onstrates that detection of ecDNA-amplified PDGFRA has the potential to be a predictive biomarker of future PDGFRα-targeted therapies.

CSIG-07. C-SRC PHOSPHORYLATES AND INACTIVATES THE CIC TUMOR SUPPRESSOR PROTEIN IN GLIOBLASTOMA

Severa Bunda[1], Pardeep Heir[1], Annie Li[1], Mamatjan Yasin[1], Gelareh Zadeh[2], and Kenneth Aldape[3]; [1]University Health Network, Toronto, ON, Canada, [2]Princess Margaret Cancer Center, Toronto, ON, Canada, [3]National Cancer Institute, National Institute of Health, Bethesda, MD, USA

Capicua (CIC) is a transcriptional repressor that counteracts activation of genes in response to receptor tyrosine kinase (RTK)/Ras/MEK/ERK signaling. Following activation of RTK, ERK enters the nucleus and serine-phosphorylates CIC, releasing it from its targets to permit gene expression. We recently showed that ERK triggers ubiquitin-mediated degradation of CIC in glioblastoma (GBM). In this study, we examined whether another important downstream effector of RTK/EGFR, the non-receptor tyrosine kinase c-Src, affects CIC repressor function in GBM. We found that c-Src binds and tyrosine phosphorylates CIC on residue 1455 to promote nuclear export of CIC. On the other hand, CIC mutant allele (CIC-Y1455F), that escapes c-Src-mediated tyrosine phosphorylation, remains localized to the nucleus and retains strong repressor function against CIC targets, the oncogenic ETV transcription factors. Furthermore, we show that the orally available Src family kinase inhibitor, dasatinib, which prevents EGF-mediated tyrosine phosphorylation of CIC and attenuates elevated ETV levels, reduces viability of GBM cells and glioma stem cells (GSC), but not of their control cells with undetectable c-Src activity. In fact, GBM cells and GSC expressing the tyrosine-defective CIC mutant (Y1455F) lose sensitivity to dasatinib, further endorsing the effect of dasatinib on Src-mediated tyrosine phosphorylation of CIC. Importantly we show that combined inhibition of Src and MEK/ERK results in greatest stabilization of CIC and attenuation of proliferation compared to either inhibitor alone. These findings elucidate important mechanisms of CIC regulation and provide the rationale to target c-Src alongside ERK pathway inhibitors as a way to fully restore CIC tumor suppressor function in neoplasms such as GBM.

CSIG-08. TARGETING ION TRANSPORT-REGULATORY KINASES AS A NOVEL TREATMENT FOR GLIOBLASTOMA

Paula Schiapparelli[1], Paola Suarez Meade[1], Pierre Miranda-Herrera[2], Alexandra R Bechtle[1], Farren Issacs[3], Andre Levchenko[3], Jesse Rinehart[3], and Alfredo Quinones-Hinojosa[4]; [1]Mayo Clinic, Jacksonville, FL, USA, [2]Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [3]Yale University, New Haven, CT, USA, [4]Mayo Clinic, Jacksonville, FL, USA

Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults. Currently, treatment is focused on achieving maximal safe resection, followed by chemo- and radiotherapy. Although surgery remains the most important therapeutic component, complete surgical resection is virtually impossible, as GBM cells readily infiltrate the adjacent brain parenchyma. One fundamental mechanism for GBM cell migration is the ability of these cells to use cell volume regulation as a driving force of cell infiltration and potentially interfering with apoptosis and cell cycle progression. In this work, we targeted the master regulators of cell volume, the kinases STE20/SPS1-Related Proline-Alanine-Rich Protein Kinase (SPAK) and Oxidative stress-responsive kinase-1) (OSR1) by using a novel proprietary small molecule inhibitor. In order to study the effects of inhibiting SPAK and OSR1, we analyzed cell proliferation and migration. First, we determined the IC50 using six different patient-derived GBM cell lines; we found IC50 values ranging from 0.2–2μM. Next, cell proliferation was determined by cell cycle analysis through Edu labeling. We found a significant decrease in S phase and cell cycle arrest in G2M (p= 0.001, n=6). In addition, cell migration was determined using boyden chamber assay. We found a dose-dependent reduction in cell migration (p=0.001, n=3). These results correlated with the phenomenon observed in an orthotopic murine model of GBM, in which the inhibitor showed a decrease in tumor growth (p=0.0026, n=4) and greater survival rates *in vivo* (p=0.0046, n=8). When used in combination with radiation therapy, this small molecule inhibitor was capable of radio-sensitizing GBM cells decreasing clonogenicity (p=0.01, n=3) were observed *in vitro*. In summary, by targeting the SPAK/OSR1 kinases with a small molecule inhibitor, GBM cells become less aggressive, mainly by interfering with cell migration and proliferation and becoming more sensitive to radiation.

