Basic Science

13109

Phosphorylation state of Myristoylated Alanine-Rich C-Kinase Substrate Effector Domain mimetics determines its cytotoxicity in glioblastoma and macrophage model

Hasan Alrefai, BS, Nicholas J. Eustace, MD, PhD, Joshua C. Anderson, PhD, Patricia H. Hicks, BS, Peter H. King, MD and Christopher D. Willey, MD, PhD
The University of Alabama at Birmingham School of Medicine

ABSTRACT IMPACT: This study provides insight into how MED2 impacts the immune cells surrounding glioblastoma that help it to grow and spread; having a more complete understanding of how MED2 works will help us better develop therapies that may one day enter the clinic to improve patient outcomes in glioblastoma.

OBJECTIVES/GOALS: The purpose of this study was to determine whether the phosphorylation state of the MED2 peptide impacts its biological activity in GBM and macrophages. MED2 variants include the phosphorylatable wild-type (MED2), pseudo-phosphorylated (MED2-PP), non-phosphorylatable (MED2-NP) and control length (CTL2) peptides. METHODS/STUDY POPULATION: MED2, MED2-NP, MED2-PP, and CTL2 were screened against a panel of molecularly characterized glioblastoma patient derived xenografts and IL4/13 stimulated M2-like THP-1 macrophages. The luminescent cell viability assay, CellTiter-Glo, was used to determine viability. RESULTS/ANTICIPATED RESULTS: The proneural lines XD456 and XI441 were highly sensitive to 5 μM MED2 and 5 μM MED2NP compared to 5 μM MED2PP (p<0.001). There was no statistically significant difference between untreated, 5 μM CTL2, and 5 μM MED2PP groups or between the MED2NP and MED2 treated groups. M2-like THP-1 macrophages were highly sensitive to 10 μM MED2NP compared to 10 μM CTL2 (p<0.01) and 10 μM MED2PP (p<0.01). No statistically significant difference was observed between untreated, 10 μM MED2, 10 μM MED2PP, and 10 μM CTL2 groups. DISCUSSION/SIGNIFICANCE OF FINDINGS: The phosphorylation state of MED2 determines its toxicity. When MED2 is phosphorylated, it is nontoxic to GBM or M2-like macrophages. The non-phosphorylatable version is toxic to both GBM and M2-like macrophages. The wild-type peptide is toxic to GBM but not M2-like macrophages, suggesting that MED2 may be phosphorylated in M2-like macrophages.

21746

Antigen discovery in membranous glomerulopathy using laser capture microdissection and mass spectrometry*

Jacquelyn Fede1, Stephen Kogut2, Anthony Heyward2, John F. Stevenson1, Amy Nunn3, Julie Paut3 and Judy A. Kimberly3
1University of Rhode Island, 2Warren Alpert Medical School, Brown University and 3Brown University

ABSTRACT IMPACT: Identifying the causative antigen in membranous glomerulopathy cohorts enables the development of serum assays to detect and monitor disease progression without the need for invasive kidney biopsies. OBJECTIVES/GOALS: Primary membranous glomerulopathy is caused by the formation of autoantibody immune complexes which deposit in the glomerulus and obstruct kidney function. Causative antigens remain to be identified in roughly 20% of cases. Our goal is to identify the antigen in these cohorts, so that non-invasive assays can be developed for disease monitoring. METHODS/STUDY POPULATION: Renal biopsy tissue from known antigen cases (PLA2R, THSD7A), and unknown cases were included in the analysis. Renal biopsy tissue from formalin fixed paraffin embedded tissue was cut at a thickness of 10 μm onto Leica PET-membrane frame slides. These slides were then stained with hematoxylin. The glomeruli were microdissected into microcentrifuge tubes using a Leica DM6000B microscope. The microdissected glomeruli were lysed in 2% SDS and 0.1MDTT at 99 degrees Celsius for 1 hour and processed by filter assisted sample preparation (FASP). Digested peptides were analyzed by liquid chromatography-mass spectrometry using an Orbitrap Fusion Lumos
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 nanoparticle prophy-lactically 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 IFNγamma. 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 ICβ +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.

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

Shyamala K. Venkatesh1, Bethany L. Stangl1, Natalie A. Quijano Cardé2, Mariella De Biasi2 and Vijay A. Ramchandani2
1Section on Human Psychopharmacology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA and 2Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

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 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 quanti- tation 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 sub- sequently 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 treat- ment, and better understand disease etiology.

Regulation and function of the i6A37 tRNA modification

Joseph I Aubee1, Kinlynn Williams2, Alexandria Adigun3, Olufolakemi Olusanya2, Karl M Thompson1
1Department of Microbiology, College of Medicine, Howard University, 2Department of Biology, Claflin University and 3Department of Biology, Howard University

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 pro- moter. 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 combina- tion 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-transcription- ally will give us an increased understanding in the maintenance and regulation of the normal function of E.coli to conserve homeo-stasis and translation fidelity.

Glycolipid-loaded nanoparticles harness iNKT cells for tumor immunotherapy

Travis Shute1, Elizabeth Dudley1, Andrew Lai1, Briana Salas2, Brandy Vincent2, Daniel Angel2, Kelly Nash2 and Elizabeth Leadbetter2
1UT Health San Antonio and 2UTSA

ABSTRACT IMPACT: My work is on the development of a novel tumor immunotherapy for non-invasive assays to inform prognosis, monitor response to treatment and better understand disease etiology.