Mechanistic Basic to Clinical

DNA-PK(c)s Regulates Stability of Egr1 During T Cell Activation
Zachary Waldrip, David Harrison, Marie Burdine and Lyle Burdine
University of Arkansas for Medical Sciences, Arkansas Children’s Research Institute

ABSTRACT IMPACT: This work provides supporting evidence for the development of a novel immunosuppression therapy for transplant patients. OBJECTIVES/GOALS: Our laboratory reported that inhibition of the kinase DNA-PK(c)s in mice delays allogeneic graft rejection in part by mitigating the induction of certain cytokines. We hypothesized that this was due to an inhibition of intracellular signaling programs in T cells and designed studies to identify the mechanism(s) by which this occurs. METHODS/STUDY POPULATION: The immortalized Jurkat T cell line was used to evaluate the effect of the DNA-PK(c)s inhibitor NU7441 on T cell activation by PMA/Ionomycin or PMA/PHA. Mouse primary splenocytes also were used to demonstrate the universality and reproducibility of our observations. Initially, protein mass spectrometry of lysates from untreated and NU7441-treated Jurkat cells identified proteins of interest regulated by DNA-PK(c)s that play a role in T cell activation and cytokine production. CRISPR genome editing was used to validate a potential downstream target of DNA-PK(c)s. Western blot, ELISA, and flow cytometry were used to document changes in protein levels with respect to treatments. RESULTS/ANTICIPATED RESULTS: We observed that expression of the transcription factor Egr1 was highly induced after activation but attenuated after treatment with NU7441 in both Jurkat T cells and mouse splenocytes. Phosphorylated serine 301 of Egr1 was identified by mass spectrometry in stimulated cells and fits the kinase consensus sequence for DNA-PK(c)s. Both an endogenous CRISPR-generated sequence 301 to alanine mutant and expression of a plasmid-based S301A mutant resulted in an unstable form of Egr1 that was barely detectable. In contrast, expression of either a S301 to D or E phospho-mimetic mutant resulted in a stable form of the protein detectable by Western blot. Further evaluation of these mutants and Egr1 phosphorylation is underway to determine the mechanism by which DNA-PK(c)s kinase regulates protein stability. DISCUSSION/SIGNIFICANCE OF FINDINGS: We previously reported a role for DNA-PK(c)s in immunomodulation. We now have evidence that this occurs in part through stabilization of Egr1. We believe this novel finding will lead to uncovering a broader role for DNA-PK(c)s as a mediator of protein stability in T cells and provide support for targeting DNA-PK(c)s in immunosuppression therapy.
K$_{ATP}$ channel prodrugs as therapeutics for chronic pain and substance abuse disorders
Alexis Doucette, Kayla Johnson, Peter I. Dosa and Amanda H Klein
University of Minnesota

ABSTRACT IMPACT: Pharmacological activation of K$_{ATP}$ channels may provide analgesia and attenuate opioid tolerance and withdrawal OBJECTIVES/GOALS: Our long term goal is to develop therapeutics for the treatment of the overuse of opioids. The objective of this application is to test novel K$_{ATP}$ channel-targeting prodrugs in rodent models of neuropathic and inflammatory pain in addition to opioid tolerance after chronic morphine administration.

METHODS/STUDY POPULATION: In one study, two different measures for chronic pain were implemented in mice. Male and female mice (n=10) were subjected to spinal nerve ligation (SNL) or intraplantar injection of Complete Freund’s Adjuvant (CFA) to induce neuropathic and inflammatory pain, respectively. Administration of K$_{ATP}$ channel prodrugs (60ug, i.t.) attenuated mechanical hypersensitivity after SNL or CFA compared to vehicle (saline). In a separate study, changes in mechanical hypersensitivity were tested while mice undergo chronic morphine treatment (15mg/kg, 2x, 5 days) with administration of the prodrugs. Tolerance was measured as the loss of antinociception, and withdrawal was measured ~24 hours after the final morphine injection.

RESULTS/ANTICIPATED RESULTS: We describe and illustrate a data-driven approach to determine characteristic spatiotemporal structure in these response shapes, summarized by a set of unique ‘basis profile curves’ (BPCs). Each BPC may be mapped back to underlying anatomy in a natural way, quantifying projection strength from each stimulation site using simple metrics. Our technique is demonstrated for an array of implanted brain surface electrodes in a human patient, and our code is shared at https://purl.stanford.edu/rc201dv0636. DISCUSSION/SIGNIFICANCE OF FINDINGS: This framework enables straightforward interpretation of single-pulse brain stimulation data, and can be applied generally to explore the diverse milieu of interactions that comprise the connectome.

L-type calcium channels in cerebellar neuron development and motor learning
DeAnna O’Quinn, *Aislinn Williams, Ashley Parker, Bryn Myers, Ashley Plumb, Hsiang Wen and Marisol Lauffer
University of Iowa Institute for Clinical and Translational Science

ABSTRACT IMPACT: We aim to understand how LTCCs impact cerebellar function. OBJECTIVES/GOALS: L-type calcium channels