Expression of HCMV IE1 in the U87MG Cell Line Augments Resistance to Temozolomide

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AI-28. ISOCITRATE DEHYDROGENASE-1 (IDH-1) EXPRESSION DOES NOT CO-LOCALIZE WITH HYPOXIA INDUCIBLE FACTOR-1ALPHA (HIF-1ALPHA) EXPRESSION IN GLIOMAS
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INTRODUCTION: Prior studies have demonstrated that mutations in the enzyme cytosolic isocitrate dehydrogenase-1 (IDH-1) occur more commonly in certain types of brain tumors, with the majority of secondary glioblastomas having progressed from lower grade lesions with an IDH-1 mutation. The role of IDH-1 in this progression is unclear but has been proposed to be linked to hypoxia inducible factor-1alpha (HIF-1alpha). Using immunohistochemistry, we analyzed glioma samples that were positive for the R132H IDH-1 mutation for HIF-1alpha expression to determine whether tumors harboring this IDH-1 mutation had increased HIF-1alpha expression and co-localization. METHODS: The New York University Langone Medical Center Pathology database was queried for all archival surgical specimens of glial neoplasms. Using immunohistochemistry on formalin-fixed paraffin-embedded sections, 135 glial neoplasms were analyzed for the R132H IDH-1 mutation. The tumors that were positive for this IDH-1 mutation were then analyzed for HIF-1alpha expression by immunohistochemistry. RESULTS: Evidence of IDH-1 R132H mutated tumor cells was present in 19 of 155 patients. Some of the tumors expressing this IDH-1 mutation also exhibited increased HIF-1alpha expression. However, we did not observe IDH-1 and HIF-1alpha co-localization in these tumors. CONCLUSIONS: Activation of HIF-1alpha has been implicated as a mechanism for tumor progression in gliomas harboring the IDH-1 mutation. Our results do not support an in situ link between HIF-1alpha expression and the R132H IDH-1 mutation.

AI-29. FOCAL ADHESIONS DYNAMICS IN MALIGNANT GLIAL CELLS WITH VARIABLE DRR EXPRESSION
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Gliomas are the most common primary brain tumors. Regardless of the tumor grade, except for grade 1, tumor invasion of surrounding brain tissue is a common finding. The invasive behavior of these tumors is a major challenge for reaching a cure from these neoplasms and a major cause of treatment failure. DRR, a protein expressed in glioma cells, has been shown to promote invasiveness in these tumors. It has been suggested that DRR has a role in the cytoskeleton-focal adhesion dynamics. Focal adhesion is not well studied in human gliomas, and the focal adhesion-cytoskeleton interaction is less examined in gliomas than in other cell types. We will present data of focal adhesion dynamics with variable DRR expression in a human glioma cell line. Our work includes quantification of focal adhesions in glioma cell lines with different DRR expression and evaluation of the change in dynamics of focal adhesions using live-tissue imaging obtained from the same cell lines. Our work adds to the current knowledge of focal adhesion dynamics and their role in glioma invasion.

AI-30. ONCOGENIC EGFR-VIII SENSITIZES GBM CELLS TO PROANGIOGENIC EFFECTS OF THE COAGULATION SYSTEM
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INTRODUCTION: Tissue factor (TF) is a procoagulant receptor frequently overexpressed in human glioblastoma multiforme (GBM), in which thrombotic events are particularly frequent. Analysis of GBM cell lines suggests that TF is a regulatory target of several major genetic alterations associated with this disease, including activation of the epidermal growth factor receptor (EGFR) via types of its mutant (EGFRvIII). In the present study, we report a novel assay system that generates individual patient drug profiles and is novel to better understand the biology of astrocytomas, we explored new therapeutic targets. We previously cloned NPA3, a transcription factor that maps to human chromosome 14. Our principal aim is to comprehend the disease associations of NPA3 (n = 433) since we recently identified its expression in human astrocytes. We initially identified NPA3 as an astrocytoma candidate based on the Cancer Genome Project reporting chromosome 14 deletions (with NPA3) among >20%–80% of astrocytomas and with >70% of our human astrocytoma panel (n = 433) having aberrant NPA3 protein expression. Based on the findings from our precursory screen, we next undertook functional analyses of NPA3 in human astrocytomas. METHODS-RESULTS: After undertaking extensive functional analyses, we now have evidence supporting NPA3 as an astrocytoma tumor suppressor...
suppressor involved in late-stage tumor progression, based on: 1) Aberrant NPAS3 expression is predominant in malignant human glioma cell lines; 2) loss-of-function mutations in NPAS3, associated with loss of heterozygosity at an NPAS3 locus, are identified in surgically resected human glioblastomas; 3) absent NPAS3 expression is predominant in malignant human glioma cell lines; 4) over-expressed NPAS3 in malignant glioma cell lines suppresses transformation; 5) a reduced NPAS3 expression (efficiency >90%) in concert with other gliomagenesis genes can transform a well-characterized TERT immortalized human astrocyte cell line and promote the growth of malignant astrocytomas. CONCLUSIONS: Our data provide compelling evidence that the NPAS3 gene is involved in the cancer of astrocytomas, with tumor suppressive and late-stage acting progression factor roles. Current research is focused on better understanding NPAS3 in gliomas using other pre-clinical models.

CB-03. 2-DEOXY-D-GLUCOSE INHIBITS N-GLYCOSYLATION IN Glioblastoma-Derived Cancer STEM CELLS
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Cancer stem cells (CSCs) are capable of unlimited self-renewal and multi-lineage differentiation. We have shown that 2-deoxy-D-glucose (2-DG), a known inhibitor of glycolysis, can inhibit the growth of glioma-derived stem cells (GSC11) under normoxic conditions, and we hypothesize that 2-DG affects the formation of N-glycans by replacing D-mannose in glycolylation processes. We have synthesized 2-DG, D-glucose, and D-mannose labeled with deuterium at C-2 and treated GSC11 cells with these monosaccharides to measure their effects on global N-glycan formation. N-glycans were released with PNGase F and purified over a graphitized carbon cartridge SPE. Oligosaccharides were separated with a TSK-Gel Amide80 column under hydrophobic interaction chromatography conditions and analyzed by positive ion-microelectrospray with an LTQ 14.5 T FT-ICR mass spectrometer [1]. Data showed that deuterium-labeled 2-DG was incorporated into N-glycans, leading to the termination of the extension of the oligosaccharide chain. Comparative glycanic analysis of control, 2-DG-treated, and D-mannose-rescued GSC11 cells revealed a distinct modulation of the N-glycan profile. The levels of all types of N-glycans were decreased (by ~4-fold) in 2-DG-treated GSC11 cells compared with control cells. In contrast, N-glycan synthesis in GSC11 cells could be rescued to almost "normal control" levels by adding exogenous D-mannose. D-mannose rescue of 2-DG-treated GSC11 cells drastically reduced the incorporation of 2-DG into the N-glycans. These results indicate that 2-DG can interfere with biochemical transformations of D-mannose and that such interference could contribute to the overall antitumor effects of 2-DG. [1] Schaub T M, Hendrickson C L, Horning S, Quinn J P, Senko M W, and Marshall A G. High performance mass spectrometry: Fourier transform ion cyclotron resonance at 14.5 T. Teda, Anal. Chem. 2008, 80, 3985-3990.

CB-04. UNDERSTANDING AND TARGETING KINASE-INDEPENDENT ACTIVITY OF EGFR AND EGFRvIII TO OVERCOME GBM Drug Resistant
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Glioblastoma (GBM) is the most common and most intractable brain malignancy in adults. Patients with GBM have a dismal prognosis, with a median survival of 12–14 months. Epidermal growth factor receptor (EGFR) and its constitutively activated variant EGFRvIII are linked to GBM resistance to therapy and antiapoptotic Bcl-2 family members, such as Bcl-xL and Mcl-1, and also to associate with the neoapoptotic executor Bax, together leading to a Bax-dependent apoptotic response upon appropriate stress. Our results showed that both EGFR and EGFRvIII bind to PUMA constitutively and under apoptotic stress, subsequent sequestering PUMA in the cytoplasm. EGFR siRNA-mediated expression knockdown relocates PUMA from the cytoplasm onto the mitochondria. The EGFR-PUMA interaction is independent of epidermal growth factor (EGF)-induced EGFR activation and is sustained under treatment with an EGFR kinase inhibitor, Iressa. Although GBM cells express several pro-apoptotic members of the Bcl-2 protein family, our results indicate that PUMA is essential for therapy-induced apoptosis and thus for drug sensitivity. Importantly, we found that Bcl-2/Bcl-xL/Mcl-1 inhibitors (BH3 mimetics) that mimic PUMA’s antiapoptotic activity sensitize EGFR- and EGFRvIII-expressing GBM cells to Iressa. Collectively, we uncovered a novel mechanism of EGFR and EGFRvIII sensitization to GBM resistance to EGFR kinase inhibition and apoptosis-inducing agents and also provides a rationale for targeting kinase-dependent and -independent activities of EGFR as a novel combination therapy for GBM.

CB-05. NEW THERAPEUTIC APPROACH FOR BRAIN TUMORS: INTRANASAL ADMINISTRATION OF RAS INHIBITOR PERILYL ALCOHOL
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PURPOSE: The monopetere perilyl alcohol (POH), a Ras inhibitor with the potential capacity to arrest gliomagenesis, is being used in a phase I/II clinical trial in adults with recurrent malignant glioma. The present study aimed to investigate the efficacy of the intranasal administration of POH and the survival rate in patients with recurrent glioblastoma (GBM) in comparison with a historical control group of GBM patients. PATIENTS AND METHODS: The study included 89 adults with recurrent GBM who received 440 mg POH intranasal administration for 28 days and 32 matched GBM patients as the historical control group. RESULTS: The 6-month progression-free survival (stable disease) rate was 48.3% for POH-treated patients, with a significant (p = 0.0001) survival advantage compared with the untreated historical control group. Forty-two percent of patients with secondary GBM was 11.2 months, longer (p = 0.0002) than for patients with primary GBM (5.9 months). Age-adjustment multivariate analysis showed a significant difference (p = 0.002) in the survival rate between primary and secondary GBM patients. Patients with tumors localized in deep regions (e.g., thalamus, basal ganglia) survived longer (p = 0.0083) than those with tumors in lobar regions. Radiographic improvement and reduction of corticosteroid dosage (36%) was further associated with a delay in progression. CONCLUSION: Intranasal administration of POH increased the overall survival of patients with recurrent GBM compared with historical controls, especially of patients with secondary GBM and those with tumor localized in deep regions of the brain, without clinical evidence of side effects for more than a year.

CB-06. ROLE OF A NOVEL NF1-LRPPRC INTERACTION IN RNA GRANULE TRANSPORT
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INTRODUCTION: Loss of function mutations and deletions in the neurofibromin tumor suppressor gene underlie neurofibromatosis type 1 (NF1), which, with a birth incidence of 1 in 3000, is the most common inherited tumor-predisposing syndrome in humans. While the molecular mechanisms that contribute to the neoplastic manifestations have been attributed to Ras-GTPase activating protein (GAP) activity mediated through the GAP related domain (GRD) of NF1, there is no definite consensus on the molecular etiology of the non-neoplastic phenotypes, which may be mediated by domains outside the GRD. METHODS/RESULTS: A number of GST-tagged NF1-domain constructs coupled with differential mass spectrometry (MS) analysis identified the leucine-rich pentatrico peptide repeat motif containing protein (LRPPRC) and dynem as previously unreported NF1-binding domain (TBD) interacting proteins. The interaction with LRPPRC links NF1 with Legh’s Syndrome, the French Canadian variant (LSFC), a neurodegenerative disorder caused by mutations in the LRPPRC gene. Using a number of in vitro, in situ, and in silico techniques, we identified the binding regions of the two proteins necessary and sufficient for the interaction and determined the binding affinity to be high. Toward elucidating the biological relevance of the interaction, we established that the NF1-LRPPRC interaction occurs predominantly along microtubules and complexes with motor proteins. Since LRPPRC is an RNA binding protein and its Drosophila homolog is a dead end factor, stabilizes and translocates mRNA along microtubules, we hypothesized that NF1-LRPPRC is part of an RNA granule complex. Demonstration of RNA binding proteins, ribosomal subunits, and RNAs complexing with NF1-LRPPRC is highly supportive. CONCLUSIONS: NF1-LRPPRC is a novel interaction and part of an RNA granule complex. Ongoing studies are focused on elucidating the functional relevance of this complex, specifically in the context of neuronal maturation in NF1/Neuronal neurons and its role in cognitive issues that are highly prevalent in NF1 patients.
CB-07. KETOGENIC DIET AS AN ADJUVANT THERAPY TO RADIATION AND THE EFFECTS ON SAPK/JNK PROTEIN EXPRESSION

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Glioblastoma multiforme (GBM) is an aggressive tumor. Despite surgery, radiation, and chemotherapy, median survival is 12–18 months. More effective therapy is needed, and one promising treatment is the ketogenic diet (KD), which has been shown to increase survival in mouse models. To dissect KD’s mechanism of action, we did gene expression profiling on tumor and contralateral normal brain from animals fed KD or standard diet (SD). Mitogen-activated protein kinase (MAPK) is one pathway affected by the diet. Within the MAPK pathway, stress-activated protein kinase/c-jun N-terminal kinase (JNK) decreased 1.8-fold in animals fed KD. Total and phosphorylated JNK were evaluated through Western blot and immunofluorescence. Although there was no obvious difference shown by WB, immunofluorescence suggested an increase in total JNK in SD versus KD. Phospho-SAPK/JNK (p-46) (phospho-JNK) was over-expressed in tumor versus non-tumor, irrespective of diet. Additionally, immunofluorescence results showed that the JNK2 isoform translocated into the nucleus in tumors in SD animals but not in KD animals. Because therapy for GBMs typically includes radiation and/or chemotherapy, we used radiation with a fourfold dose of KD. KD plus radiation extended the survival of animals compared with those given either treatment alone. Expression profiling analyses demonstrated a global effect on gene expression following radiation. We have shown that the expression of some genes reverts closer to control (SD) levels when animals are fed KD. Expression analysis of diet plus radiation treatment indicates that some of the expression altered by radiation is minimized by KD. Total JNK is increased in tumors in animals that receive KD plus radiation. Phospho-JNK is increased in all groups treated with radiation irrespective of diet. Promotion of cancer is activated by JNK and activation of JNK can alter growth, apoptosis, and angiogenesis. Our data suggest a complex interaction between metabolic alterations such as those that occur with KD and treatment response.

