using data-dependent acquisition. RESULTS/ANTICIPATED RESULTS: Mass spectrometry data collected from the laser captured glomeruli was searched against the human proteome fasta database from Uniprot using MaxQuant. IBAQ values were used for quantitation and statistical analysis. Null hypothesis significance testing was performed for each protein by comparing each sample group to the rest of the samples in the data set. In the control groups, the causative antigens PLA2R and THSD7A were detected and quantified with the largest magnitude fold change in their respective category, validating the experimental design. Using this approach, the proteins SAP, NELL1, and NCAM1 were identified and subsequently validated as causative antigens in distinct patient cohorts. DISCUSSION/SIGNIFICANCE OF FINDINGS: Here, we share the results of our efforts to comprehensively identify the spectrum of causative antigens in membranous glomerulopathy. In this context, antigen discovery is an essential first step for the development of non-invasive assays to inform prognosis, monitor response to treatment, and better understand disease etiology.

22511

Glycolipid-loaded nanoparticles harness iNKT cells for tumor immunotherapy

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ABSTRACT IMPACT: My work is on the development of a novel tumor immunotherapy to treat various types of cancer OBJECTIVES/GOALS: As iNKT cells can have direct and indirect killing effects on tumor cells, we propose a novel strategy for activating iNKT cells, via a PLGA nanoparticle delivery platform, to promote anti-tumor immune responses. METHODS/STUDY POPULATION: Poly-lactic-co-glycolic acid (PLGA) nanoparticles can be reproducibly loaded with an iNKT cell glycolipid agonist, alpha-galactosylceramide (αGalCer), and a tumor associated antigen, ovalbumin (OVA). We then test our nanoP prophylactically and therapeutically against a murine model of melanoma, B16F10-OVA. RESULTS/ANTICIPATED RESULTS: These dual-loaded PLGA nanoparticles rapidly activate iNKT cells in vivo to produce IFNgamma. Furthermore, in an in vivo model of melanoma, using B16F10-OVA cells, both prophylactic and therapeutic administration of nanoparticles containing αGalCer and OVA led to decreased tumor cell growth and increased survival. We also show our nanoparticle therapy has synergistic potential with clinically used immune checkpoint blockade (ICB) therapies, anti-PD-1 and anti-CTLA-4, indicated by the significance increase in survival and lower tumor growth rate of ICB +nanoP treated mice compared to either ICB or nanoP alone. DISCUSSION/SIGNIFICANCE OF FINDINGS: This novel delivery system provides a platform with tremendous potential to harness iNKT cells for cancer immunotherapy purposes against many cancer types.

31547

Regulation and function of the i6A37 tRNA modification

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ABSTRACT IMPACT: MiaA has a human homolog known as TRIT1. Mutations in TRIT1 have been associated with rare diseases such as MELAS and MERRF syndromes. These diseases are associated with mitochondrial disfunction.Understanding the mechanisms of bacterial sRNAs, and the miRNAs associated with these diseases could potentially afford the insight into effective cures. OBJECTIVES/GOALS: The aim is to investigate the regulation and function of tRNA isopentyladenine transferase enzyme in Escherichia coli. We aimed to execute screens for the identification of small RNA regulators of MiaA. The study will also investigate if i6A tRNA modification is necessary for the expression of major heat shock and mitochondrial proteins. METHODS/STUDY POPULATION: We constructed a chromosomal miaA-lacZ translational fusion driven by the arabinose responsive PBAD promoter and used it to screen against an Escherichia coli small RNA library. Using CsrB, one of our candidate sRNA regulators from our genetic screen, we measured the steady state levels of MiaA by Northern Blot in a PBAD-miaA2(P2HS)-lacZ translational fusion strain whereby pBR-pLac-csrB, pBR-pLac-csrA and the pBR-pLac vector are over-expressed, and under the control of an IPTG inducible promoter. Additionally, and in the same PBAD-miaA2(P2HS)-lacZ translational fusion strain background, we measured the steady state levels of MiaA in the wild type, csrA:zeo mutant strain, and csrA:zeo pBR-pLac-csrA complementation strain to determine if a combination of the pair would restore the wild-type genotype. RESULTS/ANTICIPATED RESULTS: Upon measuring the effect of small RNAs on miaA expression using quantitative b-galactosidase assays, we saw a 5-fold decrease in the expression of MiaA in the miaA-lacZ translational fusion containing sRNA CsrB, suggesting that this sRNA may play a role in the regulation of post-transcriptional expression of MiaA.From our northern blotting analysis, we observed a 6-fold decrease in MiaA expression in the absence of csrA, suggesting that csrA is essential for MiaA expression. DISCUSSION/SIGNIFICANCE OF FINDINGS: Identifying, mapping and characterizing how MiaA is regulated post-transcriptionally will give us an increased understanding in the maintenance and regulation of the normal function of E.coli to conserve homeostasis and translation fidelity.

