to enhance tumour growth and proliferation, particularly within the characteristic hypoxic tumour microenvironment (TME) of GBM. I hypothesize that the expression of ICAM1 on the surface of TAMs contributes to GBM cell invasiveness, especially in the hypoxic TME, by enhancing the interaction between tumour cells and macrophages, thereby facilitating the migration and invasion of the tumour cells. METHODOLOGY: Assess the expression levels of ICAM1 in primary and immortalized human and mouse macrophages under hypoxic conditions. Analyze the effect of ICAM1 deficiency on macrophage behaviour including migration, proliferation, and adhesion to tumour cells. Intracranially inject GL261 glioma cells in ICAM1 deficient and wild type mice. RESULTS: ICAM1 is highly expressed in different cell types within the GBM microenvironment, including TAMs. The expression is particularly enhanced when primary or immortalized macrophages are treated with tumour cell-conditioned medium and is further exacerbated upon incubation of these cells in hypoxic conditions. The migration levels of bone marrow derived macrophage mouse cell type is higher in wild type cells than in ICAM1 deficient cells and higher when co-cultured with tumour cell condition media. ICAM1 deficient mice succumbed to GBM more quickly compared with wild type. CONCLUSIONS: It is evident that the hypoxic tumour microenvironment increases the expression of ICAM1 in macrophages. The tumour microenvironment increases migration levels of macrophages. The expression of ICAM1 in TAMs in hypoxic TME promotes GBM cell invasiveness, proliferation, aggressiveness and migration.

BSCI-15

INVESTIGATING CD8 T CELL EXHAUSTION STATES WITHIN THE TME AND DRAINING LYMPH NODE OF PRIMARY BRAIN TUMORS AND BRAIN METASTASES

Jessica Waibl Polania, Alexandra Hoyt-Miggelbrink, William Tomaszewski, Peter Fecci; Duke University, Durham, NC, USA

Brain metastases affect nearly 20% of all cancer patients. Likewise, glioblastoma (GBM) is the most common primary brain cancer in adults and remains universally lethal. Current immunotherapeutic efficacy is hindered by immunosuppression present in the brain tumor microenvironment (TME). T-cells, critical for tumor clearance, take on a functionally exhausted phenotype. Importantly, two exhaustion states, progenitor (Tpe) and terminal (Tte), have been identified in models of chronic infection and cancer. This distinction is particularly relevant, as Tpe can remain responsive to immune checkpoint blockade (ICB), while Tte cannot. To date, the dynamics and characteristics of these exhausted populations in primary tumors and brain metastases remain unclear. Using intracranially implanted murine models of GBM (CT2A) and metastatic melanoma (B16F10), Tpe and Tte were identified by flow cytometry as PD1+SLAMF6+ and PD1+TIM3+, respectively. Functional differences between subsets were evaluated via intracellular staining of IFNγ, TNFα, IL2, CD107a, and Ki67. To determine the role of antigen, we performed adoptive lymphocyte transfers of tumor-specific and non-tumor-specific transgenic T-cells into a TRP2 or OVA overexpressing intracranial CT2A or B16 tumor, respectively. Tte displayed higher cytotoxic molecule expression than Tte, consistent with chronic infection models. Key exhaustion-associated transcription factors were identified in exhaustion subsets, including Tox, Tcf7, T-bet, and Eomes. Tox and Tcf7 expression identified Tpe within tumor-draining lymph nodes, suggesting a potential origin outside of the tumor, and the capacity for rescued function. We observed a decline in the Tpe population over time, with an associated rise in Tte within both tumor types. Notably, the ratio of Tpe to Tte was higher at all time points in B16F10 compared to CT2A. Tte appeared only in tumor-specific T-cells of the TME, further confirming the tumor-antigen dependence of T cell exhaustion. Tpe may arise outside the TME in tumor-specific T-cells. Further study may reveal a means and time window for rescuing T-cell function with ICB for brain tumors.

BSCI-17

TOPIRAMATE DECREASES RADIATION-INDUCED CYTOTOXIC EDEMA IN HER2+ BRAIN METASTASES VIA AQUAPORIN 4 INHIBITION.

Maria J Contreras-Zárate, Karen Alvarez-Eraso, Zachary Littrell, Gina Kwak, Ryan Ormond, Sana D Karam, Peter Kabos, Diana Cittelly; University of Colorado, Denver, CO, USA