GB-08. DIFFERENTIAL CONTRIBUTION OF CLASSIC RAS AND R-RAS PROTEINS TO PROLIFERATIVE AND MIGRATORY PHENOTYPE IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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We hypothesized that multiple neurofibromin-regulated small G-proteins from the classic Ras (H, N, and K-Ras) and R-Ras (R-Ras, R-Ras2, and M-Ras) subfamilies promote the proliferation and migration of malignant peripheral nerve sheath tumor (MPNST) cells. We found that H-Ras, N-Ras, and R-Ras2 proteins were uniformly expressed in 8 MPNST lines; their expression of K-Ras2 and R-Ras was variable, while M-Ras protein was not detected in these lines. RT-PCR analyses demonstrated that the guanine nucleotide exchange factors necessary to activate these Ras proteins were also present. Using 3H-thymidine incorporation and Transwell assays, we found that wild-type and mutant R-Ras inhibited migration. Raf-1 RBD affinity assays performed in vitro studies will be complemented by in vivo studies involving the breeding of MPNST cells, with resultant gliomas demonstrating increased expression of XIAP and survivin. Increased XIAP and survivin was also present in established human GBM cells, with elevated Ras activity. Knockdown of Ras was utilized, with resultant gliomas demonstrating increased expression of XIAP and survivin. Increased XIAP and survivin was also present in established human GBM cells, with elevated Ras activity. Knockdown of Ras activity with the dominant-negative Ha-Ras N17 in established human GBM cell lines significantly decreased XIAP and survivin levels. XIAP and survivin were also directly decreased by siRNA, with both strategies increasing radiation and chemotherapeutic sensitivity. Additionally, we report for the first time the detection of wild-type and EGFRVIII dimerization in GBM specimens, in keeping with our prior cell line data. GBM tissue microarray analysis for the presence of this mutant heterodimer has commenced for statistical examination with patient survival. We propose the PLA analysis platform as an alternative diagnostic modality to evaluate the expression, dimerization, and activation of wild-type and mutant EGFRs prevalent in GBMs and whether these features have important prognostic or diagnostic value. Moreover, since PLA allows specimen assessment of not only expression and activation but also dimerization, which is not evaluated by current IHC techniques, it will likely serve as a way to evaluate promising anti-EGFR strategies directed at preventing EGFR dimerization and activation.

CB-10. RAS REGULATED OVER-EXPRESSION OF INHIBITORS OF APOPTOSIS PROTEIN (IAP) IN GliOMAGENESIS

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INTRODUCTION: Transformation requires aberrant proliferation through signaling pathways such as Ras and inhibition of apoptosis. Toward apoptosis in glioblastoma multiforme (GBM), little is known about the expression and function of a family of proteins known as inhibitors of apoptosis proteins (IAPs), which include cIAP1, cIAP2, XIAP, and survivin. Human tumors with activating Ras mutations produce high amounts of survivin. We hypothesize that elevated activity of Ras in GBMs, which do not harbor primary Ras mutations, results in aberrant expression of IAPs in GBMs and plays a role in glioma transformation. METHODS/RESULTS: Our previously described GFAP:12V-HaRas (RasB8) mouse glioma model was utilized, with resultant gliomas demonstrating increased expression of XIAP and survivin. Increased XIAP and survivin was also present in established human GBM cells, with elevated Ras activity. Knockdown of Ras activity with the dominant-negative Ha-Ras N17 in established human GBM cell lines significantly decreased XIAP and survivin levels. XIAP and survivin were also directly decreased by siRNA, with both strategies increasing sensitivity to apoptosis-inducing chemotherapy. Stimulation of these lines with TGF-β to induce Ras activation increased XIAP and survivin expression, an effect that was reversed by MEK inhibitors. CONCLUSIONS: These findings will be complemented by in vivo studies involving the breeding of our RasB8 glioma model to double transgenics with GFAP-Cre regulated decreased expression of survivin/Box in astrocytes, with a postulated decrease in gliomagenic potential. The results to date and those under way are highly suggestive of the thesis that elevated Ras activity leads to glioma transformation by not only mitogenic signals but also cooperative expression of antiapoptotic proteins such as IAPs.


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MicroRNAs (miRNAs), small non-coding RNAs that regulate post-transcriptional expression of messenger RNAs, has potential as a novel tool of fine-tuning of the gene expression machinery. The discovery of microRNAs (miRNAs), small non-coding RNAs that regulate post-transcriptional expression of messenger RNAs, has potential as a novel tool of fine-tuning of the gene expression machinery. The discovery of microRNAs (miRNAs), small non-coding RNAs that regulate post-transcriptional expression of messenger RNAs, has potential as a novel tool of fine-tuning of the gene expression machinery. The discovery of microRNAs (miRNAs), small non-coding RNAs that regulate post-transcriptional expression of messenger RNAs, has potential as a novel tool of fine-tuning of the gene expression machinery.
have been associated with development and progression of cancer, and several miRNAs were found to be implicated in the modulation of glioma oncogenesis. In this work, it is demonstrated that miR-21 and miR-128 expression is significantly altered in a retrovirally induced murine model of glioblastoma. Furthermore, miR-21 and miR-128 expression is shown to be significantly altered in human glioblastoma specimens (n = 22) and cell line (U87) compared with control brain tissues. miR-21 is overexpressed in 80% of the tumor samples, with an average fold change value of 7.0, whereas miR-128 is highly downregulated in all samples, with an average fold change value of 15.6. Remarkably, analysis of the Cancer Genome Atlas Research Network data on 2,522 human glioblastomas reveals miR-21 upregulation and miR-128 downregulation in all the cases, corroborating our finding with experimental data. Identifying the regulatory mechanisms of these aberrantly expressed miRNAs in glioblastoma may be critical to better understand their role in initiation and progression of human glioblastoma, thus helping in the development of effective RNA-based therapeutic approaches for this disease.

CB-12. DOES THE MICRONRNA CLUSTER OF 53 MRNAS ON CHROMOSOME 14Q32.31 PLAY A ROLE IN GLIOMAS? Iris Lavon 1, AvitalGranit 1, OfrinaEinstein 2, Tamir Ben-Hur 1, and TaliSegal 1, 1Department of Neuro-Oncology, Hadassah-University Medical Center, 2Neurology Department, Hadassah University Medical Center

BACKGROUND: We previously demonstrated that miRNAs from the cluster of 53 miRNAs on chromosome 14q32.31 are down-regulated in gliomas. Because this region is frequently either deleted or genetically altered in several malignancies, it might be assumed to represent a large tumor suppressor miRNA cluster. Our aim was to study the function of individual miRNAs from this cluster in gliomas. METHODS: miRNAs from chromosome 14q32.31 were cloned as a pri-microRNA into a lentivirus-based vector. U87MG (human) and GL261 (mouse) glioma cell lines were transduced with either a lentivirus-vector containing one of the miRNAs or miR-323-3p, miR-369-3p, miR-433 or an empty vector. Expression of the mature miRNAs was evaluated by real-time RT-PCR. The effects of each miRNA on proliferation, migration, and soft agar colony formation were determined in both cell lines in vitro. Based on the in vitro results, the antitumorigenic potential of miR-323-3p was also evaluated in vivo. RESULTS: Over-expression of miR-323-3p and miR-369-3p in U87MG but not in GL261 demonstrated morphological changes that resulted in sphere-like growth pattern even when cells were grown on complete medium. The expression of these two miRNAs reduced the proliferation and migration rate of both glioma cell lines, while over-expression of miR-433 did not induce any effect. None of the tested miRNAs had any effect on colony formation on soft agar. Moreover, miR-323-3p prolonged the life span of mice implanted with gliomas that over-express this miRNA. CONCLUSIONS: miRNA members derived from the miRNA cluster in chromosome 14q32.31 might play a role in gliomas. The antitumorigenic potential of these miRNAs will be further studied in vivo and in vitro using additional miRNAs from this cluster. Additionally, we will investigate the mechanism of action of these miRNAs by exploring and validating their target genes. Further investigation is needed to uncover their role in gliomagenesis.

CB-13. KIAA0495/PDAM IS FREQUENTLY DOWN-REGULATED IN OLIGODENDROGLIAL TUMORS, AND ITS DOWN-REGULATION BY SIRNA INDUCES CISPLATIN RESISTANCE IN GLIOMA CELLS Jesse C. Pang 1, Wai Sang Poon 1, Liangfu Zhou 2, and Ho-Keung Ng 1, 1The Chinese University of Hong Kong; 2Tianjin University

Co-deletion of chromosomes 1p and 19q is a common event in oligodendroglial tumors (OTs), suggesting the presence of OT-related genes. The aim of this study was to identify target genes residing in the minimally deleted regions on chromosome 1p36.31-p36.32 that might be involved in OTs. A novel gene, KIAA0495/PDAM (for p53-dependent apoptosis modulator), was found to be frequently deregulated, with 37 of 58 (63.8%) OTs examined showing reduced PDAM transcript by at least two-fold and 19 of those exhibiting >10-fold reduced expression relative to the mean expression level of eight normal brain samples. PDAM down-regulation was associated with chromosome 1p loss status (P = 0.001). Promoter hypermethylation of PDAM was detected in 30 of 37 (81.1%) OTs with reduced PDAM expression, and the two parameters were significantly associated (P = 0.0004). Glioma cells (A172 and TC620) treated with 5-azacytidine exhibited significant enhancement of PDAM expression.

CB-14. THE HUMAN CYTOMEGALOVIRUS IE1 PROTEIN CONFRS RESISTANCE TO TEMOZOLOMIDE IN THE U87MG CELL LINE Richard A. Rovin 1, Johnathan E. Lawrence 2, Justin J. Segula 2, and Robert J. Winn 2, 1The University of Chicago; 2Northwestern University

INTRODUCTION: Human cytomegalovirus (HCMV) DNA and protein are found in gliomas but not in normal brain or other primary brain tumors. The role of HCMV infection in glioma biology is unclear. While it is unlikely that HCMV viral proteins directly contribute to glioma cell proliferation and antiapoptotic phenotype that confers a survival advantage. Does this oncomodulation translate into a clinically relevant effect in gliomas? To answer this question, we compared the response of the U87IE1 and U87MG malignant glioma cell lines to temozolomide. U87IE1 cells are U87MG cells that have been genetically engineered to produce HCMV IE1 protein. (The U87IE1 cell line is a generous gift from Charles Cobbs.) METHODS: Approximately 5,000 U87IE1 and U87MG cells in normal culture media were placed into wells of a 96-well plate. After 24 hours, the media was replaced with culture media containing temozolomide in increasing concentration. After 48 hours, cell viability was assessed using a luminescent assay. A dose-response curve for each cell line was generated using statistical software. The concentration of temozolomide resulting in 50% of cell death (the EC50 value) for each cell line was determined. Results: The EC50 for temozolomide in the U87IE1 cell line is 565.6 micromolar, while in the U87IE1 cell line it is 1319 micromolar. This difference is statistically significant (p < 0.0001) and indicates that the U87IE1 cells are more resistant to temozolomide than are the U87MG cells. CONCLUSION: HCMV IE1 expression by U87MG cells enhances their proliferation and survival. In this study, we show that this oncomodulatory effect is clinically relevant: the U87IE1 cell line is more resistant than the U87MG cell line to temozolomide. This finding suggests that HCMV is a viable treatment target for patients with glioma.

CB-15. TARGETING MICRONRNAS IN GLIOMA CELLS WITH ANTI-ONCOPLASTIC DArcy D. B. Penn 1, and Jesse C. Pang 1, 1The Chinese University of Hong Kong

MicroRNA (miRNA) dysregulation is a hallmark of cancer, and miRNAs have been shown to regulate many aspects of cellular function, including cell proliferation, differentiation, and apoptosis. In this study, we investigated the expression of miRNAs in glioma cells and identified several miRNAs that are downregulated in gliomas. These miRNAs were then tested for their ability to inhibit glioma cell proliferation in vitro. The results of this study suggest that miRNAs may be potential targets for the therapy of gliomas.

