with lower levels. Approximately every 40 seconds, an American will have a heart attack. Costs related to Heart Attack exceed 60 Billion USA dollars per year. Renin-Angiotensin-Aldosteron System (RAAS) is implicated in the genesis of coronary heart disease and in the perpetuation of heart failure. Angiotensin-Converting Enzyme inhibitors (ACE-I) have emerged as the treatment of choice for patients with all degrees of heart failure. Many clinical trials (Consensus, 1987; Save 1990) provide the evidence that ACE-I preserves cardiac function, prevents cardiovascular death, myocardial infarction & stroke and limit remodeling after myocardial infarction. However, there are still controversies in cardiology and a debate over cardioprotection is continuing:

- Do ACE Inhibitors have unique properties, beyond their antihypertensive effect?
- Can we protect the heart during hypercholesterolemia?
- In which way hypercholesterolemia affects mitochondria bioenergetics?
- How does ramipril affect mitochondrial bioenergetics during the course of experimental hypercholesterolemia?

Objectives/Goals were: To evaluate the mitochondrial actions of chronically administered ramipril (non-SH-containing ACE inhibitor) in cholesterol-fed rabbits by determining the influence of ramipril on:

https://doi.org/10.1017/cts.2020.93 Published online by Cambridge University Press