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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