several mutations of high frequency, in comparison with systemic diffuse large B cell lymphoma (DLBCL)s. Consequences of these specific mutations in PCNSL are unknown. In this study, we have analyzed the functional consequence of mutations in the *PIM1* gene, observed in 100% of PCNSL patients, which encodes a serine/threonine kinase and is known to drive tumorigenesis in several malignancies. METHODS: Four most frequent mutations of *PIM1* in PCNSL, *S77N*, *K115N*, *P216S*, *L275F*, were chosen from our previous study, and each mutant was generated by site directed mutagenesis in *PIM1* cDNA cloned in an expression vector. Resulting vectors were transiently transfected into human cancer cell lines. Cell death of the cells expressing each mutant was evaluated by dye-exclusion method under treatment of chemotherapeutic agents. Alteration of molecular signaling was evaluated by immunoblotting. RESULTS: Among the four mutants, increased phosphorylation of BCL-2 associated death promoter (BAD) at Ser112, which is a phosphorylation target of Pim-1, was observed by expression of *K115N* mutant compared with wild type *PIM1* in Nagai and Hela cells expressing endogeneous BAD. Decreased cell death under campthotethin treatment was also observed in *K115N* mutant expressing Nagai cells compared with wild type *PIM1*-expressed cells. Moreover, we observed a significant shift in subcellular localization of Pim-1 carrying *K115N* mutant; from the nucleus, main sublocalization for wild type Pim-1, into the cytosol determined by immunocytochemistry and immunoblotting of nuclear and cytosolic fraction of the cells. DISCUSSION: It is suggested that *PIM1 K115N* mutant may drive chemoresistance through increased BAD phosphorylation that suppresses cell death compared with wild-type *PIM1* through modification of its subcellular localization.

CSIG-32. DUAL PI3K/Akt INHIBITION TO OVERCOME THE P-gp/BCRP DRUG EFFLUX SYSTEM FOR IMPROVED DRUG DELIVERY IN GLIOBLASTOMA THERAPY

Anika Hartz, Julia Schulz, Brent Sokola and Bjoern Bauer; University of Kentucky, Lexington, KY, USA

The blood-brain barrier is a primary obstacle for effective anticancer drug therapy of patients with glioblastoma multiforme (GBM). On a molecular level, failure of anticancer drug treatment is largely due to the blood-brain barrier efflux transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP). P-gp and BCRP (P-gp/BCRP) work together to restrict anticancer drugs from crossing the barrier and from entering the brain to reach tumor targets. We found that PI3K/Akt regulates P-gp/BCRP in brain capillaries of the rodent and human blood-brain barrier. Our *in vivo* data show that combination treatment with LY294002 (PI3K inhibitor) and triciribine (Akt inhibitor) downregulates P-gp and BCRP protein expression and transport activity in brain capillaries. We also have evidence from brain capillaries isolated from GBM mice and GBM patients showing that GBM induces P-gp/BCRP overexpression in capillaries in the brain hemisphere that is *contralateral* to the primary tumor. These findings indicate that P-gp/BCRP overexpression in brain capillaries protects invasive tumor cells that are scattered throughout the brain from being targeted by anticancer drugs. To overcome this obstacle, we are currently developing a novel therapeutic strategy by targeting PI3K/Akt to transiently decrease P-gp/BCRP expression and activity, thus, creating a "window-in-time" during which anticancer drugs can enter the brain.

CSIG-33. ONCOLYTIC HERPES VIRUS TREATMENT INDUCED NOTCH SIGNALING VIA HSV-1 microRNA H16 AND IT INVOLVED IN TREATMENT RESISTANCE

Yoshihiro Otani[1], Ji Young Yoo[1], Tae Jin Lee[1], Joseph Liu[2], Alena Cristina Jaime-Ramirez[2] and Balveen Kaur[1]; [1]Department of Neurosurgery, University of Texas Health Science Center at Houston, Houston, TX, USA, [2]Department of Neurological Surgery, James Comprehensive Cancer Center, The Ohio State University Wexner Medical Center, Columbus, OH, USA

