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Modulating decreases in Superior Colliculus activity through optogenetic activation attenuates seizures in the WAG/Rij genetic model of Absence Epilepsy

Devin D. Palmer[1], Carolina Campos-Rodriquez[2] and Patrick A. Forcelli[2]
[1]Georgetown-Howard Universities and [2]Georgetown University

OBJECTIVES/GOALS: While anti-seizure medications are effective, nearly one third of patients have seizures that go untreated. Prior studies using evoked seizure models have shown that activation of the Superior Colliculus (SC) display anti-seizure effects. Here we monitored and modulated the DLSC to suppress spontaneous seizures in a genetic model of epilepsy. METHODS/STUDY POPULATION: WAG/Rij rats (4 months old) were employed as study subjects. Animals were surgically prepared for virus injection (ChR2 excitatory opsin, or control vector), fiber optic implantation and cortical EEG for optogenetic studies. For In vivo electrophysiology, animals were implanted with a 16 wire multi-electrode array into the DLSC. In optogenetic experiments, we compared the efficacy of continuous neuromodulation to that of on-demand neuromodulation (real time detection of seizures) paradigms on a within-subject basis. We compared three stimulation frequencies on a within-subject basis (5, 20, 100 Hz). We quantified the number and duration of each spike wave discharge (SWD) during each two-hour-long trial. Electrode array single units were sorted and analyzed for activity before, during and after seizures. RESULTS/ANTICIPATED RESULTS: In vivo electrophysiology found there to be a significant decrease in single unit activity leading up to the start of SWDs. Interestingly, on-demand neuromodulation was effective in both females and males – where the greatest reduction in seizure duration was under 100 Hz light delivery. As expected, male and female animals injected with a control vector did not show a reduction in seizures in response to light delivery. In the open-loop (continuous) stimulation paradigm, optogenetic activation of the DLSC was without effect on the number or duration of SWDs at any of the frequencies examined. DISCUSSION/SIGNIFICANCE: SC activity is significantly decreased prior to the start of seizures. Furthermore, activation of the SC displays anti-seizure effects in a model of spontaneous seizures. A striking difference between open and closed-loop neuromodulation approaches underscores the importance of stimulation paradigm in determining therapeutic effect.

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Repurposing FDA-approved PI3K/Akt Inhibitors to Improve Anti-Cancer Drug Brain Uptake in Glioblastoma Resection Models

Louis Rodgers[1], Yuma Tega[1], Julia A. Schulz[1] and Anika M.S. Hartz[2], Bjoern Bauer[1]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY, USA and [2]Sanders-Brown Center on Aging and Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky, Lexington, Kentucky, USA

OBJECTIVES/GOALS: We have shown that glioblastoma upregulates blood-brain barrier drug efflux transporters via a mechanism that likely involves TNFÎ± and PI3K/Akt. Our goal is to repurpose FDA-approved PI3K/Akt inhibitors to increase anticancer drug brain concentrations, which holds the potential for translation into the neuro-oncology clinic. METHODS/STUDY POPULATION: GL261 Red-FLuc and MBR525-1 Red-FLuc cells (2Î¼l; 2.5K cells/Î¼l; 1Î¼l/min) were injected into the right hemisphere of 8-week old female J:NU mice (coordinates relative to bregma: AP -2 mm, ML -2 mm, DV -3 mm). Tumor burden was assessed weekly with IVISÂ® Spectrum in vivo imaging; tumor volume and invasiveness were measured by MRI and histopathology, respectively. On day 14 post-injection, mice received 5-ALA (200 mg/kg ip), and tumors were resected with a 2 mm punch biopsy tool and surgical fluorescence microscope (ex/em: 405/635nm). Drug efflux transporter expression and activity in isolated brain capillaries were determined by Western blot and substrate fluorescence assays, respectively. Cytotoxicity was assessed after 48-hour drug incubation using CyQuant MTT Cell Proliferation Assay kits. RESULTS/ANTICIPATED RESULTS: IC50 values of temozolomide, lapatinib, alpelisib, and miltefosine were N/A, 32, 20, and 190 Î¼M for GL261 Red-FLuc cells and N/A, 49, 36, and 148 Î¼M for MBR525-1 Red-FLuc cells, respectively. Median survival of GL261 Red-FLuc mice was 26.5d and significantly increased to 34d with resection (p=0.116). In GBM mice, drug efflux transporter expression and activity levels in brain capillaries isolated from the contralateral hemisphere were significantly upregulated compared to sham controls. Furthermore, treatment with FDA-approved PI3K/Akt inhibitors, alpelisib and miltefosine, significantly reduced drug efflux transporter expression and activity to control levels. In PK and survival studies, we expect that PI3K/Akt inhibition will increase brain uptake of anticancer drugs and prolong GBM mouse survival. DISCUSSION/SIGNIFICANCE: We have previously shown that PI3K/Akt inhibition reduces P-gp/BCRP levels in brain capillaries. Here, we vertically extend this strategy by repurposing the FDA-approved PI3K/Akt inhibitors alpelisib/miltefosine to improve brain uptake of anticancer drugs in GBM resection models.