CSIG-09. ATRX DEFICIENCY IN GLIOMA IMPACTS TRANSCRIPTIONAL PROFILES AND THE IMMUNE MICROENVIRONMENT IN VIVO

David Irvin[1], Hannah Roberts[1], Vladislav Sharin[1], Ahsan Farooqi[1], Sharvari Dharmaiah[2], Christian Alvarez[1], and Jason Huse[3]; [1]MD Anderson Cancer Center, Houston, TX, USA, [2]MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA, [3]Department of Translational Molecular Pathology, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Current treatment for diffuse astrocytoma fails to address its underlying molecular mechanisms leading to inevitable disease progression and eventual patient death. Genomic studies have implicated ATRX alterations as critical to low grade glioma biology. Our lab has previously shown in vitro that ATRX influences glioma motility, cellular differentiation state, and epigenetic programming, however, the influence of ATRX alterations in vivo remains unclear. Here, we leveraged an RCAS/tva mouse tumor model to probe the role of ATRX deficiency in glioma. Atrx deficient murine tumors exhibited lower histopathological grade and were associated with longer survival than Atrx-intact counterparts, and syngeneic allografts of cell lines derived from primary tumors mirrored the differential degrees of aggressiveness seen in primary tumors. Tumor-derived Atrx-deficient cell lines showed increased susceptibility to G-quadruplex stabilizing compounds, pointing to increased replication stress and recapitulating a key phenotype of ATRX-mutant gliomas in humans. Transcriptional profiling revealed enrichments in MYC target genes, E2F targets as well as G2/M checkpoint pathways in Atrx-intact tumors and cells, and enrichment in RAS signaling in Atrx-deficient tumors and cells. Finally, Atrx deficient murine gliomas displayed increased levels of NK cells, a phenotype recapitulated in ATRX-mutant human gliomas, and primary Atrx-deficient glioma lines exhibited increased levels of NK cell-attracting cytokines. These latter findings suggest that ATRX deficiency could influence interactions between glioma cells and their immune microenvironment by way of phenotypically relevant molecular mechanisms.

CSIG-10. GENOTYPE – KINOME GUIDED DEVELOPMENT OF PRECISION EGFR-TARGETED THERAPEUTICS FOR GLIOBLASTOMA

Erin Smithberger[1], Abigail Shelton[2], Madison Butler[3], Allie Stamper[4], Ryan Bash[1], Steven Angus[5], Michael East[6], Gary Johnson[6], Michael Berens[7], Frank Furnari[8], and C. Ryan Miller[4]; [1]University of Alabama Birmingham, Hoover, AL, USA, [2]University of North Carolina, Alabaster, AL, USA, [3]National Cancer Institute, Bethesda, USA, [4]University of Alabama Birmingham, Birmingham, AL, USA, [5]Indiana University, Indianapolis, IN, USA, [6]University of North Carolina, Chapel Hill, NC, USA, [7]The Translational Genomics Research Institute, Phoenix, AZ, USA, [8]University of California, San Diego, San Diego, CA, USA

Glioblastoma (GBM) is an aggressive primary brain tumor with poor survival and limited treatment options. However, it is an attractive candidate for precision therapeutic approaches due to the frequency of amplification and/or activating mutations in the epidermal growth factor receptor (EGFR) gene and the availability of several brain penetrant second- and third-generation EGFR tyrosine kinase inhibitors (TKI). We used comprehensive molecular profiling of a panel of genetically engineered mouse astrocyte models to examine whether mutational profiles, particularly EGFR and PTEN status, could be used to identify kinases upregulated in specific mutational backgrounds. Using RNA-seq and multiplex inhibitor bead/mass spectrometry (MIB-MS) to analyze the kinase transcriptomes and proteomes, respectively, we have identified several potential targets for combination therapy. Overexpression of wild type EGFR in immortalized, *Cdkn2a-/-* astrocytes resulted in mild rewiring of the GBM kinome. Only 5 kinases aside from EGFR itself were overexpressed on either the transcript or protein levels. One overexpressed kinase, *Hck*, has been shown to be involved in cell survival, proliferation, adhesion, and migration. In contrast, overexpression of EGFRvIII, a constitutively active, extracellular domain truncation mutant of EGFR, resulted in significant alteration of the GBM kinome – 81 kinases showed differential expression, with 27 upregulated. One potentially attractive target among these was *Cdk6*, a drug-targetable, prognostically significant cyclin-dependent kinase implicated in proliferation, migration, and invasion. Finally, overexpression of EGFRvIII in cells lacking *Pten* dysregulated 46 kinases, including 15 upregulated. One particularly interesting target in these cells was *Ddr2*, a tyrosine kinase involved in migration, invasion, and extracellular matrix remodeling. We conclude that Hck, Cdk6, and Ddr2 represent attractive targets for therapeutic intervention in their relevant genetic contexts. These findings also suggest that molecular diagnostics for EGFR and PTEN status may be useful in guiding development of rational, EGFR TKI-centric drug combinations.

CSIG-11. TARGETING PD-L1 IN GLIOBLASTOMA USING NANOPARTICLE-BASED GENE EDITING

An Tran, Javier Fierro, and Huanyu Dou; Texas Tech University Health Science Center - El Paso, El Paso, TX, USA

Glioblastoma multiforme (GBM) is an astrocyte derived brain tumor with very poor prognosis, usually with a less than one year survival rate. Immunotherapy has shown promising therapeutic potentials in research and