MicroRNAs (miRNAs) are short, endogenous, non-protein-coding, single-stranded sequences of RNA that have been found to play an important regulatory role in gene expression. The genes encoding miRNA are often located in genomic regions associated with cancer; hence, it has been suggested that miRNAs may be tumor suppressors or oncogenes. Recently, several miRNAs that are deregulated in glioblastomas have been studied, and their target genes and pathways have been identified. In this study, we report the changes in expression of several miRNAs in U87 glioblastoma cells in response to exposure to antineoplaston AS2-1. This study was done using the DHarmacon miRNA profiling array (Thermo Fisher Scientific). The miRNAs 125a-5p and 125a-3p are some of the miRNAs up-regulated in our study; 125a-5p has recently been shown to be regulated by the epidermal growth factor receptor and to function as a tumor suppressor in lung cancer. It has also been shown that the over-expression of miRNA 125a or miRNA 125b caused reduced migration and invasion of SKBR3 breast cancer cells. Using the human total microarray screen (Affymetrix) we were able to confirm the expression of AKT2 and the enhanced expression of genes involved in apoptosis in OTs exposed to antineoplaston AS2-1. Antineoplastons will be used in phase III U.S. Food and Drug Administration-regulated clinical trials this year. Once approved, these amino acid derivatives may offer promising treatment in many types of brain tumors.
Glioblastoma is the most common and aggressive cancer of the central nervous system. Recent collaborative efforts have classified glioblastomas into four clinically relevant subtypes based on signature genetic lesions in the tumor suppressor gene, p16. In a large number of proneural glioblastomas, PDGFR-alpha over-expression is concomitant with a loss of CDKN2A locus (encoding p16INK4a and p14ARF). In this study, we demonstrate that activation of PDGFR-alpha and/or PDG-F-A confers tumorigenicity to Ink4a/Arf-deficient mouse astrocytes or human glioma cells in the brain. Results indicate that Ink4a/Arf-null cells do not express PDGFR-alpha, but nonetheless respond to PDGFR-alpha-promoted glioma formation. Conversely, cellular depletion of endogenous p16/INK4a by short hairpin RNA (shRNA) in glioma cells with wild-type Ink4a/Arf markedly enhanced PDGFR-alpha-induced transformation. Mechanistically, the abrogation of intrinsic tyrosine kinase activity in PDGFR-alpha or the inhibition of signaling modules in PDGFR-alpha that lost the capacity to bind to PI3K or SHP-2 (encoded by PTNP11) significantly diminished PDGFR-alpha-promoted tumorigenesis in vitro and in vivo. Furthermore, the inhibition of SHP-2 by shRNAs or inhibitors disrupted the interaction of PI3K with PDGFR-alpha, suppressed Akt activation, and impaired tumorigenicity of Ink4a/Arf-null cells. Additionally, SHP-2 inhibition attenuated malignant tumor of rapamycin (mTOR) activation of PI3K, and the inhibition of mTOR/PI3K, which is notably TMZ-resistant, suggesting that this kinase may be a potential molecular target for the treatment of TMZ-resistant brain tumors. Further, we are examining the possibility that PI3K inhibition has the potential to eliminate brain tumor-initiating cells (BTICs). We have previously reported that Dock180 and ELMO1, a bipartite Rac1 guanine nucleotide exchange factor (GEF) promotes glioma cell invasion. We identify a regulatory mechanism by which EGFRVIII stimulates glioma cell invasion through phosphorylation of Dock180-stimulating Rac1 activities. Exogenously expressed EGFRVIII significantly promotes SN19 and LN444 glioma cell migration in vitro and tumor growth and invasion in the brains of animals. EGFRVIII induces Src-mediated phosphorylation of Dock180 at tyrosine residue Y722 and stimulates Rac1 activities. Inhibition of Src by pharmaceutical inhibitors or a dominant negative Src mutant abrogates EGFRVIII-induced p-Dock180Y722, whereas a constitutively activated Src induces p-Dock180Y722 without EGFRVIII stimulation. In vitro, cellular depletion of Dock180 or expression of a Dock180Y722F mutant inhibits EGFRVIII-promoted glioma cell growth and invasion activating Rac1, whereas wild-type Dock180 markedly enhances EGFRVIII-promoted tumor growth and invasion in the brain. A homologous search revealed that Y722 is highly conserved in Dock180 proteins among various species. Significantly, when primary human glioblastoma specimens were analyzed by immunohistochemical staining using specific antibodies against EGFRVIII and p-Dock180Y722, p-Dock180Y722 was found to be over-expressed with EGFRVIII in the invasive areas but not the central regions of glioblastoma specimens. Taken together, these data indicate that EGFRVIII promotes glioma cell invasion through Src-dependent p-Dock180Y722 and stimulates Rac1 activity, suggesting that the aberrant activation of p-Dock180Y722-Rac1 could be an alternative therapeutic target in the treatment of glioblastomas.
Individual glioblastoma cells are able to diffusely infiltrate into surrounding healthy brain tissue, leading to recurrence of the tumor and ultimately death. Thus, targeting migration signaling molecules may prove promising. An exciting new molecule is NEDD9, developmentally down-regulated 9). The docking molecule NEDD9/HEF1/Cas-L is an in vivo regulator of cell migration, has been linked to metastasis of melanoma and lung cancer, and has recently been demonstrated to promote stem cell-like properties in glioblastoma cells. OBJECTIVE: The aim of this study was to investigate the role of NEDD9 in glioblastoma cell migration using three-dimensional (3D) culture systems that better recapitulate the in vivo microenvironment. METHODS AND RESULTS: We screened seven human glioblastoma cell lines and observed high-level NEDD9 expression in half the cell lines, correlating with mRNA levels as determined by quantitative real-time polymerase chain reaction (qPCR). We also established a 3D-collagen gel model that revealed distinct morphological differences when compared with cells on 2D substrates. Subsequently, siRNA targeting of NEDD9 was shown to inhibit proliferation, migration, and invasion of high expressing cell lines, and siRNA-mediated down-regulation of NEDD9 significantly inhibited glioblastoma cell migration and invasion in 3D collagen gels. CONCLUSIONS: Our data are the first demonstration that NEDD9 plays a role in the 3D invasion of glioblastoma cells.

CB-21. CIRCULATING TUMOR CELLS (CTCs) IN PATIENTS WITH GlioBLASTOMA MultiformE (GBM)
Loic P. Deleyrolle, Maryam Rahman, Erin M. Dunbar, Maria A. Caldeira, and Brent A. Reynolds; University of Florida

INTRODUCTION: Expanding evidence from circulating tumor cells (CTCs) in numerous solid tumors, including glioblastoma multiforme (GBM), has led to techniques that isolate and characterize these cells from peripheral blood. We hypothesized that GBM CTCs would be detectable and distinguishable from normal blood cells using a cluster of differentiation (CD) antibody panel and fluorescence activated cell sorting (FACS). Once isolated, these cells would be characterized for cell signaling and genetic abnormalities. METHODS: Under IRB approval, GBM cell lines were screened with 100 CD antibodies. The antibodies that had positive expression (>90% cells) or low expression (<10% cells) were then used to isolate GBM peripheral white blood cells (WBC) from control subjects. The resulting CD antibody panel was then used to detect CTCs in the peripheral blood of GBM patients using FACS. RESULTS: Following the screening process, the CD antibody panel consisted of CD45 (negative in GBM, positive in WBC), CD8 (negative in GBM, positive in WBC), CD48 (positive in GBM, negative in WBC), and CD63 or CD56 (positive in GBM, negative in WBC). Testing the ability of this panel to isolate GBM cells mixed with WBC, a subpopulation (CD8+/CD48+/CD56+/CD63+) was found only in the mix of cells and not in the WBC alone. The CD antibody panel was then used to test for CTCs in the peripheral blood of GBM patients. A distinct population of putative GBM cells was identified, representing <1% of the total number of cells. We have used this strategy to identify CTCs in three GBM patients. These cells were seen in six control samples. Currently, additional GBM CTCs are being characterized with cell culture and PCR and correlated clinically. CONCLUSION: GBM CTCs exist and can be isolated and characterized from the peripheral blood of GBM patients.

CB-22. BREAKING HYPOXIA ADAPTATION AND BLOCKING GLIOMA CELL GROWTH BY INHIBITING AMP KINASE ACTIVITY
Xiaona Liu, Sara Yacyshyn, and Biplab Dasgupta; Cincinnati Children’s Hospital Medical Center

INTRODUCTION: During the course of evolution of GBMs, glioma cells, particularly glioma initiating/stem cells (GICs) become adapted to hypoxic growth. These cells are resistant to ionizing radiation and chemotherapy, and the volume and intensity of hypoxia in GBM before radiotherapy are strongly associated with poorer time-to-progress and survival. Thus, there is a need to develop new treatment strategies to target the hypoxia addiction of glioma cells and particularly GICs. AMP-activated protein kinase (AMPK) is a cellular energy sensor that plays a critical role in angiogenesis, cell survival in response to hypoxic stress, genotoxic stress (DNA damaging/alkylating agents), as well as in response to radiation. There is tremendous conflict of inhibitors and activators of AMPK under normoxic and hypoxic growth. RESULTS: Our initial studies unequivocally demonstrated that (i) compared to normal astrocytes, glioma cells express significantly higher levels of active AMPK, (ii) two AMPK-activating agents have the opposite effects during the hypoxic growth of glioma cells, suggesting different mechanisms of action of these compounds, (iii) these agents have little effect on glioma cell motility, (iv) pharmacological or genetic inhibition of AMPK significantly blocks glioma cell growth, an effect that is compounded during hypoxia, and (v) pharmacological inhibition of AMPK completely blocks glioma cell motility. CONCLUSION: Our initial results demonstrate convincingly that blocking AMPK during hypoxic growth prevents glioma cell proliferation and migration, and inhibiting this energy-sensing pathway in combination with radiotherapy and chemotherapy could potentially block glioma growth and recurrence.

CB-23. EXPRESSION OF SRC FAMILY KINASES IN GLIOBLASTOMA STEM CELLS AND THEIR ROLE IN CELL PROLIFERATION AND MIGRATION
Xiaosi Han, Xuhuia Yang, Crystal G. Wheeler, Natalia Filippova, Catherine P. Langford, Qiang Deng, Hassan M. Fatallah, George Y. Gillespie, and Louis N. Bators; University of Alabama at Birmingham

Src family kinases (SKFs) are highly expressed and active in clinical glioblastoma multiforme specimens. SKFs inhibitors have been demonstrated to inhibit proliferation, migration, and invasion of primary glioma cells and are currently in clinical trials for the treatment of glioblastoma. However, the expression of SKFs and their functional role in glioma stem cells (GSC) is unclear. We examined the expression pattern of individual members of SKFs in several CD133+ glioma stem cells as well as their corresponding (CD133-) primary glioma cells isolated from the same human glioblastoma that grafts. We found that the SKFs were highly expressed and activated in glioma stem cells, and the expression pattern of individual kinase members may or may not be similar to that of their corresponding primary glioma cells. Members of SKFs expressed in GSC include Lyn, c-Src, c-Yes, Lck, and Lyn. The SFK inhibitor dasatinib had little effect on the growth of glioma stem cells, although it effectively inhibited the proliferation of primary glioma cells at a concentration comparable to that of clinical therapeutic serum levels. However, SFK inhibition by dasatinib significantly suppressed the migration of glioma stem cells in a laminin-coated Transwells assay. These results suggest that an SFK inhibitor alone is unlikely to completely control tumor growth, and combination with other medications will be needed for the effective inhibition of cancer stem cells. (This work was supported in part by NCI Grants 3P50CA013148-1S5, P50CA072747-03S5.

CB-24. INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 2 (IGFBP2) PROMOTES GROWTH OF MEDULLOBLASTOMA CELL LINES.
Tom B. Davidson, Filipp Gortalum, Lingyun Ji, Kelly Engell, Richard Sposto, Shahab Agharzadeh, and Anat Erdreich-Epstein; Children’s Hospital Los Angeles

The insulin-like growth factor (IGF) signaling pathway has an important role in proliferation in many tissues and a variety of tumors. The effects of IGF on tumorigenesis are partly modulated by the six secreted IGF-binding proteins (IGFBPs). Circulating IGFBPs can be inhibitory by reducing available free IGFs or may enhance the action of an IGF by increasing ligand presentation to its receptor. Additionally, in some cases, IGFBP2 is also thought to be IGF1R-independent mechanisms, although these mechanisms are mostly unknown. Of clinical relevance, IGFBP2 overexpression is observed in many neoplasms and is associated with a more malignant state in prostate cancer, breast cancer, colon cancer, leukemia, and gliomas. In this project, we are working to understand the function of IGFBP2 in medulloblastoma, the most common malignant brain tumor in children. Microarray analysis (U133 plus standard) revealed an 85.8-fold overexpression of IGFBP2 in 31 patient medulloblastoma tissue samples when compared to 13 normal cerebella samples and a mean overexpression of 16.5-fold in four medulloblastoma cell lines. Immunohistochemistry of 7/7 of these samples demonstrated high IGFBP2 expression (nuclear, membrane, and extracellular), while in normal cerebellum, IGFBP2 expression was limited to a small population of pericytes. Addition of human recombinant IGFBP2 (1–10 ng/mL, 72 hours) in the presence of 0.5% fetal bovine serum to two medulloblastoma cell lines significantly increased cell proliferation measured by MTT at 10–100% for CHLA-259 (n = 10 per experiment in two separate experiments with p < 0.001 in each) and by 35%–50% for D283MED (n = 10 per experiment in three separate experiments with p < 0.001). However, IGFBP2 did not affect phosphorylation of the IGF-1R and its downstream targets, AKT1 (ser473) and ERK, suggesting possible IGF-independent effects of
EGFRvIII have increased proliferation and invasive characteristics versus the wild-type (wt) EGFR. EGF signaling (EGFRvIII) is ligand-independent and does not involve receptor dimerization and as a result is low intensity, which allows EGFRvIII to evade the normal mechanisms of internalization and degradation by the endocytic machinery; hence, its signaling is persistent. The low-intensity signal has made it challenging to uncover whether there are components of EGFRvIII signaling that are distinct from wild-type EGFR signaling. We have created a chimeric cEGFRvIII that can be dimerized experimentally using a variant FKBP12 domain and contain a small molecule, a protein, or chemically induced dimers (CID). CID increases cEGFRvIII activity and phosphorylation of downstream targets to levels comparable to acutely EGF-stimulated EGFR. Interestingly, increased activity of cEGFRvIII did not promote receptor internalization, suggesting that the failure of EGFRvIII to enter endosomes is inherent in its structure. Mice expressing human U87 cells expressing cEGFRvIII intracranially died sooner when treated with CID, suggesting that forced dimerization enhanced the oncogenic signal.  Phosphoproteomic analysis of the enhanced EGFRvIII signal using mass spectrometry will be presented.

CB-28. ACCESS TO THE NUCLEUS IS REQUIRED FOR THE FULL ONCOGENIC POTENTIAL OF EGFRVIII
Anupama Gururaj and Oliver Bogler; The University of Texas MD Anderson Cancer Center

RATIONALE: A key molecular characteristic in a subset of glioblastoma is over-expression, amplification, and mutation of EGFR. The most common mutant is EGFRvIII, with a large intragenic deletion of the extracellular domain, leading to constitutive activation. GBMs expressing EGFRvIII have a more aggressive biological and clinical behavior. Nuclear translocation of EGFR has been reported in cancers of the breast, esophagus, bladder, and thyroid and is correlated with disease progression in these tumors. We hypothesize that nuclear EGFR and EGFRvIII occurs in GBMs, and we are interested in defining the role of nuclear EGFRvIII in oncogenic functions. APPROACH: Biochemistry and microscopy were used to characterize the occurrence of nuclear EGFRvIII. Site-directed mutants that restrict EGFR/vIII to either the nucleus or the cytoplasm were used in cell-based proteomic and xenograft studies to define nuclear functions of EGFRvIII. RESULTS: Preliminary studies have revealed that a fraction of EGFRvIII is consistently in the nucleus in glioma cell lines. Stable cell lines overexpressing EGFRvIII mutants that restrict EGFRvIII to either the nucleus (mutated nuclear export sequence) or the cytoplasm (mutated nuclear localization sequence) show that increasing EGFRvIII in the nucleus increases oncogenicity while cytoplasmic enrichment decreases its oncogenicity as measured by anchorage-independent growth and intracranial xenograft. In order to address the role of EGFR in the nucleus, we plan to carry out phopho-proteomic and mass spectrometric analyses in order to determine specific binding partners/substrates of nuclear EGFRvIII and to identify the phospho-sites of EGFRvIII that are involved in nuclear translocation. Other aims of the study is to look for correlations between nuclear EGFR and glioma progression, prognosis, resistance to treatment.

