Abstracts

BSCI-11. STROMAL PLATELET DERIVED GROWTH FACTOR RECEPTOR-B (PDGFRB) PROMOTES BREAST CANCER BRAIN METASTASIS

Katie Thib1, Anisha Hammer1, Blake Hildreth II1, Luke Russell1, Steven Sizemore1, Anthony Trimboli1, Raleigh Kladney1, Sarah Steck1, Manjusri Das1, Chelsea Bolyard1, Robert Pilarski1, Maria Cutino2, Christopher Kovisto3, Lynn Schuenfeld1, Jose Otero1, Arnab Chakravarti1, Matthew Ring0, Jinwo Li1, Balveen Kaur1, Gastavo Leone1, Michael Ostrowski2, and Gina Sizemore1

1Ohio State University, Columbus, OH, USA, 2Medical University of South Carolina, Charleston, SC, USA, 3UT Health Science Center, Houston, TX, USA

Stromal platelet-derived growth factor receptor-beta (PDGFRβ) has emerged as an actionable mediator of breast tumor-stromal communication. As a receptor tyrosine kinase, PDGFRβ is activated by its ligand, PDGF, which is released by neighboring tumor epithelium and endothelium. However, how PDGFR signaling mediates breast cancer (BC) initiation, progression, and metastasis remains unclear. To evaluate PDGFRβ in this disease, we developed a mouse model of stromal-specific PDGFRβ activation using the Fip-cre transgene previously published by our group. Mesenchymal-specific activation of PDGFRβ promotes preferential experimental brain metastasis of PDGFRβ-expressing mammary tumor cells when injected intravenously and accelerates intracranial tumor growth of these cells. Mammary tumor cells expressing low levels of PDGFRβ do not exhibit a similar increase in brain metastases in PDGFRβ mutant mice. To our knowledge, this is the first example where genetic manipulation of the stroma leads to an increased incidence of BCBM. Our pre-clinical data suggests that primary breast tumors that express high PDGFRβ could preferentially metastasize to the brain. To test this hypothesis, we analyzed PDGF expression in a tissue microarray comprised of HER2-positive and triple negative BC primary tumors that express high PDGFB could preferentially metastasize to the brain. The most common alterations which we observed were EGFR and NRAS mutations. These data indicate that PDGFRβ promotes preferential experimental brain metastasis and may be an actionable mediator in breast cancer brain metastasis.

BSCI-12. COMPREHENSIVE GENOMIC ANALYSIS OF BRAIN METASTASES FROM MULTIPLE CANCER TYPES

Kazutaka Fukumura1, Xueqin Mao1, Xinghui Song1, Grant Fischer1, Jie Yang2, Erik Sulman2, Michael Davies3, Jianhua Zhang4, and Jason Huse5

1UT MD Anderson Cancer Center, Houston, TX, USA, 2NYU Langone Medical Center, New York, NY, USA

PURPOSE: Brain metastases occur in approximately 8–10% of patients with cancer, and the incidence has increased over the past decades. The most common primary tumors responsible for brain metastases are lung cancer, melanoma, renal cell carcinoma (RCC), breast cancer and colorectal cancer. The molecular mechanisms by which genomic and transcriptional abnormalities drive the formation of brain metastases remain unclear. Here, we conducted comprehensive genomic and transcriptional analysis with paired primary tumor tissue (or extracranial metastasis tissue) and brain metastases tissue using whole-exome sequencing (WES), mRNA-Seq and global methylation profiling. METHODS: Frozen, paired brain metastasis tissue and primary tumor tissue (or extracranial metastasis tissue) and white blood cells were acquired from RCC (n=12), breast cancer (n=17), lung cancer (n=15) and melanoma (n=14) patients, followed by extraction of DNA and RNA. WES and mRNA-Seq were performed on the Illumina HiSeq4000 platform. For methylation profiling, DNA was analyzed using Illumina Infinium MethylationEPIC Beadchip arrays. RESULTS: Somatic mutations or methylation of VHL gene were identified in 81.8% of RCC patients. Gene Set Enrichment Analysis revealed significant enrichment for hypoxia pathway transcripts in RCC brain metastases relative to primary tumors. The most common alterations in RCC and lung cancer patients were TP53 mutations with frequencies of 50.0% and 73.5%, followed by ERBB2 alterations (43.8%) in breast cancer patients and mutually exclusive alterations of EGFR (33.3%) and KRAS (26.7%) in lung cancer patients. Mutually exclusive alterations of NRAS (42.9%) and ERBB2 (42.9%) in melanoma were also observed. A causal association was observed in glioblastoma and pancreatic adenocarcinoma and epigenetic analysis revealed characteristics of brain metastases depending on primary cancer types. CONCLUSIONS: Comprehensive genomic analysis of brain metastases from four different cancer types revealed that brain metastasis tissues have unique genomic, transcriptional and epigenetic profiles according to histopathology groups. Therefore, the therapeutic strategies should be designed based at least in part on tumor histogenes.

