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PKM2 mediates anti-tumor immunity and T cell dysfunction

Geoffrey Markowitz[1], Yi Ban[1], Michael Crowley[1], Diamile Tavarez[1], Stephen TC Wong[2], Kenneth Chang[3], Andrea Schietinger[4], Nasser Altorki[1] and Vivek Mittal[1]
[1]Weill Cornell Medicine, [2]Houston Methodist, [3]Cold Spring Harbor Laboratories and [4]Memorial Sloan Kettering Cancer Center

ABSTRACT IMPACT: T cell dysfunction is a dominant suppressor of anti-tumor immunity, reducing immunotherapeutic efficacy and benefit to patients; our work will identify novel mediators of this process for both therapeutic potential and underlying mechanism, allowing for both potential immediate clinical utility and identification of future targets based on new mechanistic insights. OBJECTIVES/GOALS: T cell dysfunction is a dominant suppressor of anti-tumor immunity, reducing immunotherapeutic efficacy and clinical benefit to the majority of patients. We aim to interrogate a novel mediator of dysfunction identified from transcriptome analyses, pyruvate kinase muscle isozyme isoform 2 (PKM2), for therapeutic utility and underlying mechanism. METHODS/STUDY POPULATION: Transcriptome analyses of CD8+ lymphocytes from tumor-bearing lungs from both murine KrasG12D p53-/- and human non-small cell lung cancer (NSCLC) patients were performed, and differentially expressed genes identified. Flow cytometric analyses for PKM isoform expression and effects of target knockdown on accumulation of dysfunctional characteristics, including checkpoint and transcription factor expression, proliferation, and cytokine production, were performed using an in vitro co-culture of murine antigen-specific T (OT-I) cells and antigen-expressing NSCLC (HKP1-ova) cells. In vivo examination of the same was performed using adoptive transfer of OT-I cells into immunocompetent recipient mice with engraftment of HKP1-ova cells, and subsequent evaluation of mouse survival and T cell phenotypes. RESULTS/ANTICIPATED RESULTS: Transcriptome analyses demonstrated that PKM expression was upregulated in dysfunctional T cells from both murine and human samples. This was confirmed both in vitro with co-culture and in vivo with adoptive transfer approaches, with both activated and dysfunctional OT-I cells expressing higher levels of isoform 2 of PKM than naive OT-I cells. Expression of PKM2 mimicked the kinetics of the transcription factor Tox, a known driver of dysfunction, and knockdown of PKM2 resulted in reduced granzyme B expression, and increased proportions of progenitors with fewer terminally differentiated dysfunctional cells. Knockdown of PKM2 in adoptively-transferred OT-I cells led to enhanced tumor control; results are being extended to other tumor models, and T cells metabolically profiled with PKM2 manipulation. DISCUSSION/SIGNIFICANCE OF FINDINGS: This work identified a novel mediator of dysfunction whose targeting has the potential to enhance anti-tumor immunity. Mechanistically, targeting PKM2 led to altered T cell differentiation to a dysfunctional state, linking metabolic phenotypes to these traits and underlining the importance and therapeutic potential of T cell metabolic pathways.

54101

Characterizing Microbiota Features of Clostridioides difficile Infections

Sarah Tomkovich, Krishna Rao, Vincent B. Young, and Patrick D. Schloss
University of Michigan

ABSTRACT IMPACT: Our goal is to identify bacterial biomarkers of adverse Clostridioides difficile infection outcomes OBJECTIVES/GOALS: We characterized microbiota features of Clostridioides difficile infections (CDIs) and will investigate the association between bacterial taxa and adverse outcomes, which includes severe and recurrent CDIs. METHODS/STUDY POPULATION: 1,517 stool samples were collected from patients diagnosed with a CDI at the University of Michigan along with 1,516 unformed and 910 formed stool control samples. We characterized the microbiota of the 3,943 stool samples by sequencing the V4 region of the 16S rRNA gene and used the Dirichlet Multinomial Mixtures method to cluster samples into community types. Severe CDI cases were defined using the Infectious Diseases Society of America criteria and recurrent CDIs were defined as CDIs that occurred within 2-12 weeks of the primary CDI. We will use machine learning to examine whether specific bacterial taxa can predict severe or recurrent CDIs. We will test 5 machine learning models with 80% training and 20% testing data split. RESULTS/ANTICIPATED RESULTS: Similar to findings from a previous study with 338 samples, we found there was no difference in diversity between CDI cases and unformed controls (Inverse Simpson index, $p > 0.5$) and samples from the 3 groups (CDIs, unformed controls, and formed controls) clustered into 12 community types. To investigate the bacterial taxa that are important for predicting adverse CDI outcomes, we will select the best machine learning model based on performance and training time and examine how much each feature contributes to performance. We anticipate the large number of CDI cases in our cohort and robust machine learning approaches will enable us to identify more bacteria associated with adverse outcomes compared to other studies that have attempted to predict CDI recurrence with fewer CDI cases. DISCUSSION/SIGNIFICANCE OF FINDINGS: Adverse CDI outcomes are a significant source of the morbidities, mortalities, and healthcare costs associated with CDIs. Identifying bacterial biomarkers of severe and recurrent CDIs could enhance our ability to stratify patients into risk groups and may lead to the development of more targeted therapeutics.

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miR-338-5p as a Biomarker of Neuropathic Pain After Spinal Cord Injury*

Jesse Kowalski[1], Nguyen Nguyen[1], Ricardo Battaglino[1], Scott Falci[2], Susan Charlie[3] and Leslie Morse[1]
[1]University of Minnesota, [2]Swedish Hospital and [3]Craig Hospital

ABSTRACT IMPACT: Identification of microRNA (miRNA) associated with neuropathic pain after spinal cord injury (SCI) will elucidate underlying epigenetic mechanisms contributing to its development and identify targets for intervention to optimize treatment strategies and outcomes. OBJECTIVES/GOALS: Approximately 70% of individuals with SCI experience neuropathic pain, which is refractory to pharmacologic intervention, and can reduce overall health and wellbeing. This study aims to identify predictive miRNA biomarkers of neuropathic pain after SCI to identify targets for the development of efficacious interventions. METHODS/STUDY POPULATION: Blood samples and clinical outcome measures were collected from adult participants with SCI with neuropathic pain ($n = 28$) and without neuropathic pain ($n = 15$). The sample population consisted of a mean age of 39 ($SD = 12.12$), 8 female (20%), with 13 classified as acute SCI (within 3 months post injury) and 30 as chronic SCI (> 3 years post injury). Pain presence, type, and intensity were assessed with the International Spinal Cord Injury Basic Pain Dataset (ISCIBPDS). Serum miRNA sequencing counts were produced from blood samples. Fold change and independent t-tests assessed differential expression between those with

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