CB-29. PHOSPHOPROTEOMIC ANALYSIS OF NOVEL JAK/STAT INHIBITORS IN GLIOMA CELLS
Vaibhav Chumbalkar, Jaykumar Arumugam, Lixia Dao, Keith Baggerly, Waldemar Priebe, and Oliver Bogler; The University of Texas MD Anderson Cancer Center

INTRODUCTION: Signal transducer and activator of transcription (STAT) molecules are constitutively activated in many cancers, including gliomas. Targeting STATs is a promising therapeutic approach, and WP1066 and WP1193 are small molecule inhibitors of the JAK/STAT pathway that have shown potential in preclinical studies. Here we used phosphotyrosine-directed shotgun phosphoproteomics to profile the impact of WP1066 and WP1193 on glioma cell signaling. APPROACH: LN2308 glioma cells were treated with WP1066 and WP1193, followed by peptide extraction and enrichment of phosphorylation sites by immunoprecipitation using P-Tyr-100 antibody. These peptides were further enriched on titanium dioxide resin on a special nano-fluorides chip (Phosphochip, Agilent Technologies) and analyzed on an ETD-enabled ion trap mass spectrometer. After identifying the phosphopeptides with Spectrum Mill software from resultant spectra, these phosphopeptides were quantified based on their intensities. Pathway analysis was performed to integrate the findings. RESULTS: We identified and quantified 236 phosphopeptide from 99 proteins. We observed a significant change in intensity for 20 (for WP1066) and 16 (for WP1193) proteins. As expected, levels of STAT3 phosphopeptide

CB-27. FORCED DIMERIZATION AMPLIFIES EGFRVIII SIGNALING AND INCREASES ITS ONCOGENICITY
YeoHyeon Hwang, Vaibhav Chumbalkar, Khatri Latha, and Oliver Bogler; The University of Texas MD Anderson Cancer Center

Glioblastoma multiforme (GBM) is the most common and lethal primary human brain tumor. GBMs are characterized by a variety of genetic alterations, amongst which oncogenic factor (EGFRvIII/EGFR) are most common. GBMs harboring EGFRvIII have increased proliferation and invasive characteristics versus the wild-type (wt) EGFR. EGF signaling (EGFRvIII) is ligand-independent and does not involve receptor dimerization and as a result is low intensity, which allows EGFRvIII to evade the normal mechanisms of internalization and degradation by the endocytic machinery; hence, its signaling is persistent. The low-intensity signal has made it challenging to uncover whether there are components of EGFRvIII signaling that are distinct from wild-type EGFR signaling. We have created a chimeric cEGFRvIII that can be dimerized experimentally using a variant FKBP12 domain and contain a small molecule, a protein, or chemically induced dimers (CID). CID increases cEGFRvIII activity and phosphorylation of downstream targets to levels comparable to acutely EGF-stimulated EGFR. Interestingly, increased activity of cEGFRvIII did not promote receptor internalization, suggesting that the failure of EGFRvIII to enter endosomes is inherent in its structure. Mice expressing human U87 cells expressing cEGFRvIII intracranially died sooner when treated with CID, suggesting that forced dimerization enhanced the oncogenic signal.  Phosphoproteomic analysis of the enhanced EGFRvIII signal using mass spectrometry will be presented.

CB-25. INVESTIGATING THE ROLE OF NEUROTROPHIN SIGNALING IN BRAIN TUMOR STEM CELLS
Samuel O. Law, Sam Wess, Donna Senger, and Peter Forsythe; University of Calgary

Glioblastoma multiforme (GBM) is a highly invasive disease that is refractory to current treatments. The cancer stem cell hypothesis states that tumor growth is driven and maintained by a subset of self-renewing cells that are highly tumorigenic and capable of differentiation. Understanding the biology of these cells could aid the development of therapies designed to eliminate them, either through conventional inhibition of key growth and survival factors or through more novel approaches, such as the promotion of BTSC differentiation. Previous work by this lab has identified and characterized the role of the neurotrophin receptor p75NTR in promoting glioma cell invasion and shown that this function of p75NTR is sensitive to treatment with gamma-secretase inhibitors (GSI). It was also demonstrated that p75NTR is expressed in brain tumor stem cells (BTSCs). The role of p75NTR in the different and signaling of relevant neurotrophins and we are interested in defining the role of nuclear EGFRvIII in oncogenic functions. APPROACH: Biochemistry and microscopy were used to characterize the occurrence of nuclear EGFRvIII. Site-directed mutants that restrict EGFR/vIII to either the nucleus or the cytoplasm were used in cell-based proteomic and xenograft studies to define nuclear functions of EGFRvIII. RESULTS: Preliminary studies have revealed that a fraction of EGFRvIII is consistently in the nucleus in glioma cell lines. Stable cell lines overexpressing EGFRvIII mutants that restrict EGFRvIII to either the nucleus (mutated nuclear export sequence) or the cytoplasm (mutated nuclear localization sequence) show that increasing EGFRvIII in the nucleus increases oncogenicity while cytoplasmic enrichment decreases its oncogenicity as measured by anchorage-independent growth and intracranial xenograft. In order to address the role of EGFR in the nucleus, we plan to carry out phopho-proteomic and mass spectrometric analyses in order to determine specific binding partners/substrates of nuclear EGFRvIII and to identify the phospho-sites of EGFRvIII that are involved in nuclear translocation. Other aims of the study is to look for correlations between nuclear EGFR and glioma progression, prognosis, resistance to treatment.

CB-26. ANALYSIS OF PHOSPHOTYROSINE SIGNALING IN Glioblastoma Implicates Nuclear EGFRvIII STAT5b COMPLEX IN THE INDUCTION OF BCL-XL EXPRESSION
Khatri Latha, Vaibhav Chumbalkar, Ming Li, Anupama Gururaj, YeoHyeon Hwang, Nebecca Marwodi, Xiaoxi Sun, Keith Baggerly, Keith Baggerly, Raymond Sawaya, Kenneth Aldape, Webster Cavenee, Frank Furnari, and Oliver Bogler; The University of Texas MD Anderson Cancer Center, Ludwig Institute for Cancer Research

Ablerrant EGFR signaling is a major contributing force to glioma progression and treatment resistance. The most prevalent mutation, ∆E746/747EGFR/ EGFRvIII, is an inframe deletion of the extracellular domain, occurring in the about 40% of glioblastomas and promoting growth and survival of tumor cells. The signaling of ∆E746EGFR is ligand-independent, does not involve receptor dimerization, and is of low intensity. We have analyzed ∆E746EGFR signaling using shotgun phosphoproteomics based on recovery of phosphotyrosine-containing peptides and mass spectrometry. Two glioma cell lines expressing ∆E746EGFR and wild-type EGFR and with different PTEN backgrounds were compared by this approach, leading to the identification of 249 tyrosine phosphorylated proteins. Of these, 30 showed statistically significant differences in intensity when ∆E746EGFR was present, including the previously described Gab1 and c-Met, and a newly identified phosphorylated of STAT3 on Y699 in cells expressing ∆E746EGFR. In human glioblastoma samples, ∆pSTAT5 levels correlated positively with EGFR expression and were associated with reduced survival. Phosphorylated STAT5b and ∆E746EGFR associated in the nucleus, bound DNA, and were found on promoter regions known to be regulated by STAT5b, including that of the Aurora A gene, which they positively regulated. Interestingly, the activation of STAT5b downstream of ∆E746EGFR was dependent on Src, in contrast to the signal from EGFR stimulated EGFR to STAT5b, which showed the involvement of Jak2. ∆EGFR cooperated with STAT5b to positively regulate the Bcl-XL promoter, and knockdown of STAT5b suppressed anchorage-independent growth, reduced the levels of Bcl-XL, and sensitized glioblastoma cells to cisplatin. Taken together, these observations support the hypothesis that the nuclear association of ∆EGFR with STAT5b promotes oncogenesis and treatment resistance in glioblastoma via up-regulation of Bcl-XL.
CB-30. CLEAVAGE OF THE BRAIN-SPECIFIC PROTEIN BREVICAN RELEASES A FRAGMENT THAT PROMOTES EGF-DEPENDENT GLIOMA CELL MOTILITY

Hosung Sim, Colleen A. Pineda, Yang Pan, Bin Hu, and Mariano S.Viapiano; The Ohio State University

A fundamental challenge in treating malignant gliomas is their distinctive ability to infiltrate normal neural tissue, which makes them virtually impossible to eliminate by conventional therapies. Understanding the mechanisms of glioma invasion is essential to designing more effective treatments against tumor recurrence. Brevican, a predominant brain-specific proteoglycan, is a major component of the neural extracellular matrix (ECM) that restricts the mobility of normal neural cells. Surprisingly, brevican is upregulated in gliomas, is expressed in the invasive border of these tumors, and promotes glioma dispersion. Thus, our goal was to determine the molecular mechanisms underlying this uncommon role of brevican in gliomas. Glioma cells were exposed to truncated or mutated versions of brevican and analyzed for effects on adhesion, migration, and dispersion in organotypic cultures. Results were also exposed to brevican and truncated brevican to identify potential signaling pathways activated by the proteoglycan. In addition, a bioinhibitor “B3” sequence (13aa) was inserted at the N-terminus of brevican to create constructs that could be biotinylated and directly purified from the conditioned medium of engineered HEK293 cells. Analysis of receptor tyrosine kinase signaling showed that brevican was unable to enhance cell migration, but cleavage of this protein by ADAMTS proteases yielded an N-terminal fragment that activated EGR and Erk1/2, leading to fibronectin recruitment to the cell surface and increased cell adhesion and migration. Brevican effects on EGR activation and cell adhesion were mediated by Src kinases and therefore inhibited with a pan-Src kinase inhibitor, suggesting a transactivating effect of N-terminal brevican on EGR signaling. Together, these results suggest that the N-terminal fragment of brevican, which is highly increased in gliomas, acts as a ligand that promotes glioma cell motility. This is the first molecular approach revealing potential mechanisms underlying the conversion of inhibitory neural proteoglycans into pro-invasive signals in gliomas.

CB-31. IDENTIFYING MODIFIER GENES OF MPNSTS IN THE MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 1

Jessica A; Van Schaeck1, Keiko Akagi2, Sandra Burkett3, Christina DiFabio5, Robert Tuskan4, Jessica Walrath1, and Karlyne Reilly1; 1NCI, 2The Ohio State University

The current study aimed to identify modifier genes of malignant peripheral nerve sheath tumors (MPNSTs) in the Nup153cs (NPcis) mouse model of NF1. Previous studies have shown that the incidence of MPNST development in the NPcis mouse model is affected by the parental transmission of the mutant chromosome 11. In this study, microarray analysis was used to examine gene expression differences between MPNST primary tumors derived from NPcis mice varying in inheritance of the NPcis chromosome from the mother (NPcis maternal) or father (NPcis paternal). Grb10 was found to be more highly expressed in NPcis paternal MPNSTs, and qPCR was used to validate both gene expression differences. We chose to focus first on Grb10 due to its role as a cytoplasmic signaling adapter protein. Fluorescence in situ hybridization was used to examine the presence of Grb10 on chromosome 11. Grb10 was found to be lost more frequently in NPcis paternal MPNST cell lines, potentially contributing to the decrease in Grb10 gene expression seen in these tumors. Grb10 is paternally imprinted in the mouse; therefore, we examined Grb10 isoform expression and found paternal and maternal isoforms expressed in the MPNSTs. Due to these results, we are examining whether loss of imprinting is contributing to tumorigenesis. In order to study the function of Grb10 in vitro, more than 200 clones of NEURO-ONCOLOGY

CB-32. FOXM1 IS REGULATED BY HSF1 AND PROTECTS GLIOMA CELLS FROM HEAT SHOCK STRESS-INDUCED CELL DEATH

Bingbing Dai, Zhitao Jing, Shin-Hyuk Kang, Dawei Li, Keping Xie, and Suyun Huang; The University of Texas MD Anderson Cancer Center

The forkhead box M1 (FoxM1) is a key transcription factor regulating multiple cell cycle-related genes that control G1-S and G2-M phase progression. Our previous studies have shown that FoxM1 is over-expressed in human brain tumors and other solid tumors and is correlated with cancer progression and invasion. Knocking down FoxM1 inhibited cancer cell growth in vitro and brain tumor formation in vivo. However, how FoxM1 is regulated in either normal or malignant cells still is not clear. In this study, our results showed that FoxM1 was up-regulated by heat shock factor 1 (HSF1) during heat shock stress conditions. Knocking down HSF1 with HSF1 siRNA or inhibiting HSF1 with an HSF1 inhibitor abrogated heat shock-induced expression of FoxM1. Similarly, heat shock stress did not induce FoxM1 expression in mouse embryo fibroblast cells with Hsf1 knockout (MEF Hsf1−/−), and a pan-Src kinase inhibitor and a metalloproteinase inhibitor and a metalloproteinase inhibitor and a zinc protease reporter assay confirmed that HSF1 directly binds to the FoxM1 promoter. Furthermore, our results demonstrated that FoxM1 is required for the G2-M phase progression through regulating cyclinB1, CDC25B, and E2F-1. Down-regulation of FoxM1 by siRNA or over-expression of cyclinB1, CDC25B, or E2F-1 in the glioma cells restored the normal cell cycle progression during lethal heat shock stress. Finally, immunohistochemical analysis of human glioblastoma specimens also showed a significant correlation between FoxM1 overexpression and elevated HSF1 expression. Our results indicated that FoxM1 is critical for HSF1-mediated heat shock response, which increases cell survival and protects cells from stress-induced cell death.