INTRODUCTION: Glioblastomas (GBMs) are resistant to traditional therapies. Thus, the development of novel treatment strategies is urgently needed. NOTCH signaling is activated in GBM and important for mediating proliferation, angiogenesis, and resistance to therapy. In this study, we uncovered the mechanism by which oncolytic herpes simplex viruse-1 (oHSV) therapy induced NOTCH activation in the tumor microenvironment (TME). We also investigated the therapeutic benefit of combining oHSV therapy with Gamma-secretase inhibitor (GSI), a NOTCH inhibitor. METHODS: Real time Q-PCR, NOTCH reporter assay, and bioluminescence mice imaging were used to test oHSV-induced NOTCH activation. Screening of HSV-1-encoded microRNAs (HSVmiRs) and genes were performed to identify the mechanism which regulates the NOTCH signaling. Intracranial glioma-bearing xenografts were used to evaluate the anti-tumor efficacy of combination of GSI and oHSV. RESULTS: oHSV infection induced gene expression of NOTCH ligands resulting in activation of NOTCH signaling in adjacent cells in primary GBM derived cells. This was mediated by HSV encoded miRH16. HSVmiRH16 targets and suppresses Factor Inhibitor Hif1-1 (FIH-1) expression which is known to inhibit NOTCH activation. Consistent with this both overexpression of HSVmiRH16 and knockdown of FIH-1 significantly induced NOTCH signaling. Treatment of mice bearing intracranial glioma with GSI and oHSV therapy significantly enhanced survival compared to that with monotherapy. CONCLUSION: We identified oHSV-induced NOTCH signaling activation, via HSV encoded miRH16. We further find that GSI treatment in combination with oHSV therapy demonstrated improved efficacy. To our knowledge this is the first report investigating NOTCH signaling in conjunction with oHSV therapy.

CSIG-34. PI3 KINASE PATHWAY ACTIVATION PROMOTES MALIGNANT PROGRESSION IN OLIGODENDROGLIAL TUMORS

Kensuke Tateishi[1], Taishi Nakamura[2], Alexandria Fink[3], Nina Lelic[4], Yuko Matsushita[5], Mara Koerner[3], Hidetoshi Murata[1], Koichi Ichimura[5], Tracy Batchelor[6], Tetsuya Yamamoto[1], Andrew Chi[7], John Iafrate[8], Hiroaki Wakimoto[3] and Daniel Cahill[9]; [1]Yokohama City University, Yokohama, Japan, [2]Department of Neurosurgery, Yokohama City University, Yokohama, Japan, [3]Massachusetts General Hospital, Boston, MA, USA, [4]Massachusetts General Hospital, Pittsburgh, PA, USA, [5]Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Tokyo, Japan, [6]Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA, [7]NYU Langone Health, New York, NY, USA, [8]Department of Pathology, Massachusetts General Hospital, Boston, MA, USA, [9]Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Oligodendroglioma (OD) is a subtype of adult diffuse glioma defined by IDH1/2 gene mutation and co-deletion of chromosomal arms 1p and 19q. Although prognosis in OD tumors is initially relatively favorable, the majority of OD develop outgrowth of a subclone that has undergone malignant transformation. Modeling the molecular mechanisms of this tumor progression is crucial to identify therapeutic targets for malignant disease. However, there are few available patient-derived OD xenograft models, which limit preclinical investigations. Here, we present novel patient derived anaplastic oligodendroglioma (AOD) xenograft models. In a panel of OD at different stages of disease, we harvested two distinct cell samples: those with and without PIK3CA mutation. From the tumor that subsequently rapidly progressed and had a *PIK3CA* mutation, we established a xenograft model that was lethal to the mouse and retained the *PIK3CA* mutation. In contrast, xenograft did not form from the other tumor that was clinically stable after resection and had wild-type *PIK3CA*. We confirmed AOD phenotype and the presence of IDH1 mutation and 1p/19q co-deletion in xenograft tissue, indicating successful capture of these signature OD genetic alterations. We also tested to see if PI3K/AKT/mTOR gene mutation could induce patient-derived OD xenograft formation. In our attempts to establish xenograft models, the presence of activating mutations in PI3K/AKT/mTOR pathway was consistently associated with successful xenograft establishment. OD/AOD tumors that did not form xenograft did not have mutation in the PI3K/AKT/mTOR pathway Importantly, we found progressive tumor cells that harbor mutant *PIK3CA* were vulnerable to alkylating agents and PIK/AKT/mTOR pathway inhibitors. These findings suggest there is a critical role of PI3K/AKT/mTOR pathway activation in driving progression and xenograft formation in oligodendroglial tumors. Our xenograft models will facilitate dissection of the mechanism of malignant transformation, contributing to the identification of optimal therapeutic strategies for patients with oligodendroglial tumors