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COVID19 disease severity influences the expression of markers of durability in memory B cells*

Raphael Reyes[1], Kathleen Clarke[1], Angelene M. Cantwell[1], Gabriel Catano[1], Robin E. Tragus[1], Thomas F. Patterson[1], Sebastiaan Bol[1] and Evelien M Bunnik[1]
[1]The University of Texas Health Science Center at San Antonio

OBJECTIVES/GOALS: Studies have shown that SARS-CoV-2 specific memory B cells can be maintained at least a year after exposure. However, reports show an altered B cell response during infection in severe COVID-19 cases. This study aims to describe the B cell response during COVID-19 convalescence with a focus on signatures that contribute to durable and robust immunity. METHODS/STUDY POPULATION: Our study cohort consisted of individuals who had recovered from non-severe (hospitalized) or severe (hospitalized and requiring invasive mechanical ventilation) COVID-19. In our comparative analysis, samples from both groups were carefully matched to fall within 4-5 weeks post-symptom onset. We also performed a longitudinal analysis of non-severe patients with sampling ending 5 months post-symptom onset. Using high parameter flow cytometry, we characterized the phenotype of memory B cells using 19 distinct cell markers and fluorescently labeled probes to identify B cells reactive with SARS-CoV-2 spike and receptor-binding domain protein. Additionally, serum collected from individuals was used to quantify antibody titers. RESULTS/ANTICIPATED RESULTS: The frequency of spike-specific B cells

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and serum antibody titers were similar between severe and non-severe groups. However, we observed that individuals recovered from severe COVID-19 have a significantly reduced frequency of spike specific IgG + memory B cells expressing Tbet and FcRL5 (markers associated with long lived immunity). In the non-severe patients, we observed IgG +Tbet+ B cells targeting the spike protein peak at 2-3 weeks post-symptom onset, decrease by almost fifty percent 4-5 weeks post-symptom onset, and return to baseline 5 months post-symptom onset. Our study also validated previous findings of a short-lived primary response of IgM+ B cells targeting the spike protein. DISCUSSION/SIGNIFICANCE: Our findings highlight potential implications for long-term immunity against re-infection or severity of the resulting disease in patients with severe COVID-19. Further investigation will be necessary to determine whether the maintenance of immunological protection is hindered in patients who overcame severe COVID-19.

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Post-translational role of RNA modifications in sRNA chaperone Hfq

Jalisa Nurse[2], Joseph[l], Aubee[2] and Karl M Thompson[1]
[1]Department of Microbiology, Howard University College of Medicine, Washington DC and [2]Howard University

OBJECTIVES/GOALS: The goal of this study is to determine the role of the tRNA modifications in the translation of Hfq. Hfq is an RNA chaperone that acts as a co-factor for the action of the largest class of small RNAs in E. coli. RNA modifications have been known to play critical roles in the translational fidelity of many cellular proteins in bacteria. METHODS/STUDY POPULATION: In this study, we used an hfq-lacZ translation fusion to screen several RNA modification mutant genes to uncover additional RNA modifications that may play a role in Hfq translation. We measured hfq-lacZ activity in genetic backgrounds mutated for several additional RNA modification enzymes previously untested for Hfq effects. RESULTS/ANTICIPATED RESULTS: We identified 5 RNA modification genes that were defective for hfq-lacZ fusion activity, and we subsequently performed western blot analysis on the Hfq protein in the absence of these modification mutant genes to determine the effect of these mutants more directly on Hfq protein levels. We identified 2 out of these 5 RNA modification mutants that also affect Hfq protein levels. DISCUSSION/SIGNIFICANCE: Since Hfq is critically important for small RNA function is a wide range of bacteria, it is possible tRNA modifications regulate Hfq expression in other bacteria. These processes, when further investigated, could provide us with the basic information to develop new antibiotics needed to address emerging antibiotic resistance.