36344

Effect of CHRNA5 genetic variation and smoking on alcohol related phenotypes in healthy adult drinkers

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ABSTRACT IMPACT: Understanding the influence of genetic variation and smoking on alcohol consumption helps in improving the treatment strategies for alcohol addiction OBJECTIVES/GOALS: Variation in the nicotinic receptor gene CHRNA5 (rs16969968) is associated with nicotine use and dependence, however its role in alcohol consumption is unclear. This study examined the effects of rs16969968 and smoking on alcohol related phenotypes in people without alcohol use disorder (AUD). METHODS/STUDY POPULATION: The study included 1,037 healthy adult drinkers without AUD (201 smokers, 836 non-smokers). A subset (n=161) participated in an Intravenous Alcohol Self-Administration (IV-ASA) laboratory session. Alcohol-related measures included Timeline Followback (TLFB), which measures drinking quantity

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and frequency in the past 90 days, and the Alcohol Use Disorders Identification Test (AUDIT), which measures alcohol use and consequences. IV-ASA measures included average and peak breath alcohol concentration (BrAC). The effect of rs16969968 was tested using a dominant model based on the presence of the A allele, and the influence of the rs16969968 polymorphism and smoking on alcohol phenotypes was assessed using t-tests and two-way ANOVA. RESULTS/ANTICIPATED RESULTS: There was a main effect of rs16969968 genotype with A-allele carriers (AA/AG) showing higher AUDIT-Dependence scores compared to the GG group. A main effect of smoking was observed on all the TLFB and AUDIT measures, with smokers showing greater alcohol consumption and problems compared to non-smokers. In the rs16969968 AA/AG group, smokers reported significantly more drinking days ($p<0.0001$), and greater number of drinks ($p<0.0001$), as well as higher AUDIT scores than non-smokers. IV-ASA measures did not show any difference between genotype groups or between smokers and non-smokers. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study identifies both independent and interactive effects of CHRNA5 gene variation and smoking on alcohol drinking measures and provides strong evidence for the effect of smoking on alcohol drinking and its consequences.

52500

Characterization of a Series of 1,4-diaryl-pyrazolo-pyridinones as Anti-Leishmanial Agents*