CSIG-35. MST4 PHOSPHORYLATION OF ATG4B REGULATES AUTOPHAGIC ACTIVITY, TUMORIGENICITY, AND RADIORESISTANCE IN GLIOBLASTOMA

Tianzhi Huang[1], Chung Kwon Kim[1], Angel Alvarez[1], Rajendra Pangeni[1], Xuechao Wan[1], Song Xiao[1], Taiping Shi[1], Yongyong Yang[1], Namratha Sastry[1], Craig Horbinski[2], Songjian Lu[3], Roger Stupp[4], John Kessler[2], Ryo Nishikawa[5], Ichiro Nakano[6], Erik Sulman[7], Xinghua Lu[8], C. David James[4], Xiao-Ming Yin[9], Bo Hu[2] and Shi-Yuan Cheng[2]; [1]Northwestern University, Chicago, IL, USA, [2]Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA, [4]Northwestern University, Feinberg School of Medicine, Chicago, IL, USA, [5]Department of Neuro-Oncology/Neurosurgery, Saitama International Medical Center, Saitama Medical University, Hidaka-city, Saitama, Japan, [6]Department of Neurosurgery, The University of Alabama at Birmingham, Birmingham, AL, USA, [7]University of Texas M. D. Anderson Cancer Center, Houston, TX, USA, [8]Dept. of Biomedical Informatics, University of Pittsburgh, Pittsburgh, PA, USA, [9]Indiana University, Indianapolis, IN, USA

Autophagy is a conserved catabolic process that maintains homeostasis by regulating the energy balance of the cell. Cancer cells use autophagy to re-

move damaged organelles and aggregated proteins, and to recycle nutrients in high demand to support tumor growth. Radiation therapy (RT) and temozolomide (TMZ), are front-line treatments for glioblastoma (GBM), the common and most malignant brain tumors in humans. However, RT and TMZ are known activating the autophagic response in tumor cells, which protects GBM cells from therapy-induced cell death. Thus, improved understanding of mechanisms regulating autophagy could reveal targets for selective and specific inhibition, which would enhance the anti-tumor activity of RT and TMZ while reducing toxic effects of treatment. In this study, we determined the roles of MST4, a less known protein serine/threonine kinase in its cellular functions in regulation of GBM tumorigenicity and therapy responses through activating autophagic activities. By using proteomic, biochemical and genetic approaches, we identified ATG4B as a novel substrate of MST4. ATG4B is a key regulator that facilitates autophagic process through reversible modification of ATG8/LC3. MST4 phosphorylates ATG4B at serine residue 383, which stimulates ATG4B enzymatic activity towards LC3, increasing autophagic flux. Inhibition of MST4 or ATG4B activities suppresses autophagic activities and tumorigenicity of patient-derived glioma stem cells (GSCs) in vitro and in the brain of mice. Furthermore, RT induces MST4 expression, ATG4B phosphorylation and autophagic activity. Inhibiting ATG4B by using a novel inhibitor NSC185058 in combination with RT in treating mice with intracranial GBM tumor xenografts markedly slows tumor growth and provides significant survival benefit to animal subjects. This study not only describes a novel regulatory mechanism by which the MST4-ATG4B axis accelerates autophagic process, regulates GBM tumorigenicity, and responses to RT, but also explores imminent clinical utility of combination of ATG4B inhibition with RT to suppress orthotopic GBM tumor xenografts.

