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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 + /−) 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 (piminidazole), 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 triplicate. 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, implementation of known pericyte targeted therapies, such as imantinib, 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.

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15N-Leucine transport across the blood brain barrier is significantly impaired in the glutamine synthetase-inhibited brain

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OBJECTIVES/SPECIFIC AIMS: Astroglial 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 infusing 0.5 μL/min of artificial extracellular fluid. Dialysate samples were collected every 30 minutes for the duration of the experiment. A 113 mM 15N-Leucine (3.6 mL/h) and 2 M 2–13C-sodium acetate (0.0633 μL/g/min for $t = 0$–5 min, 0.0316 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 ($p =$ ns.). 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.

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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

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