BSCI-19. EFFECT OF STEREOTACTIC RADIOSURGERY ON NON-SMALL CELL LUNG CANCER BRAIN METASTASIS: CORRELATIVE RADIOBIOLOGIC ANALYSIS FROM PHASE-II CLINICAL TRIAL NCT03398694

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BACKGROUND: Stereotactic radiosurgery (SRS) is an increasingly common modality used with or without surgery for brain metastases (BM). However, biological effect of SRS to tumors in vivois not known. METHODS: Patients were treated with SRS prior to surgery as per the clinical trial protocol. The resected tumor was divided into two groups: 'center' and 'periphery' with respect to the center of SRS treatment with periphery within 50%-isodose-line. Tissue was analyzed by whole exome sequencing (WES) and compared between the two groups as well as to non-radiated control tissues. RESULTS: All sequenced samples contained greater than 95% clean reads with an average read density of 100 base pairs and mapping efficiency of >99%. Preliminary analysis focused on SNP and Indel detection. In pooled groups with n=7 replicates there was no statistically significant differences in total mutation burden in either SNP's or Indels compared between controls and both treatment locations. Delving deeper intronic, frameshift, missense, and nonsense mutations all also showed insignificant changes between controls and center or peripheral tumor locations ($p > 0.5$, $p > 0.1$, $p > 0.4$, $p > 0.3$ respectively) hinting that at a pooled biological level there are not significant mutational burdens between treatment locations. However, at the individual level, matched comparison of SRS samples originating from the center or periphery of the same tumor showed total mutational burden differences. 6 out of 7 (86%) patients showed decreased total number of indels in peripheral vs. center and 5 out of 7 (71%) patients showed decreased number of SNP's in peripheral vs. center locations. CONCLUSION: Ultimately, these data demonstrate the power of matched patient controls over bulk analysis in order to elucidate smaller but possibly biologically meaningful effects, and point to a possible location dependency in treatment associated mutation burden within a single patient and single tumor that may be masked at a population level.

BSCI-20. THERAPEUTIC TARGETING OF HLA-G IN BRAIN METASTASES

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Brain metastases (BM) are the most common brain tumor in adults, with an incidence ten times greater than that of primary brain tumors. The most common sources of BM in adult cancer patients include cancers of the lung, breast and melanoma, which together account for almost 80% of all BM. Current clinical modalities for BM include surgery, whole brain radiation therapy and stereotactic radiosurgery but these therapies still offer limited efficacy and reduced survival of only months in treated patients, emphasizing the need for novel BM research approaches and better therapeutic strategies. Our laboratory recently discovered that stem-like cells exist in patient-derived BM from lung, breast and melanoma cancers, which we termed "brain metastasis-initiating cells" or BMICs. Through clinically relevant human-mouse xenograft models established with these patient-derived BMICs, we captured lung, breast and melanoma BMICs at pre-metastasis – a key stage where circulating metastatic cells extravasate and initially seed the brain, prior to organization into micro-metastatic foci. Transcriptome analysis of pre-metastatic BMICs revealed a unique genetic profile and several genes commonly up-regulated among lung, breast and melanoma BM, including the non-classical human leukocyte class I antigen-G (HLA-G). Loss of HLA-G in lung, breast and melanoma BMICs using two HLA-G specific shRNAs attenuated sphere formation, migratory and tumor initiating abilities of lung, breast and melanoma BMICs compared to control BMICs. HLA-G knockdown also resulted in reduced phospho(p)-STAT3 expression in patient-derived BMICs suggesting a potential cooperative role between HLA-G and pSTAT3 in BM. Since HLA-G is highly expressed at the cell surface in control tumors, ongoing experiments are focused on developing HLA-G specific chimeric antigen receptor -T cells (CAR-Ts) and determining their efficacy in targeting lung-, breast- and melanoma-BM as blocking the brain metastatic process will markedly extend patient survival and ultimately transform a fatal systemic disease into a more treatable one.

