oxic oxygen conditions from cells in solitary or co-cultures were analyzed using a Bruker Avance III HD spectrometer at 600 MHz for comparison over time using PCA analysis of metabolic intermediate differences. After 16h, there was observable differences in produced metabolites between the T cells cultured alone or co-culture with GSCs, compared to the GSCs alone or media alone controls. Quantifiable changes in glucose, lactate, fumarate, acetate and pyruvate, among others, indicated a large shift in T cell metabolism dependent on oxygen conditions and co-culture interactions, while GSCs are less metabolically responsive to culture conditions. Ongoing experiments will examine precise changes in UDP-GlcNAc and glycosylation precursors in T cells and CAR-T cells via targeted NMR analysis, which we expect will help us understand energy dependent mechanisms of T cell exhaustion and lead to development of novel strategies to sustain T cell function in the hostile TME.

TAMI-10. CIRCULATING ANGIOGENIC CELLS (CACS) IN GLIOBLASTOMA: TOWARDS DEFINING CRUCIAL FUNCTIONAL DIFFERENCES IN CAC-INDUCED NEOPLASTIC VERSUS REACTIVE NEOVASCULARIZATION

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In order to identify suitable therapeutic targets for glioma anti-angiogenic therapy, the process of neovascularization mediated by circulating angiogenic cells (CACs) needs to be scrutinized. In the present study we compared the expression of neovascularization-related genes by three circulating CAC subsets (HPCs, $CD34^+$ and KDR^+ cells; internal controls: PBMCs and circulating endothelial cells) of treatment-naïve patients with glioblastoma (GBM) to those of patients undergoing reactive neovascularization (myocardial infarction (MI). CACs from umbilical cord (representing developmental neovascularization) and healthy subjects served as controls. Fluorescent activated cell sorting was used to isolate CACs, RT-PCR to determine the expression levels of a panel of 48 neovascularization-related genes, Luminex assays to measure plasma levels of 21 CAC-related circulating molecules. We found essential differences in gene expression between GBM and MI CACs. GBM CACs had a higher expression of pro-angiogenic factors (esp. *KITL, CXCL12* and *JAG1*); growth factor and chemotactic receptors (*IGF1R, TGFbR2, CXCR4* and *CCR2*); adhesion receptor monomers (*ITGA5* and *ITGA6*) and matricellular factor *POSTN*. In addition, we found major differences in the levels of neovascularization-related plasma factors. A strong positive correlation between plasma MMP9 levels and expression of *CXCR4* in the CAC subset of hematopoietic progenitor cells (HPCs) was found in GBM patients. Our findings indicate that CAC-mediated neovascularization in GBM is characterized by more efficient CAC homing to target tissue and a more potent pro-angiogenic response than in physiologic tissue repair in MI. Our findings can aid in selecting targets for therapeutic strategies acting against GBM-specific CACs.

TAMI-11. IMPACT OF OHSV ACTIVATED NOTCH SIGNALING IN TUMOR MICROENVIRONMENT, AND ITS IMPACT ON ANTI-TUMOR IMMUNITY

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NOTCH signaling is a method of cell-cell communication where membrane bound NOTCH ligands on signal-sending cells can bind to and initiate cleavage of the NOTCH receptor, releasing NICD which can initiate signal transduction in adjacent "signal-receiving" cells. We have recently shown that oHSV treatment of GBM cells induces NICD cleavage and NOTCH activation in adjacent uninfected glioma cells. RNA sequencing of GBM cells post-infection also uncovered Gene Ontology NOTCH signaling pathway to be significantly upregulated. This activation was induced by viral miRNA-H16, which represses FIH-1 expression. FIH-1 was found to be a negative regulator of Mib1, a ubiquitin ligase, which activates NOTCH ligand-mediated activation of adjacent signal-receiving cells bearing the NOTCH receptor (Otani et al Clin. Can. Res. 2020). Here we have investigated the impact of oHSV-induced NOTCH signaling on the tumor microenvironment. Treatment of brain tumors in immune competent mice with oHSV and NOTCH blocking gamma secretase inhibitor (GSI) induced an anti-tumor memory immune response. Long term survivors in mice treated with the combination also completely rejected subsequent tumor re-challenge in the other hemisphere. UMAP of flow cytometry of tumor-bearing hemispheres and functional analysis of isolated cellular fractions from treated mice showed a significant influx of MDSC cells after oHSV treatment that was rescued in mice treated with oHSV and GSI. Ongoing mechanistic studies are uncovering a significant induction of NOTCH in tumor associated macrophages that aids in recruitment of MDSC cells. Overall these studies have uncovered a significant impact of oHSV therapy on GBM tumor microenvironment and presents opportunities for combination therapies that can help improve therapeutic benefit and anti-tumor immunity.

TAMI-12. 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.

TAMI-13. IMPORTANCE OF CD38 INHIBITION IN PRE-RECURRENT GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is the most aggressive CNS neoplasm with a mere 15 month progression-free survival following current standards-of-care. Furthermore, conventional therapies have been effective, in part, by inducing a senescent-like phenotype in at least a proportion of glioma, as well as within the tumor-adjacent, healthy brain cells. Through an N-glyco-capture large-scale screening method, we were able to identify 25 genes exclusively overexpressed on glioma cell surfaces. Of those 25 targets, CD38 was an attractive target due to pre-existing FDA approved therapeutics. Recent studies have shown, however, that senescent cells, such as those induced via chemo- and radiotherapies, secrete a pro-inflammatory array of cytokines, known collectively as senescence associated secretory phenotype (SASP), that are capable of increasing CD38 expression in monocytes. CD38 functions as an ectoenzyme to convert extracellular NAD+ to nicotinamide (required for glioma cell salvage pathway NAD+ synthesis) and ADPR/cADPR (a cellular proliferation signal). To examine the effects of radiation on human glioma tissue, we performed reverse cyclase assays (RCA) on paired primary and recurrent human glioma tissue samples and found an increase in CD38 activity in post-irradiated tissues (recurrent glioma) compared to pre-irradiated (primary glioma). Through proliferation assays, we also found an increase in glioma cell growth following treatment with cADPR compared to untreated. Our results demonstrate that CD38 activity is tumorgenic, and furthermore that conventional chemo- and radiotherapies increase this CD38 activity. This indicates that treating CD38 with previously FDA approved therapeutics may provide hope for increasing