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ABSTRACT IMPACT: The first-line chemotherapies used to treat leishmaniasis are highly toxic intravenous antimonials yet drug resistance has begun to develop, causing the use of oral treatment options with high price tags; there is a strong need for new, safe, and effective chemotherapeutic agents to treat leishmaniasis. OBJECTIVES/GOALS: This study was conducted in order to identify novel chemical compounds that exhibit anti-leishmanial activity and to further characterize their efficacy and toxicity in in vitro and in vivo systems in the hopes of future chemotherapeutic developments. METHODS/STUDY POPULATION: A total of 28 unique 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP) compounds were synthesized and anti-leishmanial efficacy and host cell toxicity were determined using L. donovani mCherry-expressing amastigotes and THP-1 macrophages. Additional pharmacokinetic analyses of a potent 1,4-DAPP compound were conducted, revealing a potential metabolite structure. A select group of the novel compounds were screened in a cutaneous leishmaniasis (CL) murine model using L. major mCherry-expressing parasites and female Balb/C mice. The treatment consisted of 10 intralesional injections of compound over a period of 4 weeks, while lesion growth was monitored via fluorescence and manual measurements. RESULTS/ANTICIPATED RESULTS: Four experimental compounds had IC50 values less than 5 micromolar, providing similar anti-leishmanial activity to Miltefosine. Compound 9279817 had a clearance almost twice the rate of normal hepatic blood flow and had a relatively high volumes of distribution, indicating this compound is rapidly cleared and distributes into tissues. In vitro rat liver microsome assays suggest a rapid metabolism of 9279817 and MS/MS results suggest this metabolite is most likely formed via oxidation of the sulfur on the lower aryl ring. This sulfoxide metabolite has similar efficacy as the parent compound and does not exhibit toxicity in vitro. Three of the experimental compounds behaved similarly to the antimony positive control in the murine CL model. DISCUSSION/SIGNIFICANCE OF FINDINGS: This study revealed a novel structural class of compounds that have anti-leishmanial activity. Experiments show compounds with similar efficacy to Miltefosine while having significantly less cytotoxicity, suggesting that the 1,4-DAPP structural class could be further developed as a potential chemotherapeutic.

60404

HIV Tat Induced Neuroinflammation

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ABSTRACT IMPACT: Demonstrate the role of astrocyte released MMPs in response to pathogenic HIV protein Tat. OBJECTIVES/GOALS: In the presence of the pathogenic HIV protein Tat, astrocytes have been demonstrated to adopt an inflammatory phenotype as well as release extracellular matrix degrading enzymes, MMPs. Our work aims to identify whether MMPs alter perineuronal net integrity and working memory in a mouse model of Tat-induced neuroinflammation. METHODS/STUDY POPULATION: Stereotaxic Injection: C57BL6/J mice were injected bilaterally with HIV-1 IIIB Tat 5ug in 5uL or Vehicle (0.2M KCl, 5mM DTT, 50mM Tris, pH 8.0), into the hippocampus (CA1; -1.9mm AP, ±1.6mm ML, -1.5mm DV from pial surface). All outcome measurements were performed 14-days post injection. Behavior: T-maze was used to assess working memory following Tat exposure. qRT-PCR: TaqMan probes were used according to manufacturer on extracted whole hippocampus mRNA. IF: GFAP and CD68 immunofluorescence was used to determine inflammation post injection. Inhibitory interneurons (parvalbumin positive) and peri-neuronal nets (WFA positive) were quantified. WB: Synaptosomes from whole hippocampi (Syn-PER) were isolated and synaptic excitatory markers were quantified (PSD-95, synaptophysin, GluR2a). RESULTS/ANTICIPATED RESULTS: Tat exposure resulted in impairments in working memory as measured by T-maze alternations and an increase in hippocampal mRNA expression of MMP-13 and IL-1β, indicative of neuroinflammation. We also noted an increase in GFAP+ injection site width 14 days post-Tat injection, suggesting robust gliosis. While there were no changes in the excitatory pre and post synaptic markers we found a significant decrease in the percent of PV+ interneurons with peri-neuronal nets (PNNs) following Tat exposure. Taken together, this preliminary data supports a role for inflammation and PNN integrity in Tat-induced alterations in working memory. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our findings suggest that Tat contributes to cognitive impairment and that astrogliosis with elevated MMP-13 facilitates the degradation of peri-neuronal nets (PNNs) within the hippocampus. Since PNN degradation can alter neuronal circuitry future studies will focus on Tat-induced changes in hippocampal signaling.

66108

Central Cholinergic Synapse Formation in Optimized Primary Septal "Hippocampal Co'' cultures

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ABSTRACT IMPACT: Optimization of primary septal-hippocampal co-cultures facilitates studying central cholinergic synapse formation and dysfunction OBJECTIVES/GOALS: Septal cholinergic

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