CSIG-36. INVOLVEMENT OF microRNAs 221/222-3p IN THE REGULATION OF PROGRAMMED CELL DEATH 10 (PDCD10) GENE IN GLIOBLASTOMA

Nargis Malik, Parthaprasad Chattopadhyay, Chitra Sarkar, Ashish Suri, Subrata Sinha and Kunzang Chosdol; All India Institute of Medical Sciences, Delhi, India

INTRODUCTION: FAT1 gene is localized at chromosome 4q35.2 encoding a 506KDa. Here in our study we are characterizing the role of FAT1 in primary brain tumors. MiR-221-3p/222-3p reported to have oncogenic role and targets tumor suppressors (e.g. PDCD10, PTEN, PUMA etc.) in many cancers including GBM. Here, we have analyzed the role of FAT1 gene in the regulation of miRNAs in GBM. METHODOLOGY: In-silico analysis of miR targets was done by target prediction software miRDB, TargetScan, miRTarBase. FAT1 knockdown was done using FAT1 specific siRNA and mRNA expression analysis done by gene specific primers and for miR-221/222-3p using LNA-primers in GBM cell lines (U87MG, U373MG, A172 and LN229). Expression and Spearman correlation analysis of FAT1 and miR-221-3p was done in GBM tumor samples (n=30). RESULTS: We have observed increased expression of FAT1 and miRNAs (miR221-3p/miR222-3p) in GBM cell lines (U87MG, U373MG, A172 & LN229). On FAT1 knockdown, by siFAT1 we observed significantly reduced expression of miR-221/222-3p. In-silico analysis identified, TIMP3, PDCD10, PUMA and PTEN as potential targets of miR-221/222-3p. Furthermore, FAT1 knocked-down cells showed significantly augmented expression of PDCD10 in all studied glioma cell lines. In order to validate our in-vitro observation and its clinical relevance, we have done expression and correlation study in GBM tumor samples. We observed significant positive spearman correlation between FAT1 and miR-221-3p ($r=0.5669$, $p\leq0.0011$) and negative correlation of FAT1 with PDCD10 ($r= -0.3492$, $p\leq0.0585$),) and miR-221-3p with PDCD10 ($r=0.526$, $p\leq0.0028$). These results suggest that FAT1 expression positively regulates the expression of miR-221-3p leading to downregulation of miR 221-3p target (PDCD10) in GBM cell lines and GBM tumors. CONCLUSION: Taken together our in-vitro and GBM tumor data for the first time suggesting FAT1 to be a novel molecule regulating the expression of miRNA in GBM and FAT1 may emerge as a target for therapeutic intervention.

CSIG-37. FOXR2 STABILIZES MYC AND ACTIVATES FAK/SRC SIGNALING IN A DUAL MECHANISM TO PROMOTE TRANSFORMATION IN NEURAL PROGENITOR CELLS

Pauline Beckmann[1], Jon Larson[2], Alex Larsson[1], Jason Ostergaard[1] and David Largaespada[1]; [1]University of Minnesota, Minneapolis, MN, USA, [2]St. Jude Children's Research Hospital

Medulloblastoma and central nervous system primitive neuroectodermal tumors (CNS-PNETs) are aggressive, poorly differentiated brain tumors that primarily affect children. Current treatment strategies with severe long-term treatment-related side effects and poor survival rates warrant further study into therapies with increased efficacy and lower cost to the patient. More targeted therapy represents a route to better treatments, but a barrier to identifying novel targets is a lack of animal models. We created a mouse model that developed medulloblastoma or CNS-PNET using *Sleeping Beauty* (*SB*) mutagenesis of neural progenitor cells (Nestin+). *SB*-induced tumors resembled human medulloblastoma and CNS-PNET histology. Additionally, we used RNA-Sequencing to determine that they most closely resemble human SHH, group 3, and group 4 medulloblastoma and a subgroup of CNS-PNET with *FOXR2* activation (CNS NB-*FOXR2*).Using both DNA and RNA analysis, we identified over 100 genes as candidate drivers in medulloblastoma and/or CNS-PNET. FOXR2 was identified as a proto-oncogene, with increased expression in *SB*-induced mouse tumors. FOXR2 drives colony formation in soft agar and tumor formation *in vivo* when overexpressed in a mouse neural progenitor cell line. We found that FOXR2 binds N-MYC and increases C-MYC stability in 2 neural cell lines. We also found a novel role for FOXR2 in activating the FAK/SRC signaling pathway. Increased FOXR2 drove FAK/SRC activation, in a MYC interaction-independent manner, and *FOXR2* KO decreased FAK/SRC activation. Interestingly, increased FOXR2 expression conveyed resistance to a SRC family kinase inhibitor (Dasatinib) in a MYC-dependent manner, indicating overlap between these two apparently distinct effects. Further studies into the mechanism of FOXR2-driven tumorigenesis may provide a novel route for therapy in treating patients with medulloblastoma and CNS-PNET with high FOXR2 levels.