BSCI-13. TUMOR-SPECIFIC TGLI1 TRANSCRIPTION FACTOR MEDIATES BREAST CANCER BRAIN METASTASIS VIA ACTIVATING METASTASIS-INITIATING CANCER STEM CELLS AND ASTROCYTES IN THE MICE BRAIN STROMA

Shereena Sirkoon1, Richard Carpenter1, Tadas Rimkus1, Daniel Doheny1, Dongqin Zhu1, Neah Aguayo1, Marilyn Anguelov1, Austin Arriaga1, Angelina Regua1, Fei Xing1, Michael Chan1, Linda Metheny-Barlow1, Kounosuke Watabe1, and Hui-Wen Lo1

1Wake Forest School of Medicine, Winston Salem, NC, USA, 2Indiana University, Indianapolis, IN, USA

Breast cancer is the second leading cause of brain metastases in women; patients with breast cancer brain metastasis (BCBM) survive only 6–18 months after diagnosis. Mechanisms in BCBM remain unclear and contribute to ineffective treatments and dismal prognosis. Truncated glioma-associated oncogene homolog 1 (GTLI1) belongs to the GLI family of zinc-finger transcription factors and functions as a tumor-specific gain-of-function mediator of tumor invasion and angiogenesis. Whether tGTLI1 plays any role in metastasis of any tumor type remains unknown. Using an experimental metastasis mouse model, via intracardiac implantation, we showed that ectopic expression of tGTLI1, but not GLI1, promoted preferential metastasis to brain. Conversely, selective tGTL1 knockdown using tGTL1-specific antisense oligonucleotides led to decreased metastasis of intracardially inoculated breast cancer cells. Furthermore, intracranial implantation mouse study revealed tGTLI1 enhanced intracranial colonization and growth of breast cancer cells. Immunohistochemical staining of patient samples showed that tGTLI1, but not GLI1, was increased in lymph node metastatic tumors compared to primary tumors. Whether tGTLI1 plays any role in radiosensitivity is unknown; we found radioreistant BCBM cell lines and patient specimens expressed higher levels of tGTLI1 than radioreistant normal breast counterparts. As a tumor-specific gain-of-function mediator of tumor invasion and angiogenesis, tGTLI1 may be an important target to develop novel combination therapies for breast cancer brain metastasis.

BSCI-14. SYNTHETIC METASTATIC BRAIN DISEASE MRI IMAGES CREATED USING A GENERATIVE ADVERSARY NETWORK TO OVERCOME DEEP MACHINE LEARNING CHALLENGES IN HEALTHCARE

