glycolytic profile that are thought to be responsible of the resistance of GBM to treatments. Metabolic reprogramming allows tumor cells to survive in unsupportive microenvironments. Manipulating tumor metabolism to counteract GBM resistance arises as a powerful approach with minimum effects in normal counterparts. At pharmacological concentrations, melatonin displays oncostatic properties. This is thought to be due to an increase in mitochondrial oxidative phosphorylation through the effects of melatonin in mitochondria, key organelle in metabolic homeostasis. We hypothesize that melatonin could alter BTIC metabolism, by inducing an anti-Warburg effect and as consequence, melatonin will decrease the viability of GBM cells and tumor growth. We found that treatment of GBM cell lines with 3mM melatonin significantly altered tumor cell metabolism. We observed that melatonin downregulated the lactate symporter MCT4 ($p<0.002$), inducing a significant intracellular accumulation of lactate ($p<0.002$) while decreasing it in the extracellular media ($p<0.001$). This was followed by a decrease in the internal pH ($p<0.002$). These effects were compensated by an increase in the oxygen consumption rate (OCR) followed by decay that leaded to an increase in ROS production ($p<0.001$). All these changes result in a depletion of cellular ATP ($p<0.001$) and eventually drove to a decrease in the proliferation ($p<0.001$) and cell death ($p<0.001$). When applied *in vivo* we observed a significant reduction in the tumor growth ($p<0.001$), volume ($p<0.002$) and weight ($p<0.002$), as well as a drop in the proliferation marker ki67 ($p<0.001$) and a fibrosis increase in treated tumors. These results position melatonin as a strong therapeutic candidate for GBM therapy.

DDRE-34. TARGETING RESISTANCE IN MEDULLOBLASTOMA

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Medulloblastoma is the most commonly diagnosed pediatric brain tumor. Although therapeutic advances have improved survival from this cancer, they result in devastating sequelae and, additionally, have proven inadequate in metastatic disease and recurrence where survival remains <5%. Effective therapies are urgently needed to improve outcomes in medulloblastoma. Medulloblastoma development is driven by dysregulation of normal cerebellar proliferation. Mutations in the sonic hedgehog (Shh) pathway are found in ~30% of these tumors and responsible for their aggressive growth. The poor outcomes in Shh-driven medulloblastoma have prompted the evaluation of Shh-targeting agents in their treatment – with limited success likely attributable in part to the upregulation of alternate survival pathways (e.g. Ras/MAPK and HIF-1α). These alternate mechanisms stimulate glycolysis, in part by increasing the activity of the 6-phosphofructo-2-kinase/fructose-2,6 bisphosphatases (PFKFB1-4) to produce fructose-2,6-bisphosphate (F26BP), a potent activator of the rate-limiting glycolytic enzyme, 6-phosphofructo-1-kinase. In recent studies, we have determined that the PFKFB4 enzyme is highly expressed in patient-derived Shh medulloblastomas. We have found that hypoxia, through HIF-1α, strongly induced PFKFB4 expression in Shh-driven medulloblastoma cells and that silencing PFKFB4 suppressed F26BP, glycolysis and proliferation in normoxia and, more markedly, in hypoxia, indicating that PFKFB4 may be required for growth under hypoxia. We found that simultaneously silencing PFKFB4 and Shh pathway effectors significantly reduced cell survival and that co-targeting PFKFB4 (with a novel inhibitor) and Shh effectors synergistically decreased cell viability. In order to simulate Shh antagonist resistance, we have now subjected Shh medulloblastoma cells to prolonged Shh inhibitor exposure and found that these cells exhibit increased proliferation, glycolysis and PFKFB4. Studies are underway to delineate their metabolic alterations. Taken together, our data indicate that targeting PFKFB4 may be a valid therapeutic option in aggressive, treatment-resistant medulloblastoma and strongly support the further examination of PFKFB4 inhibitors in these tumors.

