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NEURO-ONCOLOGY ADVANCES

ABSTRACT CODES/CATEGORIES:

BSCI - Basic Science
LPTO - Leptomeningeal Disease
TRLS - Clinical Trials
THER - Medical Therapy (Chemotherapy, Targeted Therapy/Immunotherapy)
MLTI - Multimodality
OTHR - Other
RADI - Radiation
SURG - Surgery

BASIC SCIENCE

BSCI-01. ACTIVATION OF C-MET/B1-INTEGRIN COMPLEX RESULTS IN INCREASE OF MESENCHYMAL GENE EXPRESSION AND STEM CELL POPULATION IN METASTATIC BREAST CANCER TO THE BRAIN AND SPINE

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INTRODUCTION: C-met and β-integrins play a central role in nearly all stages of cancer metastasis. They bind at the cell surface, driving ligand independent co-activation of downstream pathways. Greater complex is seen in metastatic tumors vs. its primary tumor counterparts in patients. The molecular, cellular, and clinical effects of complex formation in metastatic breast cancer are investigated. METHODS: Utilizing variations of the MDA-231 breast cancer cell lines (standard MDA-231, inducible complex formation MDA-231, brain seeking MDA 231, lung seeking MDA 231, and bone seeking MDA-231), in vitro and in vivo studies were performed. Clinical correlates from patient samples were studied.

RESULTS: Induction of c-Met/β1 complex promotes breast cancer invasion ($p< 0.001$), migration ($p< 0.05$), circulation intravasation ($p< 0.01$), and adhesion ($p< 0.01$). These effects may be driven by the increased mesenchymal character ($p< 0.05$) and larger stem cell population ($p< 0.001$) caused by inducing c-Met/β1 complex formation. OS2966 (a therapeutic β1 integrin blocking antibody) decreases invasion ($p< 0.05$), intravasation ($p< 0.05$), and mesenchymal form factor ($p< 0.001$) and gene expression ($p< 0.001$) in MDA-MB-231 cells. Brain- and bone-seeking breast cancer cells have higher c-Met/β1 complex than parental controls and preferentially adhere to tissue-specific matrix ($p< 0.01$). In intracardiac metastasis models, complex formation resulted in significantly higher metastatic burden and shorter survival times ($p< 0.001$). qPCR data suggests that complex formation may drive exiting and colonization of cancer cells (micrometastasis) rather than tumor growth. Patient brain and bone metastases demonstrated high β1/c-Met levels. CONCLUSIONS: The c-Met/β1 complex drives intravasation and extravasation of breast cancer cells from the circulation. Preferential affinity for tissue-specific matrix enables the c-Met/β1 complex to drive formation of breast cancer metastases to the brain and bone. Pharmacological and genetic targeting of the complex with agents may provide therapeutic approaches to prevent metastases, particularly to the brain and bone.

BSCI-02. T GLI1 IS A NOVEL, ACTIONABLE TARGET FOR THE TREATMENT OF BREAST CANCER BRAIN METASTASES

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Despite improvements in early detection and intervention, breast cancer remains the second leading cause of cancer-related death in women and the second most common cancer to metastasize to the brain. Current standard of care options for breast cancer brain metastases (BCBM) include stereotactic radiosurgery, whole-brain radiotherapy, and surgical resection. Local and distant recurrences are common leading to significant morbidity; effective FDA-approved drugs for these patients remain a significant unmet need. Our laboratory discovered an alternative splice variant of glioma-associated oncogene homolog 1 (GLI1), termed truncated GLI1 (tGLI1) that is a tumor-specific gain-of-function transcription factor preferentially expressed in most BCBM samples and recurrent gliomas. Recent results established that tGLI1 promotes breast cancer stem cells (BrCSCs) and is associated with preferential metastasis to the brain and radioresistance, justifying tGLI1 as an ideal therapeutic target for BCBM patients. To identify tGLI1-targeting agents, we screened 1,520 compounds across three commercial drug libraries and found ketoconazole, an FDA-approved azole antifungal and component of previously studied anti-neoplastic regimens, selectively killed tGLI1-expressing breast cancer cells with heightened efficacy against the CSC subpopulation *in vitro*. tGLI1 knockdown abolished the ability of ketoconazole to target BrCSCs, indicating that ketoconazole effect is dependent on tGLI1. Intracardiac mouse studies showed ketoconazole selectively inhibited circulating tGLI1-positive breast cancer cells from developing into brain metastases and suppressed the progression of existing brain metastases. Mass spectrometry demonstrated ketoconazole effectively penetrated the blood-brain barrier (BBB) and blood-tumor barrier (BTB). Mechanistic studies suggest that ketoconazole-dependent cell kill is, in part, mediated through disruption of the tGLI1-STAT3 interaction. Collectively, our preclinical results demonstrate that ketoconazole is an effective inhibitor of BrCSCs and brain metastasis of tGLI1-positive breast cancer. Based on these promising preclinical data, we opened a window-of-opportunity study in patients with BCBM and recurrent gliomas to determine if ketoconazole treatment alters tGLI1 signaling in humans (NCT03796273).

