Characterization of the host pericyte role in glioblastoma angiogenesis
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OBJECTIVES/SPECIFIC AIMS: Glioblastoma (GBM) carries a prognosis of 14.6 months mean survival despite maximal surgical, chemotherapeutic, and radiation therapy. The pericyte is a recently characterized cell shown to be a critical component of cerebral vessel physiology and pathology. Importantly, alterations in pericyte densities have shown resulting changes in breast and lung tumor growth. We leverage transgenic pericyte-deficient mouse models to evaluate resulting behavior of implanted patient-derived GBM. METHODS/STUDY POPULATION: Patient-derived, green fluorescent protein labeled, GBM will be implanted in right frontal bregma of both 6-month old pericyte-deficient (PDGFR α−/−) mice and age-matched wild-type littermate controls (IACUC 20755, IRB 16-00929), which are immunosuppressed via daily intraperitoneal cyclosporine injection. In total, 30 mice of both genders are included in tumor and control cohorts. Fixed cortical sections following 3-week period will be stained for pericytes (NG2), endothelium (CD31), hypoxia (pimonidazole), and tumor size. One-way ANOVA with will used to compare groups using SAS software (Cary, NC). RESULTS/ANTICIPATED RESULTS: Feasibility studies show robust in vitro growth of patient-derived GBM cells, showing continued growth over 10 cellular division passages. Lentivirally transduced GFP reveals reliable tumor tracking both in vitro and in vivo. Transgenic mice at 6 months display reproducibly decreased pericyte and microvascular density in triplane. Wild-type mice tolerate tumor injection up to three weeks with visible tumor growth, peritumoral hypervascularity, and no evidence of mouse neural dysfunction. With current cohorts recently implanted with tumor, we anticipate a significant decrease in tumor size with Cohen’s d effect size of 0.5 in GBM implanted in pericyte-deficient mice when compared to control. Effect sizes are based moderate to large (effect size 0.5–0.8) reductions of in vitro GBM growth in vascular gene (TGF-β) knockdown studies. In addition, tagged tumor-derived pericytes should comprise a greater proportion of new vasculature in pericyte-knockdown mice to overcome host pericyte depletion. Finally, tumors in transgenic mice should show increased hypoxia from limitations in angiogenesis. DISCUSSION/SIGNIFICANCE OF IMPACT: Feasibility studies show successful tracking of fluorescently tagged-patient derived GBM samples in transgenic mice with decreased vasculature. GBM grafts show no evidence of immunogenic response with cyclosporine protocol. Successful limitation of tumor size with reduced pericyte density will provide support to increasing study of blood-brain barrier: stem cell activity and inflammatory activity of pericyte microenvironments altering GBM behavior. Furthermore, implementa-

tion of known pericyte targeted therapies, such as imatinib, can be evaluated for GBM patient treatment efficacy. Studies with assembled clinical translational research scholar mentorship team will allow the principal investigator to develop an independent career with laboratory focused on contributing to improved patient outcomes, translating successful pericyte-targeted results to patient trials.

A clinically relevant rabbit surgical model of pelvic reconstruction to evaluate the immune response to novel surgical materials
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OBJECTIVES/SPECIFIC AIMS: Pelvic organ prolapse, a disorder in which the muscles of the pelvic floor are weakened over time, affects over a million women each year in the United States. A quarter of these women undergo a reconstructive procedure, increasingly using polypropylene mesh as mechanical reinforcement to the pelvic floor. However, the number of complications such as chronic pain and mesh erosion/exposure in women with vaginal mesh implants were reported at rates as high as 10%–20%. This indicates a limited understanding of the host response to mesh in vaginal tissue and strategies to reduce these complications. Utilizing a novel surgical technique in New Zealand white rabbits, we implant mesh analogously to human implantation and evaluate