EPIGENOME, TRANSCRIPTOME, METABOLOME AND MODELING

ETMM-01. CANCER STEM CELL ENRICHMENT AND METABOLIC SUBSTRATE ADAPTABILITY ARE DRIVEN BY HYDROGEN SULFIDE SUPPRESSION IN GLIOBLASTOMA

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Glioblastoma (GBM) remains among the deadliest of human malignancies. The emergence of the cancer stem cell (CSC) phenotype represents a major challenge to disease management and durable treatment response. The extrinsic, environmental, and lifestyle factors that result in CSC enrichment are not well understood. The CSC state endows cells with a fluid metabolic profile, enabling the utilization of multiple nutrient sources. Therefore, to test the impact of diet on CSC enrichment, we evaluated disease progression in tumor-bearing mice fed an obesity-inducing high-fat diet (HFD) *versus* an energy-balanced, low-fat control diet. HFD consumption resulted in hyper-aggressive disease that was accompanied by CSC enrichment and shortened survival. HFD consumption also drove intracerebral accumulation of saturated fats, which in turn inhibited the production and signaling of the gasotransmitter hydrogen sulfide (H_2S). H_2S is an endogenously produced bio-active metabolite derived from sulfur amino acid catabolism. It functions principally through protein S-sulfhydration and regulates a variety of programs including mitochondrial bioenergetics and cellular metabolism. Inhibition of H_2S synthesis resulted in increased proliferation and chemotherapy resistance, whereas treatment with H_2S donors led to cytotoxicity and death of cultured GBM cells. Compared to non-cancerous controls, patient GBM specimens were reduced in overall protein S-sulfhydration, which was primarily lost from proteins regulating cellular metabolism. These findings support the hypothesis that diet-regulated H_2S signaling serves to suppress GBM by restricting metabolic adaptability, while its loss triggers CSC enrichment and disease acceleration. Interventions augmenting H_2S bioavailability concurrent with GBM standard of care may improve outcomes for GBM patients.

ETMM-02. PRECLINICAL MODELS REVEAL BRAIN-MICROENVIRONMENT SPECIFIC METABOLIC DEPENDENCIES IN GLIOBLASTOMA

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Metabolic reprogramming is a hallmark of cancer, and malignant cells must acquire metabolic adaptations in response to a multitude of intrinsic and extrinsic factors to fuel neoplastic progression. Mutations or changes in metabolic gene expression can impose nutrient dependencies in tumors, and even in the absence of metabolic defects, cancer cells can become auxotrophic for particular nutrients or metabolic byproducts generated by other cells in the tumor microenvironment (TME). Conventional cell lines do not recapitulate the metabolic heterogeneity of glioblastoma (GBM), while primary cultured cells do not account for the influences of the microenvironment and the blood brain barrier on tumor biology. Additionally, these systems are under strong selective pressure divergent from that *in vivo*, leading to reduced heterogeneity between cultured tumor cells. Here, we describe a biobank of direct-from-patient derived orthotopic xenografts (GliomaPDOX) and gliomaspheres that reveal a subset of gliomas that, while able to form *in vivo*, cannot survive *in vitro*. RNA sequencing of tumors that can form both *in vivo* and *in vitro* (termed "TME-Indifferent") compared to that of tumors that can only form *in vivo* (termed "TME-Dependent") revealed transcriptional changes associated with altered nutrient availability, emphasizing the unique metabolic programs impacted by the tumor microenvironment. Furthermore, TME-dependent tumors lack metabolic signatures associated with nutrient biosynthesis, thus indicating a potential dependency of these tumors on scavenging specific nutrients from the extracellular milieu. Collectively, these data emphasize the metabolic heterogeneity within GBM and reveal a subset of gliomas that lack metabolic plasticity, indicating a potential brain-microenvironment specific metabolic dependency that can be targeted for therapy.

ETMM-03. CANCER CELLS DEPLOY LIPOCALIN- 2 TO COLLECT LIMITING IRON IN LEPTOMENINGEAL METASTASIS

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The tumor microenvironment plays a critical regulatory role in cancer progression, especially in central nervous system metastases. Cancer cells within the cerebrospinal fluid (CSF)-filled leptomeninges face substantial microenvironmental challenges, including inflammation and sparse micronutrients. To investigate the mechanism by which cancer cells in these leptomeningeal metastases (LM) overcome these constraints, we subjected CSF from five patients with LM to single-cell RNA sequencing. We found that cancer cells, but not macrophages, within the CSF express the iron-binding protein lipocalin-2 (LCN2) and its receptor SCL22A17. These macrophages generate inflammatory cytokines that induce cancer cell LCN2 expression but do not generate LCN2 themselves. In mouse models of LM, cancer cell growth is supported by the LCN2/SLC22A17 system and is inhibited by iron chelation therapy. A Phase Ia/1b clinical trial focused on this novel treatment approach is underway.