Zhenzhen Dai1, James Snyder1, and Ning Wen1

1Henry Ford Hospital, Detroit, MI, USA

Deep Machine Learning (DML) in commercial applications such as recognizing animal species in photographs occurred through analyzing large volumes of public data. To achieve similar success in brain tumor imaging, additional factors must be addressed such as the need to follow strict regulatory protocols, work with limited datasets, and protect patient privacy. Generative adversary network (GAN) restricted to intracranial disease is one possibility to overcome these challenges and enable training on small annotated datasets with unique specific genetic and epigenetic alterations. In our pipeline, we use a novel framework that allows collections of DML datasets to be derived from patient MRI using GAN models encodeable DML of BM MRI studies. METHOD: We randomly selected 82 glioma
patient imaging studies from the MICCAI BrainT1S 2018 Challenge\cite{1}. All patients underwent contouring of GD-enhancing tumor (C\textsubscript{E}), peritumoral T2 (pT2), necrotic and non-enhancing tumor core [NCR\textsubscript{NET}]. Images are co-registered to the T2 FLAIR and visualized with Manfod-Cox logrank test. Our network was trained on a GAN and a discriminative network. The generative model works to synthesize patient images from labels. Labels comprise the normal brain mask as well as the contoured C\textsubscript{E}, pT2 and NCR\textsubscript{NET}. Normal brain mask is extracted from the T1 FLAIR.\cite{2} Our network was trained on a GAN and discriminative network. The generative model compares the difference between synthetic and real patient image in both pixel and perceptual difference. The generative model is trained to minimize the difference from the discriminative network. This method was refined in the obbligatoa dataset and applied to BM MRI. RESULTS: Figure 1. Synthesis of BM MRI images derived from human brain MRI studies using GAN model with four modalities (T2, T2 FLAIR, T1 contrasted image, and T1 non-Contrasted Image). CONCLUSION: Training DML in BM disease using GAN MRI models may overcome limitations in applying DML to healthcare, namely volume of high-quality data and patient privacy. GAN based modeling for BM needs to be further refined and validated.

BSCL-15. METASTATIC BRAIN TUMOR TARGETING PEPTIDES ISOLATED THROUGH PHAGE DISPLAY BIOPANNING AGAINST BRAIN METASTASIS-INITIATING CELLS

JongMyung Kim and James Liu, Moffitt Cancer Center, Tampa, FL, USA

To effectively target metastatic brain tumors (MBT), the paradigm of treating MBTs after visualization on clinical imaging needs to be shifted to understanding of the mechanisms that drive the formation and metastasis of brain tumor cells. Targeting this tumor sub-population, which may form as a result of activation of epithelial-mesenchymal transition, may allow for more effective means of isolating and targeting brain metastasis. In order to isolate BMICs, we have harvested cells from brain metastasis-specific, and human lung cancer, and cultured cells using serum-free media conditions. In vitro phage display biopanning was used to isolate 12- amino acid length peptides that specifically target BMICs. Of the peptides recovered, one peptide, LBM4, was tested for specificity of binding to MBTs through in vitro and in vivo binding assays. When comparing patient derived metastatic brain tumors cells against brain metastasis cell lines, we found that both types of cells demonstrated similar morphology when grown in serum media conditions, but when grown in serum-free media demonstrated a tumor sphere morphology, similar to a stem-cell like state. LBM4 demonstrated specific binding to MBT cells from primary lung cancer cells in vitro through flow cytometry analysis and immunocytochemistry. Fluorescent tagged LBM4 intravenously injected into mice harboring intracranial BM demonstrated peptide localization to the tumor within the intracranial cavity visualized with live animal imaging. In vivo phage display biopanning is an effective tool that is able to isolate cell specific targeting peptides. MBT targeting peptides can potentially result in a shifting of the clinical treatment paradigm of brain metastases, through the development of more effective targeted therapeutics aimed at BMICs, as well as improving detection of MBT cells which may result in earlier tumor visualization as well as delineation of tumor recurrence versus radiation effects.

BSCL-16. HEMODYNAMIC SHEAR STRESS SELECTS A SUBPOPULATION OF LUNG ADENOCARCINOMA CELLS WITH HIGHER METASTATIC CAPACITY

Keila N. Alvarado-Estrada\textsuperscript{1}, Lana Muenaco-Hilembrandt\textsuperscript{1}, David Mampre\textsuperscript{2}, Alfredo Quinones-Hinjosa\textsuperscript{2}, Rachel Sarabia-Estrada\textsuperscript{1}, Kaisorn Chaisana\textsuperscript{1}, Yu Shrike Zhang\textsuperscript{1}, and Sushila Maharjan\textsuperscript{4}; Mayo Clinic, Neurologic Surgery Department, Jacksonville, FL, USA, \textsuperscript{2}Johns Hopkins Medicine, Baltimore, MD, USA, \textsuperscript{3}Department of Medicine, Harvard Medical School; Division of Engineering in Medicine, Brigham and Women’s Hospital, Cambridge, MA, USA, \textsuperscript{4}Bingham and Women’s Hospital at Harvard Medical School, Cambridge, MA, USA