15N-Leucine transport across the blood brain barrier is significantly impaired in the glutamine synthetase-inhibited brain
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OBJECTIVES/SPECIFIC AIMS: Astrogial glutamine synthetase (GS), which metabolizes glutamate and ammonia to glutamine, is critical for the detoxification of brain ammonia, clearance of synaptic glutamate, and production of brain glutamine. Perturbations in the expression and activity of GS are thought to play a causative role in the pathogenesis of several conditions of abnormal neurotransmission. Although the long-term consequences of GS inhibition on amino acid homeostasis in the brain are unknown, it is thought that amino acid influx in the brain is tightly coupled with glutamine efflux via the L-type amino acid transporter. Both glutamine and leucine serve many critical functions in the brain including protein synthesis, gene expression, insulin regulation, and immune signaling. The objective of this study was to determine the effects of chronic GS inhibition with methionine sulfoximine (MSO) on glutamine and leucine homeostasis in the brain. METHODS/STUDY POPULATION: In total, 12 rats were surgically implanted with microdialysis guide cannulas in the bilateral dentate gyrus. Rats were randomly divided for surgical implantation of either a MSO (n = 6) or phosphate buffer saline (PBS; n = 6) pump in the right dentate gyrus. After 7 days, bilateral microdialysis probes were placed under brief isoflurane anesthesia, and microdialysis flow was established by injecting 0.5 µL/min of artificial extracellular fluid. Dialysate samples were collected every 30 minutes for the duration of the experiment. A 13 mM 15N-Leucine (3.6 mL/h) and 2 M 2H2-13C-sodium acetate (0.0633 µL/g/min for t = 0–5 min, 0.0136 µL/g/min for t = 5–10 min, and 0.0253 µL/g/min for t > 10 min) solution was infused intravenously for 300 minutes. The EZ:Faast Free Amino Acid analysis kit and ultra-performance liquid chromatography/tandem mass spectrometry was used for quantification of amino acids in the dialysate fluid. RESULTS/ANTICIPATED RESULTS: At baseline (t = 0 h), the concentrations of glutamine were significantly lower in MSO-treated rats (p < 0.001) in the ipsilateral (GS-inhibited) hippocampus. There were no differences in glutamine concentrations between MSO and PBS-treated rats in the contralateral hippocampus. In PBS-treated rats, there was a significant increase in 15N-leucine between t = 0 hour and t = 5 hour in the contralateral (p < 0.05) and ipsilateral (p < 0.05) hippocampus. In MSO-treated rats, there was a significant increase in 15N-leucine between t = 0 and t = 5 hours in the contralateral (p < 0.05) hippocampus, but not in the ipsilateral hippocampus (p = n.s.). DISCUSSION/SIGNIFICANCE OF IMPACT: This study demonstrated for the first time that basal glutamine concentrations are low in areas of the brain where GS is acutely inhibited, and that leucine uptake in these brain areas are markedly decreased. Perturbations in glutamine and leucine homeostasis have been implicated in several disease processes including diabetes, obesity, liver disease, immune system dysfunction, epilepsy, and cancer, and the glutamine-dependent leucine influx in the brain may be a novel and important therapeutic target to treat these conditions.
changes in the immunologic response at early (14d) and tissue remodeling outcomes at late stages (90 and 180d) of implantation. The mesh-tissue complex was removed from each rabbit and processed for histological staining as well as immunolabeling of immune cells, such as macrophages. Extracellular matrix protease assays and mechanical integrity of the tissue also evaluate the overall immunologic response associated with each implant. RESULTS/ANTICIPATED RESULTS: The results of this study show that implants into vaginal tissues elicited an increased host immunologic response at 14 days as compared to those in the abdominal wall. However, at chronic time points the immunologic response in the vagina was reduced as compared to that in the abdominal cavity. The present study also demonstrates the scale-up of a previous methodology for a nanoscale coating. We present a nanometer thickness, tunable, and uniform coating capable of releasing bioactive interleukin-4. We evaluated the biological nanoscale coating. We present a nanometer thickness, tunable, and uniform coating capable of releasing bioactive interleukin-4. We evaluated the biological nanoscale coating. We present a nanometer thickness, tunable, and uniform coating capable of releasing bioactive interleukin-4. We evaluated the biological nanoscale coating. We present a nanometer thickness, tunable, and uniform coating capable of releasing bioactive interleukin-4.

A novel multi-photon microscopy method for neuronavigation in deep brain stimulation surgery

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OBJECTIVES/SPECIFIC AIMS: The goal for this project is to determine the feasibility of using a novel multi-photon fiber-coupled microscope to aid surgeons in localizing STN during surgeries. In order to accomplish this goal, we needed to identify the source of a strong autofluorescent signal in the STN and determine whether we could use image classification methods to automatically distinguish STN from surrounding brain regions. METHODS/STUDY POPULATION: We acquired 3 cadaveric brains from the University of Colorado Anschutz Medical Campus, Department of Pathology. Two of these brains were non-PD controls whereas 1 was diagnosed with PD. We dissected a 10 square centimeter region of midbrain surrounding STN, then prepared this tissue for slicing on a vibratome or cryostat. Samples were immuno-labeled for various cellular markers for identification, or left unlabeled in order to observe the autofluorescence for image classification.

RESULTS/ANTICIPATED RESULTS: The border of STN is clearly visible based on the density of a strong autofluorescent signal. The autofluorescent signal is visible using 2-photon (850–1040 nm excitation) and conventional confocal microscopy (488–647 nm excitation). We were also able to visualize blood vessels with second harmonic generation. The autofluorescent signal is quantified by high concentrations of Sudan-black B (0.5%–5%), and is primarily localized in microtubule-associated protein-2 (MAP2+) cells, indicating that it is likely lipofuscin accumulation in neurons. Smaller lipofuscin particles also accumulate in microglia, identified based on ionized calcium binding adopter 1 (Iba1+) labeling. We anticipate that colocalization analysis will confirm these qualitative observations. Using 2-photon images of the endogenous autofluorescent signal in these samples, we trained a logistic regression-based image classifier using features derived from gray-level co-occurrence matrices. Preliminary testing indicates that our classifier performed well, with a mean accuracy of 0.89 (standard deviation of 0.11) and a Cohen’s Kappa value of 0.76 (standard deviation of 0.24). We are currently using coherently-stimulated Raman scattering and high-speed imaging to identify different features of myelin that can be used to distinguish between these regions and expect similar results.

DISCUSSION/SIGNIFICANCE OF IMPACT: Traditional...