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Inhibition of GPR30 Reveals Putative Genes Involved in the Pathogenesis of Inflammatory Breast Cancer

Melanie Carrion-Laureano[1], Melvyn Acosta-Montalvo[1,2], Xavier Bittman-Soto[2], Esther Peterson-Peguero[2,3] and JosuÃ© PÃ©rez-Santiago[3,4]
[1]University of Puerto Rico Comprehensive Cancer Center, [2]University of Puerto Rico Rio Piedras, Department of Biology, [3] University of Puerto Rico Comprehensive Cancer Center, Division of Cancer Biology and [4]University of Puerto Rico Medical Sciences Campus, School of Dental Medicine

OBJECTIVES/GOALS: Inflammatory Breast Cancer (IBC) is the most aggressive form of breast cancer and does not have targeted therapy. GPR30, a 7-transmembrane estrogen receptor, may play a role in regulating cell growth and proliferation of cancerous cells. Here, we evaluated changes in gene expression while inhibiting GPR30 to determine putative targets to treat IBC. METHODS/STUDY POPULATION: IBC cell lines (SUM149PT) were cultured in medium with serum stripped from growth factor and hormones for 48 hours. Cells were then exposed to either G15 (GPR30 inhibitor) at a concentration of 1ÂµM or ETOH (vehicle negative control) 3 hours in triplicates. After exposure, total RNA was extracted using the Qiagen RNAeasy Mini kit and RNA was sequenced using the Illumina NextSeq (2 X 75bp). The higher-quality reads were aligned, annotated, and quantified to the human genome (HG38) using STAR and RSEM softwares. Gene expression analysis was performed in R statistical software (packages tximport and DESeq2). Functional and enrichment analyses were performed using Metascape and STRING database, respectively. RESULTS/ANTICIPATED RESULTS: There were 656 significantly expressed genes ($p < 0.05$) between groups (G15 vs. ETOH). The top 5 significant genes include: SMIM7, FANCG, ARID1A, MAML2, and ATF3. Significantly impacted biological processes and pathways include: electron transport chain, mitotic cell cycle process, microtubule cytoskeleton organization, cellular component morphogenesis and DNA-dependent DNA replication (adj $p < 0.05$). Additionally, physical and functional interaction networks showed 3 major clusters (â‰¥ 12 genes), which contained several gene hubs including BRCA1, BRCA2, FOS (proto-oncogene), PLK1 and PAK1 (both serine/threonine-protein kinases), among others. Interestingly, the network analysis showed the previously known interaction between FANCG and BRCA2, which were both dysregulated by GPR30 inhibition. DISCUSSION/SIGNIFICANCE: Through gene expression, functional and enrichment analyses we found several targets genes that could be associated with the pathogenesis of IBC. Validation of candidates genes (qRT-PCR and Western blot), and functional assays (cell proliferation, motility, and invasion) will be performed to understand the potential of these genes in treating IBC.

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Isolation and identification of bioprospects capable of metabolizing 17-beta-estradiol and 17-alpha-ethinylestradiol using metagenomics and culture-dependent techniques in Puerto Rico

Carlos RÃos-VelÃ¡zquez[1], Melissa M. Cabrera-RuÃz[1], Kristal M. Delgado-Serrano[1] and Hailey N. Morales-Lajara[2]
[1]University of Puerto Rico at Mayaguez, Biology Department and [2]University of Puerto Rico at Mayaguez, Chemical Engineering Department

OBJECTIVES/GOALS: This research project aims to isolate and identify bioprospects capable of metabolizing estrogen using culture-dependent, culture independent methods and the identification of the gene/genes responsible for the metabolization of estrogen by the bioprospects. METHODS/STUDY POPULATION: For the culture dependent technique, samples were collected from the water treatment plant in MayagÃ¼ez, cultivated on TSA medium and selected specific and diverse colonies were patched on M-9NC (no carbon sources), M-9-glucose (M9G) and M-9-hormone mixture (M9H: 17-beta-Estradiol and 17-alpha-Ethynylestradiol). After the 48hrs incubation at 25 and 37 Celsius, growth was scored on the different media, to choose those potential bioprospects that use the hormones as the sole carbon source. For the culture independent approach, metagenomic clones from libraries generated from the

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