4321

Personalization of T cell production for cellular immunotherapy

Dennis Jinglun Yuan[1], Shuai Shao[1], Joanne H Lee[1], Stacey M Fernandes[2], Jennifer R Brown[3], and Lance C Kam[1]
[1]Columbia University; [2]Dana Farber Cancer Institute; [3]Harvard Medical School

OBJECTIVES/GOALS: Utilize polymer-based fiber scaffolds and machine learning methods applied to patient biomarker data to enhance and personalize T cell expansion and production for T cell therapy in chronic lymphocytic leukemia. METHODS/STUDY POPULATION: Scaffolds are 1) generated from a co-polymer blend of PDMS and PCL with controlled fiber diameters and pore size, 2) coated with activating antibodies to CD3 and CD28, and 3) used to stimulate T cells from both healthy donors and CLL patients. CLL patients have pre-annotated mutation burdens and clinical biomarkers. T cell populations will be analyzed for exhaustion markers and phenotypes before, during, and after expansion. Cell functionality will be measured by cytokine secretion, cell cycle analysis, and fold expansion, with respect to platform parameters, and analyzed with inputs of disease markers and exhaustion profile of isolated T cells using regression and random forest classifiers. RESULTS/ANTICIPATED RESULTS: We previously showed that engineering the mechanical rigidity of activating substrates can enhance and rescue T cell expansion from exhausted populations. Now we aim to study a broader range of compositions and geometry of scaffolds with respect to capacity to expand CLL T cells. Preliminary data with fiber diameters ranging from 300 nm to 6 um confirm the effect of geometry in modulating expansion. A biorepository of T cells from 80 CLL patients have been isolated concurrently. Anticipated results include correlating exhaustion profile of T cells with clinical biomarkers and identifying markers associated with expansion on panel of platform parameters. DISCUSSION/SIGNIFICANCE OF IMPACT: T cell therapy has shown particular promise in treating blood cancers, yet significant percentage of T cells isolated from patients undergoing treatments are unresponsive to activation. A powerful tool is to predict if and how patient T cells can be robustly expanded on a personalized approach.

4081

Quantifying pH buffering capacity and kinetics of tumor and healthy tissue to understand and exploit differences in biology

A. Colleen Crouch[1], Emily A. Thompson[2], Mark D. Pagel[2], and Erik N.K. Cressman[2]
[1]The University of Texas Health Science Center at Houston; [2]University of Texas MD Anderson Cancer Center

OBJECTIVES/GOALS: The purpose of this work is to investigate natural buffering capacity of liver tissue and tumors, to understand and exploit differences for therapy. Using this work, we will determine the concentrations of reagents (acids or bases) used in ablation treatment to optimize treatment by increasing tumor toxicity and minimizing healthy tissue toxicity. METHODS/STUDY POPULATION: For this preliminary study, two methods will be used: benchtop pH experiments *ex vivo* and non-invasive imaging using acidoCEST MRI *in vivo*. For *ex vivo*, two types of tissues will be tested: non-cancerous liver and tumor tissue from HepG2 inoculated mice (n = 10). After mice are euthanized, pH will be measured in tissue homogenates at baseline and then the homogenates will be placed in either acidic (acetic acid) or basic (sodium hydroxide) solutions with varied concentrations (0.5–10M) and time recorded until pH returns to baseline. For *in vivo* imaging, Mia PaCA-2 flank model mice (n = 10) will be imaged with acidoCEST MRI to quantify pH at baseline. Mice will then be injected intratumorally with (up to 100 μL of) acid or base at increasing concentrations and imaged to quantify pH changes in the tumor. RESULTS/ANTICIPATED RESULTS: For this study, buffering capacity is defined as the concentration threshold for which tissue can buffer pH back to within normal range. Non-cancerous tissue is likely to buffer a wider range of concentrations compared to tumor tissue. From the benchtop experiment, comparison of time-to-buffer will be made for each concentration of acid/base for the two tissue types. AcidoCEST MRI will provide *in vivo* buffering capacity and potentially demonstrate tumor heterogeneity of buffering capacity. For both experiments, a pH vs. concentration curve for the two tissue types will allow for comparison of ex vivo to *in vivo* experiments, which will differentiate contributions of local tissue buffering capacity from the full body's natural bicarbonate buffer system that depends on respiration and blood flow. DISCUSSION/SIGNIFICANCE OF IMPACT: The pH of the body must be maintained within a narrow range. With cancer, impairment in regulation of tumor metabolism causes acidosis, lowering extracellular pH in tumors. It remains unclear if pH plays a role in local recurrence or tumor toxicity. This work will determine if acidoCEST MRI can measure deliberate alteration of pH and how this change affects biology.

4335

Role of PSD95 and nNOS interaction in gene regulation following fear conditioning and implications for molecular mechanisms underlying PTSD

Jheel Patel[1], Erik Dustrude, Melissa Haulcomb, Liangping Li, Guanglong Jiang, Yunlong Liu, Yvonne Lai, Andrei Molosh, and Anantha Shekhar, MD, PhD[1]
[1]Indiana University School of Medicine

OBJECTIVES/GOALS: Normal fear learning produces avoidance behavior that promotes survival, but excessive and persistent fear after trauma can lead to development of phobias and post-traumatic stress disorder (PTSD). Our goal is to understand the mechanism and identify novel genetic targets underlying fear responses. METHODS/STUDY POPULATION: Involvement of the amygdala in fear acquisition is well established and requires activation of N-methyl-D-aspartic acid receptors (NMDARs). At a cellular level, NMDAR activation leads to production of nitric oxide (NO) by a process mediated by interaction between postsynaptic density protein 95 (PSD95) and neuronal nitric oxide synthase (nNOS). To elucidate mechanisms underlying the role of the PSD95-nNOS-NO pathway in conditioned fear, here we use rodent behavioral paradigms, pharmacological treatment with a small molecular PSD95-nNOS inhibitor, co-immunoprecipitation, Western blotting, and RNA-sequencing. RESULTS/ANTICIPATED RESULTS: We show that fear conditioning enhances the PSD95-nNOS interaction and that the small-molecule ZL006 inhibits this interaction. Treatment with ZL006 also attenuates rodent cued-fear consolidation and prevents fear-mediated shifts in glutamatergic receptor and current densities in the basolateral amygdala (BLA). With RNA-sequencing, expression of 516 genes was altered in the BLA following fear expression; of these genes, 83 were restored by systemic ZL006 treatment. Network data and gene ontology enrichment analysis with Ingenuity Pathway Analysis and DAVID software found that cell-cell interaction, cognition-related pathways, and insulin-like growth factor binding

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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

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