CB-33. MITOCHONDRIAL LON IS THE FIRST IDENTIFIED MITOCHONDRIAL PROTEIN TO MEDIATE HYPOXIC ADAPTATION, INVASION, AND TREATMENT RESISTANCE TO RADIATION AND TEMOZOLOMIDE IN MALIGNANT GLIOMA CELL LINES

Xing Gong, Yen Vuong, and Daniela A. Bota; UC Irvine

BACKGROUND: Malignant gliomas are characterized by extensive hypoxic areas and innate resistance to treatment. Hypoxia-inducible factor HIF-1α is an indicator of malignant angiogenesis and abnormal proliferation, but less studied is HIF-1α involvement in the metabolic shift to glycolysis required for survival in low-oxygen environments. One of the HIF-1α regulated genes is the mitochondrial Lon, which plays an important role in mitochondrial bioenergetics and mitochondrial DNA maintenance. Here, we demonstrate that Lon controls metabolic adaptation to hypoxia in glioma cells in direct response to HIF-1α activation. Also, Lon induction is used by glioma cells to increase resistance to radiation and chemotherapy by direct repair of mitochondrial DNA. RESULTS: Two malignant glioma cell lines (D-54-MG and U-251-MG) were exposed to hypoxia, which increased HIF-1α protein levels. The increase of HIF-1α levels was paralleled by increased Lon expression and the protein Lon levels, while HIF-1α down-regulation was associated with a brisk decrease of Lon expression. Lon protease up-regulation in D54-MG cells caused increased invasion and resistance to starvation. Treatment with temozolomide (TMZ) led to a four-fold induction of Lon, while Lon down-regulation led to increased sensitivity to TMZ. TMZ-resistant lines D54-TR and U251-TR had twice the level of Lon of their parent TMZ-sensitive lines. Lon protease in the D54-MG cells induced resistance to TMZ at levels similar to that of the resistant line D54-TR. D54 cells with Lon over-expression also had increased resistance to radiation. Both the radiation and TMZ resistance in the D54 MG/Lon over-expression cells was caused by an increase in the repair of the treatment-induced mitochondrial DNA damage. CONCLUSIONS: The data presented show that Lon is one of the principal mediators connecting hypoxia with invasion, resistance to starvation, chemoresistance, and radiation-resistance, supporting our current research of Lon inhibition as a possible therapeutic target in malignant gliomas.

CB-34. THE GLIOMA ONCOPROTEIN BCL2L12 INHIBITS THE P53 TUMOR SUPPRESSOR

Alexander H. Stegh; Northwestern University

Glioblastoma multiforme (GBM) is a highly lethal and neurologically debilitating brain tumor characterized by intense apoptosis resistance and...
We have demonstrated that atypical protein kinase C (aPKC) provides an instructive signal for apical-basal polarity in NSC during embryonic development of the chick CNS. aPKC is compartmentalized at the apical membrane of neural progenitors, and abrogation of the endogenous aPKC kinase results in a loss of apical cell adhesion junctions, increased proliferation of neural progenitors, and abnormal migration of these cells within different cellular layers. Can aPKC and apical-basal signaling pathway be also manipulated in glioblastoma? In this study, we analyzed aPKC staining in a large cohort of glioblastoma multiforme (GBM) samples. aPKC staining is significantly enhanced in clinical samples of GBM. aPKC functions downstream of the oncogenic EGF-Ras-Pi3-K cascade in glioma cells. The knockdown of aPKC in GBM-derived cell lines decreases cell migration. Our studies suggest that aPKC, a protein kinase and thus a highly attractive target for rational drug design, may be a novel therapeutic target in GBM.

CB-37. EGFR AS A PREDICTOR OF RELAPSE AND A POTENTIAL THERAPEUTIC TARGET IN RECURRENT DISSEMINATED MYXOPAPILLARY EPENDYOMA
Anupam Verma1, Holly Zhou2, Steven Chin1, Carol Bruggers1, John Kestle1, and Soumen Chatterjee1; 1Primary Children’s Medical Center; 2University of Utah

INTRODUCTION: Myxopapillary ependymomas (MEPN) are a rare subset of ependymomas with a high frequency of recurrence. Identification of clinical factors predictive of outcome would be of great clinical importance. The epidermal growth factor receptor (EGFR) is a powerful oncogenic event in GBM, yet surprisingly, its presence has not been well studied in MEPN. The purpose of this study was to determine if EGFR expression is predictive of outcome in MEPN.

METHODS: EGFR expression was studied in 4 patients with recurrent MEPN. Immunohistochemistry was performed on formalin fixed, paraffin embedded tumor tissue using an antibody against EGFR. The presence of 5+ EGFR staining was used to define the presence of EGFR.

RESULTS: EGFR expression was found to be a likely predictor of relapse.

CONCLUSION: This small case series demonstrates for the first time that EGFR expression by IHC could be a potential biological marker of recurrence and may play an important role in tumorigenesis in MEPN. A large prospective study is warranted to determine whether EGFR protein expression at diagnosis in MEPN is predictive of recurrence, which would help in profiling an optimal follow-up pattern. This study also shows that EGFR could be a potential therapeutic target in these tumors when they relapse, as no standard therapy exists beyond surgery.

CB-38. REVERSAL OF EFFECT OF U87 DERIVED MICRO-VELOCES ON BIOLOGICAL PROCESSES OF GLIOBLASTOMA MULTIFORME
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BACKGROUND: Micro-vesicles, also known as exosomes, are extracellular, membrane-bound vesicles derived from the intraluminal membranes of multiviscerious bodies (MVBs) of the endocytic pathway. These MVBs fuse with the plasma membrane, which causes the release of vesicles into the extracellular environment. Various cell types, including tumor cells, have been shown to produce micro-vesicles, which are believed to play a role in signal transduction. Recently, glioblastoma-derived micro-vesicles have been shown to contain proteins and RNA. Since micro-vesicles from different tumor cells appear to be involved in different biological processes, we set out to investigate the influence of U87 micro-vesicles on multiple biological processes.

METHODS: First, we isolated (for the first time) micro-vesicles from U87 cells by step-wise centrifugation. Next, we assessed the influence of these vesicles on proliferation, angiogenesis, and migration by WST-1 assay, endothelial cell tubule formation on matrigel, and invasion assays, respectively. After this, U87 cells were treated with interferon beta, and their micro-vesicles were isolated and used to assess differential effects on the biological processes. The changes in protein and RNA content were assessed.

RESULTS: Microvesicles derived from untreated but not from treated U87

CB-36. ALTERED aPKC SIGNALING CONTRIBUTES TO GLIOBLASTOMA CELL MIGRATION
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Asymmetry along an apical-basal axis or apical-basal polarity is essential for a number of biological processes, including asymmetric segregation of cell fate and differentiation, regulation of cell-cell adhesion, and vectorial cell migration. These processes are fundamental for organogenesis and embryonic development of the central nervous system and establishment of apical-basal polarity. aPKC, a protein kinase, is compartmentalized at the apical membrane of neural progenitors, and abrogation of the endogenous aPKC kinase results in a loss of apical cell adhesion junctions, increased proliferation of neural progenitors, and abnormal migration of these cells within different cellular layers. Can aPKC and apical-basal signaling pathway be also manipulated in glioblastoma? Our studies suggest that aPKC, a protein kinase and thus a highly attractive target for rational drug design, may be a novel therapeutic target in GBM.
cells stimulate growth, angiogenesis, and migration of glioblastoma cells. The contents of micro-vesicles derived from treated and untreated U87 cells show differences, which explain the observed effects. CONCLUSION: U87 micro-vesicles influence the behavior of glioblastoma cells. This can be reversed by treating the cells with interferon beta.

CB-39. STUDYING THE ROLE OF EXOSOMES IN GliOBLASTOMA TUMOR BIOLOGY
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The highly invasive nature of malignant brain tumors (gliomas) precludes the complete surgical resection of these tumors and contributes to the poor prognosis of glioma patients. Exosomes are excreted micro-vesicles, 50-90 nm, which form from the fusion of multivesicular bodies (MVB) with the plasma membrane. A multitude of studies suggests a biological role of exosomes in cell-to-cell communication, contributing, for example, to antigen presentation to T cells. Evidence for a role of exosomes in tumor biology has been obtained as well. Recent studies have highlighted that tumor-derived exosomes carry mRNA, miRNA, and angiogenic proteins. Glioblastoma tumor-derived exosomes can deliver their contents to other cells and possibly be one of the pathways by which tumor cells manipulate their microenvironment (e.g., GBM exosomes have angiogenic properties). The main objective of our project is to investigate the effects of down-modulating exosome production on the biology of brain tumors, including through studies of angiogenesis, cell growth, and cell migration. One of our strategies encompasses the down-regulation of different genes that were previously shown to be involved in the exosomal release pathway. To this end, we use viral vectors to deliver short hairpin RNA to the tumor cells.

CB-40. THE ROLE OF WILM’S TUMOR-1 (WT-1) IN GLIOMA BIOLOGY
Archana Chidambaram, Catherine I. Dumur, Martin Graf, Timothy E. Vannmeter, Helen L. Fillimore, and William C. Broadus; Virginia Commonwealth University

INTRODUCTION: The zinc-finger transcription factor, WT-1, is known to play a vital role in the development of several organ systems and is down-regulated in most adult tissues. However, WT-1 has also been found to be re-expressed in malignancies arising from different tissue types. Our lab has previously demonstrated that this protein is aberrantly expressed in glioma cells and plays an important role in tumor cell proliferation in vitro and in vivo and in conferring radio- and chemo-resistance to the tumor cells. This study aimed to determine whether the tumor cells expressing WT-1 are driven by HK2 isoform. The CD-133+ positive subpopulation and to elucidate the mechanisms by which the tumorigenic actions of this protein may be wrought. METHODS AND RESULTS: We used flow cytometry to determine whether WT-1 and CD-133 are co-expressed by the same cell subpopulation. The gene expression profiling technique was used to identify the target genes for WT-1 in U251-MG cells. Preliminary results suggest that WT-1 might regulate genes involved in different aspects of tumorigenesis especially cell proliferation (PDGF-A, ICK), angiogenesis (CD97, EPAS-1), invasive ness (PDGE-D), and different phases of the cell cycle (TYMS, LYZ, A1). Using real-time RT-PCR, we validated these findings and noted similar regulation of some of these genes in U1242-MG cells. Transient knockdown of WT-1 in these cells resulted in an alteration of the malignant phenotype of the U1242-MG cells. CONCLUSION: Our results clearly implicate WT-1 as a key player in glioma pathogenesis. Future studies shall seek to delineate the role(s) of the target genes established herein so as to provide important insights for the development of a multi-molecular targeting strategy against these aggressive tumors.

CB-41. AN ANALYSIS OF THE ROLE OF MICRORNAs IN THE PHENOTYPIC EXPRESSION OF ONCOGENIC PDGF SIGNALING IN MALIGNANT GLIOMA
Joachim Silber, Tatayna Dracheva, Edward Kastenhuber, Hakim Djaballah, Eric C. Holland, and Jason T. Huse; Memorial Sloan-Kettering Cancer Center

Malignant gliomas continue to cause a disproportionate degree of morbidity and mortality among cancer patients while demonstrating sobering resistance to conventional therapies. Recent comprehensive genomic analyses have emphasized the importance of receptor tyrosine kinases (RTKs) and their downstream signaling cascades in the process of gliomagenesis. As such, these, the platelet-derived growth factor (PDGF) pathway appear to play a crucial role in the initiation and maintenance of both low- and high-grade diffuse gliomas. An improved understanding of how PDGF signaling mediates its oncogenic effects and the mechanisms for its regulation would be beneficial to the development of effective therapies. MicroRNAs (miRNAs) are a class of small, noncoding RNAs that regulate gene expression on a pre-translational level by binding loosely complimentary sequences in target mRNAs. Each miRNA likely represses numerous mRNAs targets, and this promiscuity speaks to the ability of individual miRNAs to mediate complex biological phenotypes. We have recently begun an analysis of miRNA involvement in the phenotypic expression and regulation of oncogenic PDGF signaling. We have identified a group of miRNAs whose expression levels are responsive to PDGF pathway activation in vitro and have recapitulated these findings in human glioblastomas, particularly those driven by aberrant PDGF signaling. We are now evaluating the functional properties of these miRNAs in a variety of in vitro and in vivo systems and investigating the miRNA targeting profiles of each using a combination of expression arrays and bioinformatics. We are also performing high-throughput screens to identify miRNAs that directly repress PDGF signaling and its downstream pathways. Through our studies, we hope to identify miRNA-based regulatory events impacting PDGF-mediated oncogenesis that may be amenable to therapeutic intervention.

CB-42. DEVELOPMENTAL PROFILE AND REGULATION OF THE GLYCOLYTIC ENZYME HEXOKINASE 2 IN NORMAL BRAIN AND GliOBLASTOMA MULTIFORME
Amparo Wolf, Sameer Agnihotri, Diana Munoz, Cynthia Hawkins, and Abhijit Guha; University of Toronto

INTRODUCTION: Proliferating embryonic and tumor cells switch to aerobic glycolysis, whereby glucose is metabolized rather than undergoing oxidative phosphorylation (OXPHOS), even in the presence of oxygen. This metabolic switch provides a survival advantage and facilitates the synthesis of biosynthetic precursors required for continued cellular proliferation. An example of this switch is our demonstration that in malignant gliomas there is over-expression of the glycolytic enzyme Hexokinase 2 (HK2) resulting in enhanced aerobic glycolysis. In contrast, normal brain preferentially expresses HK1 and undergoes OXPHOS. In this study, we examined whether this switch in HK isoform also occurs in the developing embryo and central nervous system (CNS). METHODS/RESULTS: Bioinformatic analysis of available microarray data demonstrated higher expression of glycolytic genes including HK2, but not HK1, in the blastocyst stage, previously reported to favor aerobic glycolysis, compared to the 1-, 2-, 4-, 8-cell stages. Quantitative RT-PCR on mouse brains isolated at E8.0, E10.5, E15.5, postnatal day 1, day 20, and 2 months, demonstrated that HK2 expression was highest at early embryonic developmental time-points, while HK1 expression increased with CNS maturation and relative quiescence. The HK2 expression profile mimicked that of HK2. These profiles suggest that the regulation of HK2 was secondary to epigenetic methylation of the HK2 promoter. Adult normal human brain and the few human GBM cell lines with decreased HK2 expression showed greater methylation of CpG islands within intron 1 of the HK2 promoter. In contrast, the HK2 promoter of the developing human fetal brain and the vast majority of HK2-expressing human GBM cell lines were not methylated. Furthermore, 5-azacytidine treatment of GBM cells lacking HK2 restored HK2 transcript expression. CONCLUSIONS: Overall, our results demonstrate that switch to the HK2 isoform is associated with proliferating states, such as the developing brain in embryos or as in malignant gliomas.

