52350

PKM2 mediates anti-tumor immunity and T cell dysfunction

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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

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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.

69742

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

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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

https://doi.org/10.1017/cts.2021.632 Published online by Cambridge University Press

and without neuropathic pain, and those with chronic or acute SCI. Linear regression was performed to explore the relationship between miRNA expression and ISCIBPDS pain intensity ratings. RESULTS/ANTICIPATED RESULTS: In individuals with SCI, significant downregulation of expression of miR-338-5p was present in those with neuropathic pain compared to those without neuropathic pain (fold change = 0.81, $p = 0.04$). A significant relationship between expression of miR-338-5p and highest reported neuropathic pain intensity on the ISCIBPDS was identified ($R2 = 0.15$, $F = 7.32$, $p < 0.01$). Covariates of sex, age, and years post injury were not found to significantly influence the relationship between miRNA expression and ISCIBPDS intensity ratings. No significant differences in miR-338-5p expression were identified between participants with acute and chronic SCI, or with nociceptive pain ratings, demonstrating specificity of the relationship between miR-338-5p differential expression with pain of a neuropathic nature. DISCUSSION/SIGNIFICANCE OF FINDINGS: These findings, along with validated targets of miR-338-5p in the NF-KB neuroinflammatory signaling pathway, suggest that miR-338-5p may serve a neuroprotective role in modulating neuroinflammation, and that its downregulation may result in maladaptive neuroplastic mechanisms contributing to the development of neuropathic pain after SCI.

76938

Does Decreased Adipose Tissue Eosinophil Content Impair Adipocyte Biology in Human Subjects with Obesity and Insulin Resistance?

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ABSTRACT IMPACT: Extrapolating from mouse data we explored eosinophil content in human adipose tissue and its effect on adipocyte biology potentially leading to the discovery of novel therapeutic targets for treatment of obesity and insulin resistance. OBJECTIVES/GOALS: The interaction between immune cells and adipose tissue (AT) in obesity has not been fully elicited. Mouse models of diet-induced-obesity show AT resident eosinophils (EOS) help preserve insulin sensitivity (IS). As data in human obesity are lacking, here we explored AT-EOS content and their role in AT metabolism in subjects with and without obesity. METHODS/STUDY POPULATION: We recruited lean (L) subjects and patients with obesity (Ob) to undergo abdominal subcutaneous AT biopsy and evaluation of insulin resistance (IR) by determination of HOMA-IR. Circulating EOS were isolated from all participants under fasting conditions and exposed to high glucose (HG) or high lipids (HL) for 4 hrs. AT EOS number was assessed via FACS analysis. Circulating EOS and AT mRNA was assessed by qPCR for multiple genes involved in inflammation and cell migration. To evaluate the effect of EOS on primary human adipocytes, in vitro cultures were exposed for 4 days to either interleukin-4 (IL-4), interleukin-13 (IL-13) or to human EOS. Adipocytes mRNA levels were evaluated for genes involved in adipogenesis and lipid metabolism. RESULTS/ANTICIPATED RESULTS: 16 lean, IS subjects (BMI 22.5+ 0.4kg/m2) and 22 age-matched IR patients with obesity (BMI: 38.9 + 1.0kg/m2) participated. We observed a ratio of 2:1 in AT EOS content of L vs Ob subjects ($P<0.03$). To assess the reduced AT-EOS content in obesity, we evaluated expression of Chemokine-C receptor 3 (CCR3) in circulating EOS. We show decreased CCR3 mRNA levels in Ob vs L subjects ($P=0.006$). We expect HL in vitro experiments on peripheral EOS of L subjects to affect CCR3 mRNA levels. In AT of Ob subjects, we found a significant decreased expression of Eotaxin 2, the main EOS chemokine binding CCR3 expressed on EOS. Preliminary data from in vitro primary adipocytes culture suggest for IL-4 and IL-13 to increase mRNA level of Peroxisome Proliferator Activated Receptor Gamma (PPARG), the master regulator of adipogenesis. DISCUSSION/SIGNIFICANCE OF FINDINGS: Comparable to animal studies, we found a decrease of AT-EOS content in patients with obesity. Alterations in CCR3/Eotaxin 2 signaling may be involved. IL-4 &IL-13 are secreted predominantly by EOS and appear to directly regulate gene expression in human adipocytes. These data represent the first evidence for a novel role of EOS in human AT biology.

95332

Intestinal inflammation and altered gut microbiota associated with inflammatory bowel disease render mice susceptible to Clostridioides difficile colonization and infection

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ABSTRACT IMPACT: Use of this novel murine model of inflammatory bowel disease (IBD) and C. difficile infection (CDI) will aid in developing new clinical approaches to predict, diagnose, and treat CDI in the IBD population. OBJECTIVES/GOALS: IBD is associated with intestinal inflammation and alterations of the gut microbiota, both of which can diminish colonization resistance to C. difficile. Here, we sought to determine if IBD is sufficient to render mice susceptible to C. difficile colonization and infection in the absence of other perturbations, such as antibiotic treatment. METHODS/STUDY POPULATION: C57BL/6 IL-10-/- mice were colonized with Helicobacter hepaticus to trigger colonic inflammation akin to human IBD. Control mice, not colonized with H. hepaticus, were pretreated with the antibiotic cefoperazone to render the gut microbiota susceptible to CDI. Mice were then gavaged with spores of the toxigenic C. difficile strain VPI 10463 and monitored for C. difficile colonization and disease. The fecal microbiota at the time of C. difficile exposure was profiled by 16S rRNA gene sequencing and analyzed using mothur. Statistical analyses were performed using R. RESULTS/ANTICIPATED RESULTS: Mice with IBD harbored significantly distinct intestinal microbial communities compared to non-IBD controls at the time of C. difficile spore exposure (14 days post-IBD trigger). Mice with IBD were susceptible to persistent C. difficile colonization, while genetically identical non-IBD controls were resistant to C. difficile colonization. Concomitant IBD and CDI was associated with significantly worse clinical and intestinal disease than unaccompanied IBD. DISCUSSION/SIGNIFICANCE OF FINDINGS: Patients with IBD who develop concurrent CDI experience increased morbidity and mortality. These studies in a novel mouse model of IBD and CDI emphasize the dual importance of host responses and alterations of the gut microbiota in susceptibility to C. difficile colonization and infection in the setting of IBD.

https://doi.org/10.1017/cts.2021.632 Published online by Cambridge University Press