we performed a lentiviral CRISPR screen in the human GBM cell line LN18. A lentiviral library was used to knock out various epigenetic genes in these cells in vitro. Following transduction, we use flow cytometry to examine surface expression of antigens currently being targeted in GBM clinical trials of CAR T cells, e.g. - GD2, B7-H3, etc. Cells with increased expression of the antigens of interest were selected using FACS. Genomic DNA was isolated from these cells and sequencing was performed to determine which epigenetic genes had been knocked out. Results showed multiple genes contributing to increased surface expression of targeted antigens. Future studies will determine whether small molecule inhibitors of the identified epigenetic pathways selectively induce up-regulation of these antigens in GBM cells in vivo.

BSCI-20

STING EPIGENETIC SILENCING IN GLIOMAS CAN BE RESCUED BY METHYLTRANSFERASE INHIBITION: IMPLICATIONS FOR NOVEL THERAPEUTIC APPROACHES.

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The stimulator of interferon genes (STING) is a key component of the innate immune response to pathogenic cytosolic DNA, resulting in IRF3- and NFκB-dependent transcription of type I interferons (IFN) and pro-inflammatory cytokines. STING activation primes endogenous antitumor immunity and is disrupted in a variety of cancers. Here we investigate STING signalling in glioblastoma (GBM) patient samples. STING agonist treatment of ex vivo gliomas leads to inconsistent induction of type I IFN responses that are restricted to tumor associated myeloid cells. Indeed, single-cell transcriptome and multiplex immunofluorescence analyses demonstrate that STING expression is suppressed in neoplastic cells but not tumor-associated immune cells or stroma. Methylation analyses reveal a STING promoter region that is highly methylated in bulk tumor samples from glioma and other neuroectoderm-derived cancers, but not in most extracranial cancers. Methylation in this region strongly correlates inversely with STING RNA expression. STING epigenetic silencing is also present in normal fetal and adult brains. We demonstrate that STING expression in glioma cell lines may be rescued by decitabine, a DNA methyltransferase inhibitor (DNMTi) that is commonly used to treat hematologic malignancies. However, transduction of a STING-expressing vector into these glioma cell lines is insufficient to reconstitute STING signalling, suggesting that additional decitabine-stimulated mechanisms are necessary for STING pathway rescue. Collectively, our results suggest that epigenetic silencing of STING occurs early in brain development and may provide an immunosuppressive context for the genesis of brain tumors. Furthermore, our work raises the potential of epigenetic modulation to reconstitute STING signalling as a therapeutic strategy for glioblastoma and potentially other STING-silenced, immunologically-cold cancers.

BSCI-21

COX4I1 EXPRESSION IN BRAIN METASTASES

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BACKGROUND: COX4I1 (Cytochrome C oxidase, subunit 4, isoform 1) is a mitochondrial enzyme involved in the process of switching from glycolysis to oxidative phosphorylation. A previously published prospective biomarker study in glioblastoma cells found that Cytochrome C oxidase (CcO) activity was associated with resistance to treatment with both radiation and temozolomide (TMZ). The current study was designed to retrospectively assess COX4I1 expression in brain metastases from various primary cancers. METHODS: This single-institution, blinded, retrospective biomarker study evaluated 24 patients with paired brain metastases and primary cancers including lung cancer, malignant melanoma, breast cancer, colorectal cancer, renal cell carcinoma, and urothelial carcinoma. COX4I1 immunohistochemical expression in primary and metastatic samples was assessed using the H-score method. A paired t-test was used to assess the difference in total H-score between primary and brain metastasis tissue samples. Cox regression was used to assess the association between COX4I1 expression and overall survival (OS). For OS, time was calculated from metastatic tissue sample retrieval to death due to any cause. RESULTS: Brain metastasis tissue samples were found to have a significantly lower total H-score, on average, when compared to primary cancer tissue samples (p=0.01, mean difference of -33.3). 83.3% of tissue samples from metastases were not radiated. 58.3% of patients were on systemic treatment 6 months prior to tumor resection of brain metastases. COX4I1 expression was not associated with overall survival. CONCLUSIONS: There is significantly increased COX4I1 expression in primary cancers as compared to brain metastases. Anti-cytochrome C oxidase therapies may be beneficial for treatment of primary tumors. No patient or treatment variables were significantly associated with overall survival in paired patients. KEYWORDS: COX4I1, cytochrome c oxidase, brain metastases

BSCI-22

DETERMINING THE EFFECT OF NOVEL SMALL MOLECULE DRUGS AGAINST THE MIGRATION OF BRAIN METASTASIS INITIATING CELLS (BMICS)

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BACKGROUND: Brain metastases are secondary tumors that predominantly arise from the spread of lung, skin, and breast cancers. The current standard of care for brain metastases is complete surgical resection, with a median survival of four months. Therefore, there is a dire need to discover new therapies that effectively target brain metastases. To do this, we have identified anti-brain metastasis drugs that specifically target brain metastasis initiating cells (BMICs), a cancer stem cell population that is thought to escape standard therapies and has the ability to leave their primary tumor, seed the brain, and form a secondary brain tumor. Since the migration of the BMICs is essential to the development of brain metastases in patients, the main goal of this study was to determine the effect our anti-brain metastasis drugs have against the migration of lung, skin, and breast BMICs. METHODS: This migration assay utilizes a bi-well silicone structure which effectively establishes a 'wound' healing-like migration assay. BMICs are plated in optimized equal concentrations in each silicone bi-well structure to successfully form two cellular mono-layers that are separated by a middle silicone wall. Once cells adhere to the plate the silicone structure is removed and the area between the two cell populations is imaged over time with an in vitro imaging system. RESULTS: This optimized assay has been used to screen our anti-brain metastasis drugs against the migration of lung, breast, and skin BMICs. Thus far our drugs have been tested against lung and skin BMICs which resulted in a significant decrease in BMIC migration. SIGNIFICANCE: Since brain metastasis arises from the migration of cancer cells to a secondary organ, it is crucial to discover the effect of anti-brain metastasis drugs on BMIC migration prior to the initiation of preclinical animal trials.

BSCI-23

ANEUPLOIDY PROFILING IN GLIOBLASTOMA IDENTIFIES MECHANISMS OF DISEASE PROGRESSION AND TREATMENT VULNERABILITIES

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Glioblastoma (GBM) is the most common and malignant adult brain tumor. Despite years of research, few advancements have been made in its management. One challenging area of glioblastoma research is patient stratification in clinical trials based on genomic features. Although several regions of aneuploidy have been known to drive disease progression in GBM, the degree of aneuploidy across the genome varies widely and the significance of regions of aneuploidy has not been assessed. Using whole genome sequencing profiles for matched tumor and non-tumor samples, we were able to accurately determine the degree of aneuploidy and loss of heterozygosity for a set of primary GBM tumors. Next, using machine learning techniques, distinct patterns of aneuploidy and loss of heterozygosity emerged among a set of GBM tumors, allowing us to define distinct aneuploidy subgroups. Interestingly, these aneuploidy subgroups showed distinctly different rates of patient survival, suggesting that regions of aneuploidy may be driving disease progression. Differing rates of various GBM genomic subtypes including IDH mutation, EGFR mutation, MGMT methylation, and tumor subtypes was also seen among the aneuploidy subgroups. We were able to derive a gene expression signature for each of these aneuploidy subgroups and revealed distinct pathways that were driving tumor growth. Furthermore, using a perturbagen-response dataset we were able to predict compounds to distinctly target each subgroup. Collectively, this suggests that aneuploidy profiling provides important clues to varying mechanisms of disease progression and is a promising approach for targeted therapy in a patient-specific manner.