CSIG-38. ROBO2 SIGNALING IN INVASION OF GLIOBLASTOMA

Amber Kerstetter-Fogle[1], Harris Peggy[1], Jill Barnholtz-Sloan[2], Marta Couce[3] and Andrew Sloan[4]; [1]Case Western Reserve University, Cleveland, OH, USA, [2]Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH, USA, [3]UH Cleveland Medical Center, Cleveland, OH, USA, [4]University Hospitals-Cleveland Medical Center, Cleveland, OH, USA

Glioblastoma accounts for 54.3% of newly diagnosed glioma in the United States each year. Rapid growth and invasion confers a devastating phenotype with very limited treatment options at recurrence. Numerous studies of the molecular alterations in GBM have been conducted but few have completely characterized the invasiveness characteristic of GBM. The roundabout (Robo) family of transmembrane receptors and their Slit protein ligands, have been demonstrated to be involved in neuronal migration and outgrowth during CNS development and implicated in various cancers. We hypothesize that Slit-Robo pathway may be involved in GBM cell invasion. We reviewed numerous databases and analyzed protein and RNA from GBM samples. Analysis of the NCI Rembrandt database indicated poorer survival among glioma patients with a greater than two-fold overexpression of the Robo2 gene (17.1 months versus 37.4 months; p=1.42E-4). GBM tumor was identified prospectively on pre-operative MRI and sampled using stereotactic image-guided resection from various regions within the tumor. In tissue samples from 6 GBM and 3 control patients, indicted a 2.5 fold difference in the expression of Robo2 protein. Quantitative PCR and Western blot confirmed a 101- fold increase in mRNA expression. Knockdown of the mRNA using siRNA directed against Robo2 in U87 cells resulted in survival advantage (65.5 days versus 52.5 days; p=0.011). These data suggest a complex and heterogenous tumor microenvironment and implicates Robo axis signaling in GBM as a potential therapeutic target.

CSIG-39. HIV-1 ENVELOPE PROTEIN GP120 PROMOTES ACTIVATION OF PROTEIN SYNTHESIS IN GLIOMAS THROUGH THE ERK AND AKT SIGNALING

Gabriel Valentin-Guillama, Yuriy Kucheryavykh and Lilia Kucheryavykh; Universidad Central del Caribe, Bayamon, Puerto Rico

Patients infected with human immunodeficiency virus (HIV-1) are more prone to developing cancers, including glioblastomas (GBMs). The median survival for GBM patients with HIV is significantly shorter than for HIV-negative GBM patients, despite the fact that they receive the same treatments. This difference indicates that HIV infection is associated with more aggressive tumor behavior and with treatment resistance. Earlier we demonstrated that gp120, a main glycoprotein in the HIV shell, stimulates glycolysis and protein synthesis in glioma cells. The purpose of this study was to evaluate the underlying gp120 dependent signaling mechanism in glioma cell. Using MAPK kinase antibody array and western blot assays we have identified the activation of MAP kinase and Akt/mTOR pathways in U87, A172, and primary glioma cells treated with gp120 (100 ng/ml) for 5 consequent days. Specifically, up regulation of pMEK1/2(Ser217/221), pERK(Thr202/Tyr224), pP90RSK(Thr359), pmTOR(S2448), pAkt(pS473), and pGSK3b(pS9) have been identified. These data coincide with previously obtained results showing that glioma cells treated with gp120 exhibit higher protein synthesis and proliferation rates compared to un-treated glioma cells. The use of pharmacological inhibitors of PI3K/Akt and ERK signaling reversed the stimulatory effect of gp120 on global protein synthesis, as