Health Equity & Community Engagement

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The Bench Tutorials Program: An Essential Educational Pivot in response to COVID-19

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ABSTRACT IMPACT: The Bench Tutorials Program is an independent study course in biomedical research in which high school students are paired with graduate and post-doctoral students during the academic year. The purpose is to enhance the rigor of high school science education and build the pipeline of tomorrow's researchers. OBJECTIVES/GOALS: The Bench Tutorials Program: o Proficiency in research design, implementation, and presentation; o Acquisition of hands-on laboratory skills; o Increase in scientific literacy; o Increase in analytical skills and critical thinking; o Career in science; o Build the pipeline of tomorrow's biomedical researchers METHODS/STUDY POPULATION: High School seniors are paired with graduate and postdoc mentors through a matching process. Students spend approximately four hours/week in supervised instruction and research from a participating laboratory in addition to classroom experience at their High School. Mentors design research projects relating to the larger research framework of their laboratories. In light of COVID-19, approaches have been adjusted to maintain the program safely through a hybrid method of using the high school lab for hands-on learning and through the use of Go-Pros 's to enable our mentors to video and narrate as they conduct experiments in their own labs to teach their mentees scientific methods and processes. RESULTS/ANTICIPATED RESULTS: Since inception, more than 400 students and mentors have participated in the Bench Tutorial's program. This year we found a way to continue the program under COVID-19 restraints without putting anyone in harms way. Go-Pros have been essential for our program to maintain continuity for high school students who receive academic credit for this course. This program is also one of few in which our graduate students have the opportunity to serve as mentors in the scientific setting. Using Go-Pro's will also enable us to provide teaching videos online for other academic institutions, so even in the absence of COVID-19 in the future, the continued use of these devices will still be of great value. DISCUSSION/SIGNIFICANCE OF FINDINGS: High school students are afforded the ability to work on cutting edge research projects alongside graduate students and post-docs, who are afforded the chance to mentor and teach. Due to the COVID-19 pandemic, we have successfully adjusted our methods for teaching through the use of Go-Pro technology.

Mechanistic Basic to Clinical

10399

DNA-PK(cs) Regulates Stability of Egr1 During T Cell Activation

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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(cs) 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(cs) 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(cs) 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(cs). 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(cs). Both an endogenous CRISPR-generated serine 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(cs) kinase regulates protein stability. DISCUSSION/SIGNIFICANCE OF FINDINGS: We previously reported a role for DNA-PK(cs) 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(cs) as a mediator of protein stability in T cells and provide support for targeting DNA-PK(cs) in immunosuppression therapy.

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Circadian Disruption in Pancreatic Cancer Carcinogenesis

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ABSTRACT IMPACT: Circadian disruption is known to cause significant human pathology but has not been evaluated in pancreas cancer carcinogenesis; through understanding how disruption of circadian rhythms can lead to pancreas cancer development and spread, preventive and therapeutic strategies can be devised. OBJECTIVES/GOALS: Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer due to early spread and poor response to therapy. Identifying factors driving PDAC growth could lead to new therapeutic strategies. Thus, we evaluated the extent to which circadian rhythm disruption, a factor strongly associated with cancer formation, contributes to PDAC pathogenesis. METHODS/STUDY

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POPULATION: To achieve the objective, we evaluated mice with pancreas lineage Kras-mutation (KC mice), which are predisposed to develop the full spectrum of pancreas cancer precursor lesions (pancreatic intra-epithelial neoplasia or PANIN-1, 2, 3) and PDAC. We subjected KC mice to a light-dark phase shift protocol known to induce circadian disruption (KCCD, n = 18), and another group to standard lighting conditions (KCNC, n = 31), with equal numbers of males and females in each group. The mice were allowed access to food and water ad libitum until sacrifice at age 9 months. Histopathologic evaluation of the pancreas was then performed to assess for pancreatic inflammation, pancreatic precursor lesions (PANIN) and PDAC. Fisher's Exact Test was used to evaluate differences in incidence. RESULTS/ANTICIPATED RESULTS: As expected, both groups of mice demonstrated 100% incidence of chronic pancreatitis and PANIN-1 (low-grade precursor lesion) at age 9 months. This is consistent with the KC phenotype. However, the KCCD mice demonstrated a significant increase in acute pancreatic inflammation (61.1% vs 19.4%, $p = 0.005$) compared to KCNC mice. Furthermore, intermediate grade precursor lesions (PANIN-2) were also significantly increase in the KCCD mice (38.9% vs 6.5%, $p = 0.006$). Incidence of high-grade precursor lesions (PANIN-3, or carcinoma in situ: 22.2% vs 9.7%) and PDAC (27% vs 19%) were also increased, but these were not statistically significant. These results are notable given the established progression from higher grade premalignant PANIN lesions (PANIN-2, PANIN-3) to PDAC. DISCUSSION/SIGNIFICANCE OF FINDINGS: Insight into how circadian disruption leads to increased PANIN-2 formation and increase in acute inflammation may be advantageous for understanding circadian disruption in PDAC carcinogenesis. The circadian clock is present in immune cells and disruption can induce immune dysregulation. This mechanism will be evaluated in follow up studies.

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K_{ATP} channel prodrugs as therapeutics for chronic pain and substance abuse disorders

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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, it) 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 is measured ~24 hours after the final morphine injection. RESULTS/ANTICIPATED RESULTS: Intrathecal administration of either K_{ATP} channel prodrugs significantly attenuated mechanical hypersensitivity after SNL and significantly attenuated mechanical hypersensitivity after CFA in mice. We predict that intrathecal administration of these prodrugs will also attenuate morphine tolerance and withdrawal in mice. This hypothesis is based off our previous data indicating non-water soluble K_{ATP} channel agonists produce analgesia and attenuate morphine tolerance in mice. DISCUSSION/SIGNIFICANCE OF FINDINGS: Pharmaceutical strategies to utilize K_{ATP} channels for therapeutics have been hindered due to the low solubility and low ability to cross the neurovascular unit. Newly developed, water-soluble K_{ATP} channel openers could be useful pharmaceutical strategy to reduce chronic pain, opioid tolerance, and withdrawal in human populations.

19233

Basis profile curve identification to understand electrical stimulation effects in human brain networks

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ABSTRACT IMPACT: Brain networks can be explored by delivering brief pulses of electrical current in one area while measuring responses in other areas, and this describes an open-source novel algorithm to carry out this exploration. OBJECTIVES/GOALS: If we focus on a single brain site and observe the average effect of stimulating each of many other brain sites, visually-apparent motifs in the temporal response shape emerge from adjacent stimulation sites. There are no existing approaches to identify and quantify the spatiotemporal structure of these motifs. METHODS/STUDY POPULATION: Individual stimulation trials are correlated with one another, then a correlation-significance matrix quantifying similarity between stimulation sites is decomposed with non-negative matrix factorization, in which the inner dimension is iteratively reduced. The dimensionality reduction identifies stimulation sites that produce a common elicited temporal response, and linear kernel PCA is applied to obtain the robust profile of this response cluster. 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 generically to explore the diverse milieu of interactions that comprise the connectome.

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L-type calcium channels in cerebellar neuron development and motor learning

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

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