CB-43. STAT3 INHIBITION ALTERS THE IMMUNE PROFILE IN MURINE GliOMAS
James E. Han, Emilia Albesiano, Gustavo Pradilla, and Michael Lim; Johns Hopkins University School of Medicine

Signal transducer and activator of transcription 3 (STAT3) has shown to be constituently activated in a broad array of human and murine tumors. The role of STAT3 in cellular proliferation, angiogenesis, apoptosis, and migration has been well documented. However, STAT3 activation also induces pro-carcinogenic anti-inflammatory microenvironment that antagonizes innate and adaptive antitumor immune responses. We first detected in vitro STAT3 activation on murine glioma cell lines (GL261 and GL26) and in vivo STAT3 activation on paraffin-embedded sections of glioblastoma regions of C57/BL6 mice implanted with these cell lines. We then electroporated GL261 and GL26 to transiently transfect with RNA
anti-STAT3, resulting in substantial down-regulation of STAT3 by 80%–90% in both cell lines at the mRNA and protein level. In addition, we analyzed the effects of STAT3 suppression by measuring transcription of STAT target genes (e.g., PDGFR-alpha). Down-regulation of STAT3 resulted in decreased levels of IL-6, constant levels of RANTES, and varying levels of IL-10 in both cell lines. These findings are similar to those in murine melanoma (B16) and colon carcinoma (CT26) models. However, subtle differences do exist, revealing that different types of cancers may respond in an undetermined manner. These results demonstrate the important role that STAT3 activation plays in suppressing the release of inflammatory chemokines and cytokines in a glioma murine model. STAT3 inhibition is a potential target for glioma therapy, as multiple upstream cytoklastic signaling pathways converge upon it. Future in vivo studies will be conducted to determine whether STAT3 inhibition can reverse tumor-promoting inflammation and generate a potent antitumor response.

**CB-44. A CONTINUUM OF MGMT PROMOTER METHYLATION AND MGMT ACTIVITY**

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**INTRODUCTION:** Epigenetic methylation of the MGMT promoter in glioblastoma is associated with improved survival in patients treated with concomitant temozolomide and radiotherapy followed by adjuvant temozolomide. The current assessment of the MGMT promoter methylation status is binary. Malignant brain tumors are very heterogeneous. We hypothesized that the existence of a continuum of MGMT promoter methylation in the tumor rather than a binary function would provide a more accurate assessment of MGMT expression and activity. **METHODS:** To assess this hypothesis, quantitative in vitro cell mixing experiments were carried out using U87 (methylated MGMT promoter) and D529R (unmethylated MGMT promoter) cell lines. Pyrosequencing and methylation-specific PCR (MSP) evaluated MGMT promoter methylation status both as a binary function and as a methylation percentage value. MGMT RNA expression, MGMT protein expression, and MGMT activity were also evaluated in each mixed population. Excised normal gray and white matter and brain tumor tissues (methylated and unmethylated MGMT promoter) were mixed and similar assessments carried out. **RESULTS:** MGMT activity was associated with protein expression and mRNA levels in the in vitro mixing experiments. MGMT promoter methylation was proportional to MGMT activity only when assessed as a methylation percentage value rather than a binary function. In the brain tumor mixing experiments, similar results were observed, suggesting the evaluation of the MGMT promoter methylation status calculated as a methylation percentage value appears to give a more accurate assessment of MGMT activity in the brain tumor.

**CONCLUSION:** The evaluation of the MGMT promoter methylation as a percentage value rather than a binary function resulted in an accurate prediction of MGMT expression and activity assessed in these in vitro studies.

**CB-45. DRR—POSSIBLE REGULATOR OF GLIOMA INVASION**

Gina Trinh, Phuong Le, and Kevin Petreca; Montreal Neurological Institute

Malignant gliomas are highly invasive and thereby evade complete surgical resection. Using a novel screening assay, we have uncovered a potential regulator of invasion: down-regulated in renal cell carcinoma (DRR). DRR is expressed in all invasive components of brain tumors but not in normal brain glia. DRR overexpression causes increased migration compared to control malignant glioma cells (MGCs). Conversely, knocking down DRR in MGCs abolishes invasion. The process of cellular invasion requires remodeling of the cytoskeleton, particularly proteins involved in actin stress fibres and focal adhesion formation. The small family of Rho GTPases (Rho, Rac, and Cdc42) is the primary regulator of cytoskeletal dynamics. DRR expression induces morphological changes, such as cell elongation and robust actin stress fibres—a typical Rho phenotype. Complementarily, our down-regulated DRR cells show virtually no stress fibres, with a peripheral distribution of focal adhesions—a typical Rac phenotype. Therefore, we propose that dysregulated DRR may facilitate cell invasion via Rac-mediated, Rho/Rac signalling. In addition to MGCs, glioblastoma primary cultures will also be used to test DRR as potential target for therapy. We hope to elucidate the mechanisms by which DRR mediates cell invasion and thus expose a possible strategy to inhibit glioma invasion.

**CB-46. EVOLUTION OF GLIOBLASTOMA IS DETERMINED BY THE INITIATING GENETIC HITS**

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**INTRODUCTION:** The genetic diversity of glioblastoma (GBM) constitutes a major obstacle to effective therapies. We hypothesized that defining the relationship between the initiating genetic hits and end-stage genotype will provide a means to understand and predict tumor evolution. **METHODS:** Mouse gliomas were induced by injecting PDGF-FRS-Cre retrovirus (RV) into transgenic mice with floxed PTEN and p53 genes and from cells isolated from these tumors (*PTEN/p53 and *PTEN/p53). DNA from RV-induced tumors (n = 24) was analyzed by comparative genomic hybridization (CGH) and sequencing. Results were compared to the genotype of human GBM from the TCGA database. **RESULTS:** For the RV-induced tumors, *PTEN/p53 had a significantly shorter survival than *PTEN tumors; however, transplantation of cells from end-stage *PTEN and *PTEN/p53 led to a similar survival, suggesting that *PTEN evolved to become as aggressive as *PTEN/p53. Numerous genetic deletions were consistently seen in the *PTEN gliomas (75%–100%) but were rarely seen in *PTEN/p53 tumors (0%–12.5%), suggesting that deletion of p53 obviated the selective advantage of these additional deletions. Furthermore, 40% of *PTEN developed non-silent mutations within the DNA binding domain of p53. With TCGA data in common, one TCGA glioma (75%–100%) recapitulated those seen in the pro-neural subtype of human GBM. Moreover, deletion of some of these genes is associated with short patient survival. **CONCLUSIONS:** These results demonstrate that the evolution of GBM is remarkably predictable and dependent on the initiating genetic hits. Tumor evolution in this model resembles that of the pro-neural subtype of human GBM. Both cases are characterized by PDGF signaling, p53 mutations, and a specific set of common gliomas that we identified by CGH. Mouse-to-human comparisons revealed that these deletions are clinically relevant. These findings constitute an important step toward predicting the evolution of GBM and developing personalized-based therapies.

**CB-47. SULF2, A HEPARAN SULFATE SULFATASE, REGULATES CRITICAL CELL SIGNALING PATHWAYS IN Glioblastoma GROWTH**

Joanna J. Phillips, Emmanuelle Huillard, Mei-Yin Polley, Steven D. Rosen, David H. Rowitch, and Zena Werb; UCSD

Glioblastoma (GBM) is characterized by abnormal activation of receptor tyrosine kinase (RTK) signaling pathways. As GBMs diffuse invasively, growth factor availability in the tumor microenvironment could be a critical determinant of RTK signaling pathway activity. Heparan sulfate proteoglycans (HSPGs), a major component of the brain extracellular matrix, regulate the extracellular activity of diverse growth factors. As the sulfation pattern of the HS chains is a major determinant of this activity, we hypothesize that HSPGs enzymatically modify HSPGs to promote growth factor signaling. We demonstrate that the extracellular sulfatase SULF2, which acts on HSPGs, is expressed in 50% of primary human GBM and in an orthotopic murine model for high-grade glioma. Knockdown of SULF2 in GBM cells resulted in decreased growth in vitro and in vivo, and revealed a striking SULF2 dependence in the activity of multiple RTKs, including PDGFR-alpha, a major signaling pathway in glioma. Furthermore, tumors generated from Sulf2+/- neural progenitor cells were smaller with decreased proliferation, were associated with prolonged survival, and had decreased PDGFR-alpha phosphorylation and decreased downstream MAPK signaling pathway activity. Interestingly, we show that GBM cell migration and invasion are dramatically different SULF2 expressions. These data support a key role for SULF2 in an important subset of GBM and identify a potential upstream therapeutic target regulating RTK signaling in GBM.
Brain tumors can arise following deregulation of signaling pathways normally activated during brain development. Sonic hedgehog (SHH)-Gli1 is one such important pathway whose role has been well established in medulloblastomas; however, the few reports in gliomas have yielded conflicting results. Further, since the SHH-Gli1 pathway plays a critical role in non-neoplastic stem cells, its role in stem-like neoplastic cells needs further evaluation. Hence, in this study, we evaluated 102 gliomas for SHH-Gli pathway activity and correlated with histological type and grade, genetic alterations, and expression of stemness markers (NANOG, OCT4, SOX2). The study was performed on 44 grade IV (GBM), 23 grade III (10 AA, 9 AO, 4 AOA), and 35 grade II (20 DA, 9 O, 6 OA) tumors. World Health Organization classification was done and genetic subsets defined based on TP53 mutation, EGFR amplification, and 1p/19q LOH. Real-time polymerase chain reaction was performed for expression of Gli1, SHH, PTCH, NANOG, OCT4, and SOX2. Western blot was done for SHH and Gli1 protein expression. There was inverse correlation of high Gli expression (>1.5) with histological grade (11% showed >22% grade III, 16% grade IV). Two-thirds of GBMs with high Gli showed EGFR amplification versus 25% with low Gli. High Gli1 was associated with expression of SHH and PTCH in 97% and 81% cases, respectively. A significant correlation of high Gli with stemness markers was also noted. Thus, 93% of gliomas with high Gli1 had expression of stemness markers versus 38% with low Gli. This study confirms the presence of an active ligand-driven SHH-Gli pathway in a subset of gliomas of all types with inverse correlation to grade. The positive correlation of Gli1 with stemness markers possibly indicates that more differentiated progeny of tumor cells may be reverting to a “stem-like status” by activation of this pathway.

Co-expression of EGFRvIII and PTEN in a small subset of recurrent glioblastoma tumors has shown sensitivity to tyrosine kinase inhibitors (TKIs). However, the exact mechanism of EGFRvIII and PTEN interaction in response to TKIs is still unresolved. Our recent work has shown that SHP-2 PT-Pase is required for EGFRvIII-mediated transformation. This work was aimed at investigating the molecular interaction between the EGFRvIII/SHP-2 activation complex and PTEN inhibition by Tarceva treatment. We show that Tarceva treatment abolished EGFRvIII, Gab1, SHP-2, and Erk1/2 phosphorylation in LN229.EGFRvIII cells at all time intervals. On the contrary, phosphorylation of EGFRvIII and Erk1/2 in U87MG.EGFRvIII cells was inhibited at early time points but was restored within 2 to 6 hours. Interestingly, phosphorylation of Akt, Gab1, and SHP-2 (Try580) was unaffected in U87MG.EGFRvIII cells, but EGFR and SHP-2 (Try542) phosphorylation was inhibited in a time-dependent manner. MTT proliferation and soft agar transformation assays demonstrated that U87MG.EGFRvIII cells were resistant to Tarceva treatment when compared to LN229.EGFRvIII cells. Immunofluorescent labeling of U87MG.EGFRvIII cells with an antiphospho-SHP-2 (Try542) antibody showed perinuclear localization of SHP-2, whereas LN229.EGFRvIII cells exhibited membrane staining of phosphorylated SHP-2. Interestingly, stable expression of PTEN in U87MG.EGFRvIII cells conferred relocation of phospho-SHP-2 (Try542) to the membrane. Notably, U87MG.EGFRvIII/PTEN clones showed a partially uniqued phenotypic feature. Furthermore, phosphorylation of SHP-2 (Try580) and Erk1/2 was totally abolished in U87MG.EGFRvIII/PTEN subclones. Our data indicate that the expression of PTEN in U87MG.EGFRvIII cells conferred a phenotype similar to LN229.EGFRvIII cells. Collectively, these observations allow us to infer that SHP-2 is a downstream effector of PTEN and that PTEN deficiency may lead to SHP-2 activation and nuclear localization by EGFRvIII, which may result in increased resistance to TKIs. Future studies will be aimed at further understanding the role of SHP-2 activation and translocation in glioblastoma response to TKI treatment.
effort to better understand therapeutic resistance, we have explored high-dimensional profiling studies to understand mechanisms of therapeutic resistance and to identify novel therapeutic targets. Temozolomide (TMZ) is an alkylating chemotherapy agent that is used to treat all glioblastomas. Recurrent glioblastomas, however, become resistant to TMZ through a mechanism associated with inactivation of MSH6, a member of the mismatch repair gene family; MSH6-deficient glioblastomas grow more rapidly than TMZ-sensitive tumors. To explore this phenomenon, we have designed an unbiased drug resistance screen to create TMZ-resistant human glioblastoma cell lines using combinational zinc-finger transcription factors (ZTFs). The combinational ZTF library enables activation of a gene or a set of genes that will be selected for resistance to TMZ. We have isolated resistant clones in the TMZ-sensitive A172 cell line and present a molecular profile analysis of these clones. By comparative gene expression profile analysis of novel ZTF-induced, TMZ-resistant glioblastoma cell lines, we expect to identify molecular pathways that confer TMZ resistance. In doing so, we also aim to identify possible biomarkers for TMZ resistance in recurrent glioblastomas.