Brain metastasis (BM) occurs in 30-40 % of breast cancer patients with Her2+ tumors, and radiation is part of the standard treatment for BM. About 10% of BM patients treated with radiation develop brain edema. We have shown that combination of Ado-trastuzumab Emtansine (T-DM1)-the main targeted therapy for metastatic Her2+ Breast Cancer- and radiation increases the risk of developing radionecrosis by 13.5-fold (Stump et al., 2019). We also showed that T-DM1 enhances radiation-induced astrocytic toxicity and cytotoxic edema through upregulation of aquaporin-4 water-transporter (AQP4). Here, we determined whether blockage of AQP4 would prevent astrocytic swelling –cytotoxic edema- in vitro and in vivo models of Her2+ BM. Results: Electron microscopy of brain cortex from mice treated with 35 Gy (single dose), showed acute astrocytic end-feet swelling and a significant increase in AQP4 expression compared with non-irradiated mice. Consistent with prior findings in murine astrocytes, primary human astrocytes (huAST) also upregulated AQP4 levels 24 h post-radiation (8 Gy), and T-DM1 treatment exacerbated this effect. AQP4 upregulation was concomitant with 4.8 fold increase in the astrocytic area (indicative of cytotoxic edema). The FDA-approved anti-epileptic and migraine prevention drug, Topiramate (TPM), which works as an AQP4 inhibitor, blocked radiation-induced astrocytic swelling in huAST in vitro. Thus, we tested whether pre-treatment with TPM could prevent radiation-induced edema in a mouse model of HER2+BMs. Mice were injected intracardially JmT1BR3 brain metastatic cells and ten days later randomized based on the total head flux to (1) Radiation + vehicle, (2) Radiation + TPM (2 days prior to irradiation), (3) Non-Radiation + vehicle, and (4) Non-radiation + TPM. TPM decreased brain-water content (a marker of brain edema) in irradiated mice as compared with vehicle-treated mice, without alteration of metastatic burden 21 days post-injection. These results suggest TPM could be repurposed as a preventive agent of radiation-induced brain edema.

BSCI-18

ESTROGEN-DEPLETION DECREASES PROGRESSION OF ER⁻ BRAIN METASTASES BY PROMOTING AN ANTI-TUMORAL LOCAL IMMUNE RESPONSE

Maria Contreras-Zarate[1], Karen Alvarez-Eraso[1], Zachary Littrell[1], Nicole Tsuji[1], Sana Karam[2], D. Ryan Ormond[3], Peter Kabos[4], Diana Cittelly[1]; [1]Department of Pathology, University of Colorado AMC, Aurora, CO, USA. [2]Department of Radiation Oncology, University of Colorado, Aurora, CO, USA. [3]Department of Neurosurgery, University of Colorado, Aurora, CO, USA. [4]Department of Medicine, Division of Medical Oncology, University of Colorado, Aurora, USA

We have shown that 17-β-Estradiol (E2) promotes brain metastasis (BM) of estrogen receptor negative (ER^-) BC cells by inducing neuroinflammatory ER+ astrocytes in the brain niche to secrete pro-metastatic factors critical for early brain colonization. E2-depletion prevented brain colonization of human xenografts (MDA231BR/NSG) and syngeneic (E0711/C57Bl6, 4T1/Balb-c) ER^- models. Yet, whether E2-depletion can be used to decrease progression of established BM and how E2-dependent modulation of brain immune response contributes to the pro-metastatic effects of E2 remains unclear. To assess whether E2-depletion could decrease BM progression in a model that mimics standard of care for BM, E0771-GFP-luc cells were injected intracardially in syngeneic ovariectomized (OVX)-female C57Bl6 mice supplemented with E2. Seven days after injection (when micrometastases are established), mice received a single 15Gy dose brain irradiation and were randomized to continue receiving E2, E2 withdrawal (E2WD) or E2WD plus the aromatase-inhibitor letrozole (EWD+LET). Endpoint BM (but not systemic metastases) were significantly decreased in E2WD+Letrozole treated mice as compared to E2-treated mice. This effect was abolished when E0711 cells were injected in severely immunocompromised NSG mice or in the absence of brain irradiation, suggesting EWD+LET decreases BM progression through boosting radiation-induced anti-tumor immunity. Accordingly, there were no differences in BM progression in E2, EWD or E2WD+let treated mice in a xenograft model (F2-7 TNBC cells) in NSG mice, even in the presence of brain irradiation. Brain immune-profiling of brain irradiated E2, EWD and EWD+Let C57BL6 mice carrying E0771 BMs shows that brains of EWD+LET-treated mice had a significantly lower fraction of CD4 T cells and an increase in CD8 T cells, suggesting that EWD+letrozole decrease brain metastatic burden in part through modulation of T cells. These results suggest E2-depletion therapies could be used in combination with brain irradiation to decrease progression of BMs and promote an anti-tumoral immune response.

BSCI-19

A LENTIVIRAL CRISPR SCREEN FOR EPIGENETIC MODULATORS OF ANTIGENS TARGETED BY CAR T CELLS IN GLIOBLASTOMA.

T. Jordan Walter, Nitish Jangde, Nagi Ayad; Georgetown University, Washington D.C., USA

Glioblastoma (GBM) is the most common adult brain tumor and is very difficult to treat. One promising treatment strategy is CAR T cell therapy, in which T cells are used to target and kill cancer cells. However, CAR T cell therapy is not always effective, and more work is needed to realize its potential. One strategy for improving the efficacy of CAR T cell therapy is to increase the expression of targeted antigens on the surface of cancer cells. Our goal in these studies is to identify pathways that could be modulated to increase expression of the targeted antigens. Focusing on epigenetic proteins,