BSCI-22. IDENTIFICATION OF ACTIONABLE TYROSINE KINASE-REGULATED NETWORKS REQUIRED FOR LUNG AND BREAST CANCER BRAIN METASTASIS

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Brain metastases are a common consequence of advanced lung and breast cancer resulting in functional impairment, cranial neuropathies, and decline in quality of life. Current therapies for brain metastasis, such as whole brain radiation therapy, can result in cognitive impairment, while targeted therapies and chemotherapy are largely ineffective due, in part, to the emergence of resistance and inability to reach effective concentrations in the central nervous system (CNS). We have uncovered a role for the Abelson (ABL) family of tyrosine kinases, ABL1 and ABL2, in lung and breast cancer metastasis to the brain. We show that cancer cells increase *ABL* expression upon colonization of the brain. Notably, we found that genetic inactivation or pharmacological inhibition of the ABL kinases suppressed lung and breast cancer metastasis to the brain. ABL allosteric inhibitors effectively cross the blood-brain barrier (BBB), inhibit ABL kinases and downstream targets in brain metastases, and markedly impair metastatic colonization of the brain. Further, treatment with an ABL allosteric inhibitor increased recruitment of Iba1+ macrophages/microglia to breast cancer brain metastases. Current studies are aimed at identifying the molecular mechanisms by which ABL kinase signaling regulates the crosstalk between cancer cells and macrophages/microglia, with the aim of disrupting metastatic colonization of the brain parenchyma. These data reveal, for the first time, a role for ABL kinases in promoting brain colonization by metastatic tumors, and demonstrate that ABL allosteric inhibitors efficiently penetrate the BBB and inhibit intracranial growth of breast and lung cancer metastases.

BSCI-23. TISSUE FACTOR SIGNALING ENHANCES METASTATIC BRAIN CANCER MALIGNANCY

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Brain metastases are on the rise and remain one of the most refractory malignancies worldwide. Currently, the standard approach for therapy involves surgery and radiation. However, this approach usually produces only a modest increase in survival. We recently discovered that Tissue Factor (TF) strongly enhances the malignancy of gliomas via protease-activated receptor 2 (PAR2) signaling, though its role in brain metastases is not as well understood. In this study, we further explored the significance of TF in lung cancer brain metastases, showing that genetic and pharmacological targeting of TF-PAR2 signaling may decrease malignancy and increase the efficacy of radiotherapy. Studies were performed using patient-derived brain metastases coming from lung carcinoma. Markers of malignancy were measured by BrdU incorporation for cell proliferation, Matrigel-coated transwell migration, soft agar colony formation for anchorage-independent growth, limiting dilution assay for tumor initiation capacity, and clonogenic cell survival assay to measure radiation sensitivity. Low transcription of the TF gene is a favorable prognostic marker for overall survival in TCGA lung cancer patients (54.7 vs 41.9 months, P=0.0053), with 74% longer progression-free survival (102.7 vs 59.1 months, P=0.0012). TF knockdown significantly reduced tumor malignancy as determined by cell proliferation, invasion, colony formation, and in vivo growth. Conversely, TF overexpression increased tumor malignancy and promoted cancer stem-like behavior, as indicated by CD44 and CD133 expression, extreme limiting dilution assay, and anchorage-independent growth. A PAR2 antagonist, I-191, inhibited TF-mediated signaling and reduced cell proliferation by 51.3% ($P < 0.001$). TF knockdown and I-191 increased radiation sensitivity. Exogenous treatment of lung cancer cells with recombinant TF suppressed radiation-induced apoptosis, and this effect was blocked with I-191. These data show that TF-PAR2 signaling may represent a novel therapeutic strategy to reduce the malignancy of brain metastasis and increase the efficacy of radiation.

BSCI-25. THE ROLE OF THE IFNΓ PATHWAY IN BREAST CANCER BRAIN METASTASIS FORMATION

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In previous work, we showed the prominence of the T cell response in the formation of brain metastases of primary ER-negative breast cancers. We also showed that prior co-cultured breast cancer cells with stimulated T lymphocytes bear an overexpression of Guanylate-binding protein 1 (GBP1) and possess an increased trespassing ability through an in vitro blood-brain barrier (BBB) model. In addition, we demonstrated a predilection for metastasizing to the brain of breast cancer cells that were co-cultured with activated T cells in a mouse model. In the present work, we show that activated CD8+ cytotoxic T lymphocytes, rather than CD4+ lymphocytes, are the main cause of increasing the ability of breast cancer cells to cross the BBB. While synthetic IFNγ does not change the ability of breast cancer cells to cross the BBB, this study shows that the T lymphocyte-secreted IFNγ activates the STAT1-dependent IFNγ pathway in breast cancer cells, enabling them to cross the *in vitro* BBB. Direct inhibition of soluble IFNγ or blocking of the IFNγ-specific receptor in breast cancer cells significantly decreases their ability to cross the BBB. The results illustrate that IFNγ signaling pathway is one of the crucial pathways in the formation of brain metastasis of ER- breast cancer. The interference with the IFNγ pathway will develop preventive strategies against the formation of brain metastases of breast cancer.