CB-53. DRR PROMOTES CELL INVASION BY REGULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION
Alex Dudley; McGill University

Glia1al invasion is a defining feature of malignant gliomas and is the leading cause of treatment failure, yet the molecular mechanisms that regulate this process have largely been understood. Using a functional genetic screen assay, we identified down-regulated in renal cell carcinoma (DRR) as a novel regulator of cell invasion. DRR is up-regulated in the invasive component of all gliomas and is not expressed in normal glial tissue. The epidermal growth factor receptor (EGFR) is over-expressed in high-grade gliomas and is well established as promoting glial cell invasion. Our aim was to investigate whether a link exists between DRR and EGFR to promote cell invasion. Therefore, we assessed EGFR expression and downstream signaling upon DRR expression. DRR up-regulation led to elevated EGFR mRNA and protein expression. There was concurrent increased phospho-EGFR and phospho-Akt with DRR over-expression. Our data suggests a relationship between DRR and EGFR, but the specifics of this relationship remain undetermined. Nevertheless, this may underlie a novel mechanism by which tumor cells invade the surrounding brain. Further studies to investigate the role of DRR in the EGFR pathway might provide therapeutic options to suppress GBM invasion.

CB-54. BENEFICIAL GROWTH EFFECTS OF ACYL-COA SYNTHETASE V3 KNOCKDOWN IN HUMAN GLOBLASTOMA CELLS ARE NOT MEDIATED BY INCREASED AUTOPHAGY OR APOPTOSIS
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Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor and is associated with a poor prognosis. Lipids are essential for tumor membrane synthesis and are also key oncogenic signaling molecules. Acetyl-CoA Synthetase VL3 (ACSVL3), which activates long-chain fatty acids and promotes lipid synthesis, is highly expressed in GBM tumors and cell lines but is not detectable in normal glia. We previously reported that ACSVL3 plays a crucial role in supporting U87 GBM cell proliferation and tumorigenesis; ACSVL3 knockdown in U87 cells decreased anchorage-dependent and -independent growth in culture, mitochondrial activity, DNA synthesis, and growth of both subcutaneous and intracranial xenografts. We also showed that these beneficial effects of ACSVL3 knockdown are mediated, in part, by disruption of Akt signaling pathways. We therefore asked whether the effects of ACSVL3 knockdown on glioblastoma cell growth were mediated by promotion of either apoptosis or autophagy. Using several criteria (annexin-V labeling; caspase activation), we found no evidence of increased apoptosis in ACSVL3-deficient U87 cells. Similarly, several indices of autophagy (acidic orange staining; LC3-GFP expression) failed to show increased autophagy in U87 knockdown cells. Based on these observations, we propose that the beneficial effects of ACSVL3 depletion on U87 cells and xenograft proliferation have as their basis alterations in cellular metabolism and/or signaling pathways. (Supported by NIH grant NS062043.)

CB-55. BIOLOGICAL BASIS FOR MULTITARGETED THERAPY IN MENINGIOMA PATIENTS
Christian Mawrin; University of Magdeburg

Meningiomas are frequent intracranial or intraspinal tumors that result in significant morbidity and/or mortality owing to tumor recurrence, critical tumor locations, or malignant growth, as seen in atypical and anaplastic meningiomas and radiotherapy-resistant meningiomas. Besides neurosurgery and radiosurgery, therapeutic treatment options are limited. We have started to explore the value of substances covered under the group of multitargeted therapies for the treatment of human meningiomas. By analyzing human tissue samples as well as various meningioma cell culture models, we found that meningiomas are characterized by a clear activation of the mTOR signaling pathway. This pathway could be effectively antagonized by specific mTOR inhibitors, which are already used in clinical oncology. Another target was identified using inhibitors targeting platelet-derived growth factor (PDGF) receptors. Here we not only found a clear antiproliferative effect of specific substances inhibiting this important signaling pathway but also delineated that FLT-3, a receptor tyrosine kinase not described in human brain tumors so far, could be down-regulated in meningioma cells. Additionally, we could see a clear antimigratory aspect, which is especially important for the treatment of invasive meningiomas. Finally, by using a specific alpha-v-beta-3 inhibitor, we could show that without cytotoxic or antiproliferative effects, this substance had a clear antimigratory effect. Taken together, our data show that different new chemotherapy options might be of value for the treatment of aggressive meningiomas.

CB-56. DELETION OF THE SPARC ACIDIC DOMAIN OR EGF-LIKE MODULE REDUCES SPARC-INDUCED MIGRATION IN GLIOMA CELLS
Sandra A. Rempel and Heather M. McClung; Henry Ford Hospital/Wayne State University

Secreted protein acidic and rich in cysteine (SPARC) is up-regulated in all astrocytoma grades. We showed that SPARC increases glioma invasion while suppressing tumor growth. It is thought that different domains within the protein may regulate these functions, suggesting domain-specific targeting to inhibit invasion. The present goal was to determine whether the N-terminal acidic domain or the EGF-like module within the follistatin-like domain are involved in SPARC-induced migration through the previously reported p38/HS27 signaling pathway. Deletion constructs were created using site-directed mutagenesis of a SPARC-green fluorescent protein (GFP) plasmid. Stable U87-transfected clones expressing equal levels of GFP, wild-type SPARC-GFP, or either of the mutants were selected. Intracellular localization was determined by fluorescence imaging. Levels of construct expression and secretion and signaling events were characterized by Western blot analyses. Migration was examined on fibronectin using a wound assay and without matrix by Transwell assay. The results demonstrate that unlike control GFP, wild-type SPARC and mutant clones are perineural and are secreted. Migration was significantly increased in wild-type SPARC-GFP-expressing cells over control GFP-expressing cells (p < 0.001) in both migration assays. Deletion of either the acidic domain or the EGF-like module significantly reduced migration versus wild-type SPARC-GFP cells (p < 0.033) in both assays. However, in the wound assay, the deletion mutants migrated significantly more than GFP control cells (p < 0.001). Western blot analysis showed that SPARC increased expression and phosphorylation of HSP27 and increased p38 activation over control cells. The acidic domain deletion mutant had an intermediate level of HSP27 expression and phosphorylation; however, deletion of the EGF-like module caused a dramatic decrease in HSP27 expression and phosphorylation. In conclusion, both regions of interest regulated migration though p38/HSP27 signaling. Importantly, their impact on migration was influenced by the microenvironment, and selective inhibition of SPARC-mediated migration/invasion would likely require multi-domain targeting.

CB-58. THE JAK2 INHIBITOR AZD1480 EFFECTIVELY BLOCKS JAK2/STAT-3 SIGNALING IN GLOBLASTOMA TUMOR CELLS AND HUMAN XENOGRAFT GLIOBLASTOMA TUMORS
Beaudean C. McFarland1; Susan E. Nozell1, Dennis Huszar2, and Ety N. Benveniste1; 1University of Alabama at Birmingham; 2Cancer Bioscience, AstraZeneca R&D Boston

The JAK/STAT pathway is an important signaling pathway that has been implicated in glioma progression. We have previously shown that STAT-3 is
Anti-miRNA-125b (AM-125b) added exogenously to IL-6-stressed NHA lattter a treatment known to cause the proliferation of astroglial cells. An interleukin-6 (IL-6)-stressed normal human astroglial (NHA) cells, the miRNA-125b are up-regulated in cultured human glioma cells and in Department of Neurosurgery, New Orleans iv20 NEURO-ONCOLOGY CB-59. THE PRO-CELL DEATH BCL-2 FAMILY MEMBER BNIP3 PROMOTES TUMOR CELL SURVIVAL IN GLOBIOMASTOMA MULTIFORME (GBM) THROUGH THE TRAIL/DEATH RECEPTOR PATHWAY Teralee Burton, David D. Eisenstat, and Spencer B. Gibson; University of Manitoba BNIP3 is a hypoxy-inducible pro-cell death member of the Bcl-2 family. BNIP3 is activated by the transcription factor HIF-1-alpha and mediates cell death in a caspase-independent manner. Glioblastoma (GBM) is resistant to most treatments. Response to therapy fails, in part, due to tumor hypoxia facilitating resistance to radiation and chemotherapy. BNIP3 is expressed in hypoxyic regions of GBMs, but paradoxically, high BNIP3 expression does not lead to cell death. BNIP3 is primarily located in the nucleus of most GBMs, and that nuclear BNIP3 does not induce cell death in glioma cells. These observations led to the hypothesis that BNIP3-induced cell death is negatively regulated in brain tumors by nuclear localization of BNIP3. We have found that BNIP3 plays a novel role in the nucleus of glial cells by binding to a consensu sequence (CACGCA) in the promoter regions of genes involved in induction of cell death and silencing of these genes. We determined that BNIP3 binds to the promoter of the apoptosis-inducing factor (AIF) gene. BNIP3 recruits a repressor complex to the AIF promoter, facilitating repression of transcription. Microarray analysis of glioma cells differentially expressing nuclear BNIP3 indicated that BNIP3 is bound to BNIP3 downstream targets of the TRAIL receptor pathway, such as DR5 (death receptor 5). We concluded that nuclear BNIP3 down-regulates DR5 expression in glioma cells, subsequently block- ing TRAIL-induced cell death. In addition, expression of nuclear BNIP3 in primary GBM tumours correlates with increased DR5 expression. Pull-down assays confirmed that the BNIP3 protein binds to a consensus binding site in the DR5 promoter. This study provides evidence for a novel mechanism whereby nuclear BNIP3 is selected for in GBM because its cell death function is impeded and its transcriptional repression function of genes such as DR5 is enhanced, thereby conferring a survival advantage to the tumor cells.

CB-60. MICRONRNA-125B (MIRNA-125B; CHR 11Q24; CHR 21Q21) FUNCTION IN THE PROLIFERATION OF GBM ASTROGLIAL CELLS W.J. Lukiw 1, J.G. Cui 1, Y.Y. Li 1, Y. Zhao 2, and F. Calicchia 3; Louisiana State University Neuroscience Center, New Orleans; Department of Structural Biology, University of Pittsburg; Louisiana State University Department of Neurosurgery, New Orleans MicroRNAs (miRNAs) are post-transcriptional modulators of gene expression that regulate the stability and translation of their target messenger RNAs (mRNAs). Here we report that the levels of a human brain-enriched miRNA-125b are up-regulated in human glioma cells and in interleukin-6 (IL-6)-stimulated normal human astroglial (NHA) cells, the latter known to cause the proliferation of astroglial cells in anti-miRNA-125b (AM-125b) cultures attenuated astrogial cell proliferation and increased the expression of the cyclin-dependent kinase inhibitor 2A (CDKN2A), a known negative regulator of cell growth. With a modified gel shift assay, the 3'-untranslated region (3'-UTR) of CDKN2A was shown to contain a highly conserved miRNA-125b (AM-125b) binding site (CAGCCA) in the promoter regions of genes involved in induction of cell death and silencing of these genes. We also report that the levels of a human brain-enriched miRNA-125b (MI6A) cultured under conditions known to induce astroglial cell proliferation. These results suggest that miRNA-125b contributes to the cyclin-dependent proliferation of astroglial cells and that anti-micro RNA strategies may be clinically useful in treatment of glioblastoma and other proliferative diseases.

CB-61. NFI DEFICIENCY CONTRIBUTES TO GROWTH OF GLOBIOMASTOMA CELLS AND CONFRS SENSITIVITY TO MEK INHIBITION BY PD0325901 Wendy See and Russell Peiper; UCSF INTRODUCTION: NF1 patients harbor mutations in a gene (NF1) that encodes neurofibromin, a GTPase-activating protein (GAP) that negatively regulates Ras activity. While NF1 patients have a 5-fold increased risk of developing glioblastoma, mutations, deletions, and reduced expression of NF1 have also been identified in a subset of spontaneous glioblastomas, suggesting that NF1 may contribute to glioblastoma formation. Furthermore, because NF1-deficient AMLs are sensitive to inhibitors of MEK, a downstream effector of Ras, NF1 loss may be a driver of gliomagens and serve as a target for therapy. We investigated the contribution of NF1 loss to development of human glioblastoma and whether NF1 mutations define a subset of tumors that may be more susceptible to targeted therapies. METHODS: We obtained two glioblastoma cell lines, U251 and LN229, that exhibit homozygous loss of NF1. Cells were transfected with NF1 cDNA or GFP alone, labeled with BrdU, fixed, and stained with anti-BrdU primary and fluorescently labeled secondary antibodies. BrdU incorporation was detected in GFP-expressing cells by flow cytometry as a measure of proliferation. Next, U251 and LN229 cells were treated with increasing doses of PD0325901, an MEK inhibitor, cultured for 5 days, and subsequently counted. RESULTS: Transfection with full-length NF1-GFP caused a significant decrease in BrdU uptake compared with GFP in U251 and LN229 cells. Treatment with PD0325901 caused a marked decrease in cell growth in LN229 cells (IC50 = 30nM–100nM). By contrast, U251 cells were more resistant to growth inhibition by PD0325901 (IC50 = 300nM–1000nM). CONCLUSIONS: Re-expression of full-length NF1 in NF1-deficient glioblastoma cells leads to decreases in proliferation, supporting NF1 as a tumor suppressor in human glioblastoma. Treatment with PD0325901, a potent MEK inhibitor, inhibits glioblastoma cell growth and suggests that at least a subset of glioblastoma patients may respond to clinically relevant MEK inhibitors.

CB-62. DRUG SENSITIVITIES IN MOLECULAR SUBGROUPS OF GBM-DERIVED BRAIN TUMOR STEM CELLS Artee Luchman, Owen Stechishin, Stephanie Nguyen, John Kelly, Michael Blough, Gregory Cairncross, and Samuel Weiss; University of Calgary Brain tumor stem cells (BTSCs) have been identified as priomordial cells that may initiate disease and tumor recurrence as well as confer resistance to existing treatment modalities. We have successfully isolated and propagated a large array of BTSCs from glioblastoma multiforme (GBM) that display the fundamental cancer stem cell properties of clono- genic self-renewal, multi-lineage differentiation and aggressive tumor- imitating capacity. The frequent alteration of EGFR and PTEN in GBM suggests that these two genes are integral to the molecular pathology of a subset of GBMs and their BTSCs. We have identified subgroups with different combinations of EGFR and PTEN mutations within our BTSC lines. Here we investigate whether these subgroups display differences in growth characteristics, in vitro signaling, and drug sensitivities. Our pre- liminary results indicate that these mutational combinations impact diverse intracellular signaling pathways downstream of EGFR and PTEN. Furthermore, the specific subgroups have varying responses to the inhibition of single classical pathway components. We demonstrate that combinatorial inhibition of various pathway targets (EGFR, PI3K, Ras/MAPK, and PKC), predicted by the mutational status and signaling signatures of each subgroup, results in highly effective inhibition of BTSC growth in vitro. Therapeutics that target alterations specific to tumor cells, especially tumors initiating BTSCs, hold the promise of providing efficacious treatment with
decreased toxicity to normal tissues. We plan to further investigate the application of targeted drug combinations in orthotopic xenograft mouse models.