Patients with primary cancers often develop delayed brain metastases. One of the most common cancer types and sources of brain metastasis is lung cancer. For metastasis from lung cancer the 3-year survival is < 5%. Cancer cells in circulation are responsible for metastatic spread. The mechanical microenvironment plays an important role in cancer cell proliferation. When cancer cells reach the bloodstream they are exposed to hemodynamic shear stress. It has been shown that most of the circulating tumor cells die once they reach the bloodstream, but the biology of the survival cells is poorly understood.\cite{3} We hypothesized that microenvironmental factors can affect cancer metastasis. To test this hypothesis, human and mouse breast cancer cells were injected into young (< 6 months old) and old (> 13 months old) mice to drive the formation of lung metastasis. To gain mechanistic insight, the transcriptome of young and old mice brains were analyzed by RNAseq, the metastatic microenvironment was analyzed by laser capture microdissection and mass spectrometry, immune populations have been identified by flow cytometry, and functional immune contributions analyzed by immunodepleting antibodies. Multiple brain immune subsets were altered with age. In vivo depletion experiments showed no significant contribution of CD4+ T-cells and GR1+ myeloid cells to baseline metastatic burden. A population of microglia in adult metastatic brains had a high side scatter profile, which is consistent with published reports that aged microglia are in a “pro-inflammatory” state. The population of microglia reduced baseline brain metastasis by 50% and experiments are underway to determine their contribution to an age effect.

BSCL-18. ABLATION OF CSF2 MITIGATES RADIATION-INDUCED NEUROCOGNITIVE DECLINE INDEPENDENT OF HIPPOCAMPAL NEUROGENESIS

Solen Gokhan, Kyle Aronson, Yagiz Alrun, Violeta Chitu, Patrik Brodin, E. Richard Stanley, Mark Mehler, and Wolfgang Tomé; Albert Einstein College of Medicine, Bronx, NY, USA

PURPOSE: The results of the RTOG 0933 and NRG CC001 clinical trials have shown that physical sparing of the hippocampus during cranial irradiation (CI) is associated with preservation of memory functions at 4- and 6-months following therapy. Whereas the putative roles of protection of neural stem cells (NSCs) residing within the subgranular zone (NGZ) of the dentate gyrus are presently poorly defined, suppression of inflammation may be involved because ablation of microglia (MG) through blockade of the CSF1R or selective targeting of CCR2\textsuperscript{+} macrophages using an appropriate CCR2 inhibitor leads to the reduction of hippocampal-dependent cognitive abilities following CI. Inhibition of Colony stimulating factor 2 (CSF-2), a proinflammatory cytokine causing the proliferation and activation of microglia, may be a suitable alternative strategy to alleviate inflammation. METHODS: Our studies have evaluated the effects of ablation of CSF2 and also the inducible ablation of MG on the properties of neuroinflammation, neurogenesis and CI-associated cognitive impairments employing the requisite mouse models. RESULTS: We demonstrate that preservation of cognitive functions following CI does not require ablation of MG. In addition, the reduction in neuroinflammation following Cs2ablation was sufficient to prevent CI-induced cognitive decline. Moreover, Cs2 ablation did not prevent the deficit in neurogenesis, thereby suggesting that NSC-mediated SGZ neurogenesis prevention is not required for radiation-induced cognitive dysfunction. CONCLUSION: We have previously shown that MG play seminal roles in neural development and adult homeostasis and plasticity. Our present study demonstrates that selective modulation of MG-associated neuroinflammatory signaling without MG ablation holds promise as a novel therapeutic strategy to preserve cognitive functions following CI. These experimental observations have seminal implications for patients undergoing radiation therapy for tumors of the brain or head and neck in which the hippocampus inevitably exposed to a high dose of radiation leading to potentially debilitating and possibly avoidable cognitive deficits.