BSCI-03. T CELL EXHAUSTION SIGNATURES VARY BY TUMOR TYPE AND ARE INDEPENDENT OF INTRACRANIAL LOCATION

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T-cell exhaustion is a hindrance to the efficacy of immune checkpoint blockade. This study is among the first to examine, and credential as *bona fide*, exhaustion among T cells infiltrating murine models of brain metastasis, including breast, lung, and melanoma cancers. Furthermore, this study demonstrates the utility of a 4-1BB agonist antibody in certain tumors resistant to PD-1 blockade alone. METHODS: Tumor-infiltrating and peripheral blood lymphocytes (TILs and PBLs) were isolated from intracranial and subcutaneous immunocompetent murine models of glioma, breast, lung, and melanoma cancers. Levels of exhaustion-associated inhibitory receptors and post-stimulation levels of the cytokines IFNγ, TNFα, and IL2 were assessed by flow cytometry. Anti-PD-1 and anti-4-1BB monoclonal antibodies were utilized as a therapeutic exhaustion-countering strategy and median survival was assessed. RESULTS: Our data reveal that tumors, regardless of their intracranial or subcutaneous location, elicit unique T-cell exhaustion signatures among infiltrating T cells characterized by: (1) prominent upregulation of multiple immune checkpoints; (2) stereotyped T-cell transcriptional programs matching classical virus-induced exhaustion; and (3) notable T-cell hyporesponsiveness in tumor-specific T cells. Exhaustion signatures differ predictably with tumor identity, but remain stable across manipulated tumor locations. Anti-PD-1 monoclonal antibody alone did not improve median survival in any tumor type tested. In tumors with high levels of 4-1BB expression, anti-4-1BB and anti-PD-1 therapy resulted in improvement in median survival. CONCLUSIONS: Distinct cancers possess similarly distinct mechanisms for exhausting T cells. Each tumor type demonstrated a unique T cell exhaustion signature regardless of location. 4-1BB may serve as a therapeutic adjunct to anti-PD-1 monoclonal therapy in tumors which may be resistant to PD-1 blockade alone.

BSCI-04. TARGETING TRIPLE-NEGATIVE BREAST CANCER BRAIN METASTASES WITH A RE-ENGINEERED LUPUS AUTOANTIBODY

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An unusual lupus anti-DNA autoantibody, 3E10, has potential to be used against triple-negative breast cancer (TNBC) brain metastases. 3E10 penetrates live cell nuclei, inhibits DNA repair, and is selectively toxic to cancer cells with the PTEN and/or DNA-damage response (DDR)-deficiencies that are associated with brain metastases in TNBC. The ENT2 nucleoside transporter that 3E10 uses to cross cell membranes is highly expressed in tumors and in brain endothelial cells (BECs) at the blood-brain barrier (BBB), and 3E10 has previously delivered cargo proteins to ischemic brain in a rat stroke model. We have re-engineered 3E10 into an optimized fragment, called Deoxymab-1 (PAT-DX1), that has increased effect on PTEN/DDR-deficient tumor cells. In the present study we tested the ability of PAT-DX1 to cross the BBB and improve outcomes in a mouse model of TNBC brain metastases. PAT-DX1 crossed from apical to basolateral chambers in an hCMEC/D3 Transwell filter model of the BBB, and penetrated the nuclei of and was toxic to the brain-