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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-

seeking 231-BR subclone of MDA-MB-231 TNBC cells, which harbors a loss of PTEN compared to parental cells. Brain metastases were generated in nude mice by intracardiac injection of $1.75x10^5$ 231-BR cells engineered for expression of luciferase, as confirmed by IVIS one week after injection. Mice with brain metastases were treated by tail vein injection of control (PBS, n=7) or DX1 (20 mg/kg, n=7) 3x/week for 4 weeks. Mice were observed for behavior and weights, and brain radiance efficiency was monitored by weekly IVIS to track metastatic tumor growth. PAT-DX1 significantly suppressed growth of brain metastases based on absolute and relative radiance efficiencies in the brain, increased the median survival of the mice from 38 to 52 days ($P< 0.02$), and was well tolerated. These results provide proof of concept for use of a re-engineered autoantibody against brain metastases.

BSCI-05. HOW MICROGLIA, BRAIN RESIDENT MYELOID CELLS, RESPOND TO BREAST CANCER METASTASIS INTO THE BRAIN?

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Brain metastasis from different cancers, including lung, breast, melanoma, colorectal or renal cell carcinoma is relatively common and its frequency increases with a prolonged survival of cancer patients. New anti-cancer therapies frequently fail to reduce metastatic burden. While the important role of tumor-associated macrophages as pro-tumorigenic cells facilitating tissue remodeling, invasion and metastasis is well documented, much less is known about the immune microenvironment of brain metastases and potential mechanisms that mediate interactions of cancer cells with brain immune cells - microglia. Triple-negative breast cancer metastases to the brain were discovered in 46% of patients. We evaluated the abundance and morphology of microglia on sections from breast cancer metastases using immunohistochemistry. We found that microglia cells are activated, surround the breast tumor cells and do not infiltrate the solid tumor. Searching for a potential attractant of microglia, we determined osteopontin levels in six human breast cancer cell lines and found upregulation of osteopontin in transformed cells, with the highest level in the triple-negative MDA-MB-231 cells. MDA-MB-231 cells activated primary murine microglia cultures when co-cultured. Invasion of MDA-MB-231 cells in co-cultures with murine immortalized BV2 microglial cells and human SV40 immortalized microglia was increased, as demonstrated using Matrigel Invasion Assay. Using immunofluorescence we detected osteopontin in cancer cells in human breast cancer metastases. Moreover, we found that minocycline, a clinically used antibiotic, reduces the osteopontin production in human breast cancer cells and the most sensitive cells were MDA-MB-231 cells. Our study shows that metastatic cancer cells may employ microglia to facilitate extravasation and colonization of brain parenchyma. We postulate that osteopontin mediates interactions between microglia and metastatic cancer cells and minocycline may interfere with those interactions. Funding: TEAM TECH CORE FACILITY FNP: Development of comprehensive diagnostics and personalized therapy in neuro-oncology

BSCI-06. FREQUENCY OF BRAIN METASTASIS FROM BREAST AND LUNG CANCER IN THE UNITED STATES -- A POPULATION-BASED ASSESSMENT

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BACKGROUND: Brain metastases (BM) are the most common central nervous system tumor in the United States and occur with increasingly frequency due to improved screening and therapeutics leading to improved survival. Current estimates of frequency of BM vary significantly by cancer site and are typically not population-based. Population-based estimates of incidence have recently become possible due to collection of data on BM identified at diagnosis ("synchronous" BM, SBM). BM may occur at any point after cancer diagnosis. We report our recent population-based estimates of SBM and period incidence of BM (PBM) from breast (BC) and lung cancer (LC). METHODS: Data from Surveillance, Epidemiology, and End Results (SEER, 2010–2016 diagnoses) were used to estimate SBM and linked data from SEER-Medicare (2008–2012 diagnoses for individuals 65+, with 2007–2014 claims) were used to estimate PBM, for BC and LC overall and by BC and LC subtypes. RESULTS: Within the SEER data, 10.9% of LC cases presented with SBM (15.5% in small cell LC [SCLC], and 10.8% in non-small cell LC [NSCLC]); 0.4% of BC cases presented with SBM, 0.7% in triple negative (TNBC), 0.8% for HER2+, and 0.2% for ER+\PR+\HER2-. Within the SEER-Medicare data, 13.5% of LC overall had LBM with 23.1% for SCLC and 15.3% for NSCLC; 1.8% of BC overall had LBM with 4.2% in triple negative (TNBC), 3.1% for HER2+, and 1.1% for ER+\PR+\HER2. CONCLUSION: Frequency of synchronous and period BM varies by originating site as well as subtype. The new SBM variable in SEER allows for estimation of this important statistic, while the SEER-Medicare linked data allows for estimation of PBM, both on a population-level for the US population. These estimates are useful to clinical practice and critical for estimating morbidity and mortality due to BM.

