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

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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

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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*

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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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