BSCI-07. BONE MARROW T-CELL SEQUESTRATION IN THE SETTING OF BRAIN METASTASES

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INTRODUCTION: Brain metastases remain one of the most dreaded consequences of late stage cancer, yet their incidence has risen as survival from primary cancers has improved. We have recently reported that tumors harbored within the brain, specifically, sequester T-cells within the bone marrow as a novel mechanism of immune evasion. Sequestration results from tumor-imposed loss of S1P1 receptor from the T-cell surface. Stabilization of the receptor on T-cells frees T-cells from sequestration and licenses T-cell activating therapies for intracranial tumors. While this phenomenon was initially uncovered in glioblastoma, its role in promoting immune-evasion in brain metastases remains less clear. METHODS: Blood, bone marrow, and tumors were collected from mice bearing intracranial tumors commonly metastatic to the brain, including lung carcinoma (LLC), melanoma (B16F10), or breast carcinoma (E0771) and analyzed by flow cytometry. T-cell S1P1 levels, as well as total T-cell counts were assessed in each compartment. Correlation analyses were conducted between T-cell counts and S1P1 levels on T-cells in the bone marrow across intracranial and subcutaneous murine tumor models. RESULTS: T-cell lymphopenia and accompanying accumulation of T-cells in the bone marrow were observed in the murine models of lung carcinoma, melanoma, and breast carcinoma, but only when these tumor lines were implanted intracranially. Sequestered T-cells in tumor-bearing mice showed decreased surface S1P1 levels in a manner correlating with their sequestration. CONCLUSION: S1P1-mediated bone marrow T-cell sequestration is a novel mode of cancer-induced T-cell dysfunction in intracranial tumors. Preventing receptor internalization abrogates T-cell sequestration and licenses T-cell activating therapies in glioblastoma. Sequestration is now observed in models of brain metastases. Pharmacologic strategies to stabilize S1P1, reverse sequestration, and restore circulating T-cell numbers are anticipated to improve immunotherapeutic efficacy for brain metastases.

BSCI-09. MECHANISMS OF ENHANCED DRUG DELIVERY IN BRAIN METASTASES WITH FOCUSED ULTRASOUND-INDUCED BLOOD-TUMOR BARRIER DISRUPTION

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Blood-brain/blood-tumor barriers (BBB and BTB) and interstitial transport may constitute major obstacles to the transport of therapeutics in brain tumors. In this study, we examined the impact of focused ultrasound (FUS) in combination with microbubbles on the transport of two relevant chemotherapy-based anticancer agents in HER2-positive breast cancer brain metastases at cellular resolution: the non-targeted chemotherapeutic doxorubicin and the antibody-drug conjugate ado- trastuzumab emtansine (T-DM1). Using an orthotopic xenograft model of HER2-positive breast cancer brain metastasis and quantitative microscopy we demonstrate multifold increases in the extravasation of both agents (7-fold and 2-fold for doxorubicin and T-DM1, respectively) and we provide evidence of increased drug penetration (>100μm vs. < 20μm and 42±7μm vs. 12±4μm for doxorubicin and T-DM1, respectively) after application of FUS as compared to control (non-FUS). Integration of experimental data with physiologically based pharmacokinetic (PBPK) modeling of drug transport reveals that FUS in combination with microbubbles alleviates vascular barriers and enhances interstitial convective transport via increase in hydraulic conductivity. Combination of experimental data and PBPK modeling suggests that FUS in combination with microbubbles increases the endothelial cell transmembrane transport and uptake. PBPK modelling indicates selective increase in transvascular transport of the non- targeted small chemotherapeutic doxorubicin through small vessel-wall pores size with a narrow range (Diameter: 10-50nm). Our work provides a quantitative framework for the optimization of FUS-drug combinations to maximize intratumoral drug delivery and facilitate the development of novel therapeutic strategies against brain metastases.

BSCI-10. NEUROLOGICAL DYSFUNCTION CAUSED BY BRAIN TUMOR-GENERATED SOLID STRESS IS REVERSED BY LITHIUM

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