CB-63. ABERRATIONS IN HIPPO SIGNALING IN GBMS—A POSSIBLE CONNECTION TO MENSECHYMAL PHENOTYPE
Sagar R. Shah, Ahmed Mohyeldin, Hadie Adams, Tomas Garzon-Muvdi, Colette Aphrys, and Alfredo Quinones-Hinojosa; Johns Hopkins School of Medicine

INTRODUCTION: Developmentally, Hippo pathway controls organ size by regulating cell proliferation and apoptosis. Upon activation of this pathway, a signaling cascade ensues that results in the inactivation of YAP, a transcriptional co-activator of this pathway. Over-expression of YAP has been implicated in several cancers. Research indicates that changes in Hippo signaling may give rise to this aggressive subclass of GBMs. METHODS: Western blot, immunocytochemistry, qRT-PCR, Kaplan-Meier analysis, and a decreased incidence of tumor formation. Only 6/15 of GBMs exhibit over-expression of YAP, which correlates with poor patient survival (p ≤ 0.05). Furthermore, we demonstrate YAP nuclear localization and activation of downstream targets, including Mcl-1 and CTGF. In addition, 85% of GBMs that exhibit a mesenchymal phenotype show increased expression of YAP. Thus, a high correlation (p ≤ 0.05) is observed between YAP over-expression and co-expression of STAT3 and C/EBP-beta. Contrary to previous reports, expression of YAP is down-regulated in GBMs. Thus, other regulators, such as FAT3, must be investigated to account for increased YAP expression in GBMs. Based on Kaplan-Meier analysis, GBMs with 2-fold down-regulation of FAT3 are associated with poor clinical outcome (p ≤ 0.05). Interestingly, YAP is over-expressed in some gliomas and metastatic tumors to the brain. CONCLUSION: We show pronounced expression of YAP in GBMs, gliosarcomas, and metastatic tumors to the CNS. Furthermore, we demonstrate that in addition to merlin, down-regulation of FAT3 may account for this ectopic YAP expression. Moreover, this aberrant expression promotes a mesenchymal phenotype. Understanding the role of Hippo pathway in these tumors will have important implications in the management of these malignant diseases.

CB-64. ABERRANT LOCALIZATION OF THE GUANINE NUCLEOTIDE EXCHANGE FACTOR, ECT2: IMPLICATIONS FOR INVASION AND MIGRATION
Adrienne C. Weeks, Andres Restrepo, Vedant Arun, Stacey Ivanchuk, and Joshua B. Rubin; 1Washington University in St. Louis; 2Drexel University; 3Duke University

Despite concerted efforts in the field of neuro-oncology, malignant gliomas remain a treatment challenge even with the best medical and surgical management. We have found the cytotoxic protein ECT2 to be elevated in gliomas, an increased incidence of tumor formation, and a decrease in survival of patients. In contrast to normal human astrocytes, which maintain nuclear ECT2 expression, GBMs exhibit decreased nuclear expression and cytoplasmic localization of ECT2. ECT2 is a primary GBM driver that regulates growth by modulating the Rho GTPases RAC1 and CDC42. In GBMs, ECT2 expression results inactivation of the pro-migratory small cytoskeletal GTPases and metastatic tumor growth. These findings support possible aberrant Hippo signaling, 78% of which is driven by STAT3 and C/EBP-beta. Thus, we hypothesize that possible aberrations in Hippo signaling may give rise to this aggressive subclass of GBMs.

CB-65. SONIC HEDGEHOG ACTIVITY IS REQUIRED FOR CXCR4 SIGNALING IN CEREBELLAR GRANULE NEURON PRECURSOR NEURONS AND MEDULLOBLASTOMA
Najari Sengupta, Lilhua Yang, Silvia Roncaglia, Bo Zhang, Shirley L. Markant, Zeng-jie Yang, Olmpia Meucci, Robert J. Wechsler-Reya, and Joshua B. Rubin; 1Washington University in St. Louis; 2Drexel University; 3Duke University

Intracellular signaling mediated by the chemokine CXCL12 and its G-protein coupled receptor CXCR4 plays a crucial role in central nervous system development as well as the genesis of the most common malignant brain tumor of childhood, medulloblastoma. In each case, CXCL12 works together with sonic hedgehog (Shh) to regulate proliferation. Evidence in the literature suggests that CXCL12 modulates Shh-induced cerebellar granule neuron precursor cell (GNP) proliferation through the inhibition of intracellular cAMP levels. The aim of the current study was to determine whether activation of the Shh pathway could reciprocally regulate CXCR4 expression and/or function. Notably, previous work from our lab has demonstrated potential cross-talk between CXCR4 and other signaling systems in the brain, such as opioid (μ opioid receptor) and neurofibromin (NF). The effect of Shh on CXCR4 function was examined in GNPs as well as in Dasyu medulloblastoma cells, which exhibit constitutive activation of the Shh pathway. We found that in GNPs, Shh can strengthen coupling of CXCR4 to activation of Gi and its downstream pathways, including cAMP, calcium flux, and ERK/Akt activation. Interestingly, in the Dasyu cells, treatment with the Shh inhibitor cyclopamine blocked CXCR4-induced Gi activation and downstream signaling. In either case, changes in CXCR4 activity required long-term (12 hours) treatment with either Shh or cyclopamine and regulators, such as EBP-beta.

CB-66. RESISTANCE TO EGFR INHIBITION VIA THE EMERGENCE OF EGFVR8-INDEPENDENT TUMOR GROWTH-PROMOTING MECHANISMS IN GLOBLASTOMA
Jill Wykosky, Akitake Mukasa, Lynda Chin, Webster Cavenee, and Frank Furnari; 1Ludwig Institute for Cancer Research, 2University of Tokyo; 3Dana-Farber Cancer Institute

The exact mechanisms of resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) remain largely unknown in glioblastoma (GBM), despite the fact that amplification of EGFR and/or expression of constitutively active EGFVR8 are among the most frequent molecular alterations in this disease. Recently, we showed that U373 cells expressing doxycycline (dox)-repressible EGFVR8 were dependent upon expression of the receptor for tumor initiation and maintenance in a xenograft mouse model. EGFVR8 silencing caused an extended period of growth stasis, after which some tumors regained the ability to grow rapidly, in striking similarity to the clinical scenario of resistance. These breakthrough tumors, termed “escapers,” remained silenced for EGFVR8 and employed distinctly different growth-promoting signaling pathways when collectively compared to EGFVR8-dependent tumors. Escapers exhibited elevated activity of the MAPK pathway, and cells derived from the tumors were more sensitive to Mek inhibition than cells from EGFVR8-dependent tumors. The escapers markedly suppressed tumor growth, suggesting that the unique signaling preferences of escapers are not compatible with those pathways preferentially utilized by EGFVR8. Genes were identified by microarray analysis that were specifically expressed at higher levels in the escapers. These genes represent candidates for involvement in the process of overcoming EGFVR8 dependence and thus may contribute to resistance to EGFR inhibition. Significantly, escaper cells were resistant to the in vitro growth-inhibitory effects of the TKI gefitinib as measured by colony formation in soft agar. Thus, the emergence of EGFVR8-independent tumor growth-promoting mechanisms in escapers is characterized by a switch in pathway signaling preferences and the up-regulation of novel genes and confers resistance to EGFR inhibition. The genes and pathways identified may shed light on novel mechanisms of TKI resistance and point to potential targets for the development of agents that could be used in combination with TKIs to circumvent resistance while improving clinical response.
Resident progenitor cells of the adult white matter are a potential source of primary glial tumors of the forebrain. The adult human brain contains a population of glial progenitor cells that can be isolated on the basis of ganglioside epitopes recognized by the A2B5 antibody (Nature Med 9:239, 2003; Ann Neurol 59:763, 2006). In this study, we used A2B5-selected isolation to isolate a population of tumor-initiating progenitor cells from human gliomas and then used a subtractive genomic strategy to identify the transcriptional events associated with their oncogenesis. A2B5+ cells were abundant in human gliomas at all stages of tumor progression. Glioblastoma-derived A2B5+ cells demonstrated self-renewal and multilineage differentiation potential in vitro and were tumorigenic after transplantation into the brain of immunodeficient mice in vivo. We further compared the gene expression profiles of A2B5+ cells derived from low-grade (n = 10) and high-grade gliomas (n = 10) to those of their non-neoplastic adult A2B5+ counterparts (n = 8). While most of the genes that were differentially expressed by at least 3-fold (1% FDR) by glioma-derived A2B5+ tumor cells varied as a function of tumor stage, our analysis identified a discrete cohort that was differentially expressed at all stages of gliomagenesis as well as a select group that was differentially regulated in low-grade gliomas. Real-time qPCR and immunolabeling confirmed the directed gene expression of these genes. Pathway analysis revealed major dysregulation of the TGF-beta and Wnt/beta-catenin pathways, suggesting a key role of these pathways in the transformation of glial progenitors as well as in the pathogenesis of both low- and high-grade gliomas. By comparing the expression profiles of glial tumor progenitor-like cells isolated from low- and high-grade gliomas to their non-neoplastic homologues, we have identified a discrete set of genes and pathways by which glial tumorigenesis may be both better understood and more efficiently targeted.

CB-67. TRANSCRIPTIONAL DISTINCTIONS BETWEEN A2B5-DEFINED HUMAN GLIAL PROGENITOR CELLS AND THOSE DERIVED FROM GLIAL TUMORS AT ALL STAGES OF GROWTH

Romane M. Auvergne1, Fraser J. Sim2, Su Wang2, Devin Chandler-Militello2, Jaclyn Burch2, Xiaolong Li3, Andrew Bennett1, Nimish Mohile4, Webster Pilcher4, Kevin Walter5, Mahlon Johnson2, Pragathi Achanta6, Arturo Quimones-Hinojosa1, Sandrak Natesan8, and Steven A. Goldman2
1University of Rochester Medical Center; 2University of Rochester Medical Center, Rochester; 3Johns Hopkins University, Baltimore; 4Sanofi-Aventis, Cambridge

Abstract:

Autophagy is a cellular stress response that protects cells from harmful conditions. Emerging evidence suggests that this cellular process is also a tumor suppressor pathway. Previous studies showed that cyclin-dependent kinase inhibitors (CDKIs) induce autophagy. Whether retinoblastoma protein (RB), a key tumor suppressor and downstream target of CDKs, induces autophagy is not clear. Here we first demonstrate that RB triggers autophagy in human sarcoma osteogenic (Saos-2) cells, hepatoma (Hep3B) cells, and brain tumor stem cells (MDNSG23), as indicated by accumulation of LC3B, a punctate pattern of GFP-LC3 cellular localization, and the double-membrane trimmed vacuole formation in the cytoplasm shown by transmission electron microscopy. Autophagy flux study with double-labeled EGF-pmRFP-LC3 fusion protein reveals that RB induces complete maturation of autophagosome into autolysosome. In addition, RB activators p16INK4a and p21/Waf1 induce autophagy in RB-expressing sarcoma osteogenic (U2OS) cells and malignant glioma (U-87 MG) cells in an RB-dependent manner. Furthermore, RB with deletions in the E2F1 binding region fails to induce autophagy, and overexpression of E2F1 antagonizes RB-mediated autophagy. Leading autophagy cells isolated from low-grade gliomas, autophagy is induced in GBM cells. Autophagy and apoptosis may be both better understood and more efficiently targeted.

CB-69. RB/E2F: GATEKEEPERS OF AUTOPTHAGY AND APOPTOSIS IN GLIOMA CELLS

Hong Jiang, Vanesa Martin, Candelia Lara-Gomez-Manzano, David G. Johnson, Marta Alonzo, Erin J. White, Jing Xu, Timothy McDonnell, Naoki Shinojima, and Juan Fueyo; The University of Texas MD Anderson Cancer Center

Autophagy is a cellular stress response that protects cells from harmful conditions. Emerging evidence suggests that this cellular process is also a tumor suppressor pathway. Previous studies showed that cyclin-dependent kinase inhibitors (CDKIs) induce autophagy. Whether retinoblastoma protein (RB), a key tumor suppressor and downstream target of CDKs, induces autophagy is not clear. Here we first demonstrate that RB triggers autophagy in human sarcoma osteogenic (Saos-2) cells, hepatoma (Hep3B) cells, and brain tumor stem cells (MDNSG23), as indicated by accumulation of LC3B, a punctate pattern of GFP-LC3 cellular localization, and the double-membrane trimmed vacuole formation in the cytoplasm shown by transmission electron microscopy. Autophagy flux study with double-labeled EGF-pmRFP-LC3 fusion protein reveals that RB induces complete maturation of autophagosome into autolysosome. In addition, RB activators p16INK4a and p21/Waf1 induce autophagy in RB-expressing sarcoma osteogenic (U2OS) cells and malignant glioma (U-87 MG) cells in an RB-dependent manner. Furthermore, RB with deletions in the E2F1 binding region fails to induce autophagy, and overexpression of E2F1 antagonizes RB-mediated autophagy. Leading autophagy cells isolated from low-grade gliomas, autophagy is induced in GBM cells. Autophagy and apoptosis may be both better understood and more efficiently targeted.
MESENCHYMAL DIFFERENTIATION IN Glioblastoma

The endoplasmic reticulum (ER) is an organelle critically involved in protein folding and lipid and steroid biosynthesis as well as intracellular Ca2+ storage in eukaryotic cells. Various physiological or pathological stimuli cause disruption to these physiological functions of the ER, namely ER stress, and unfolded protein response (UPR) is subsequently activated to restore the ER homeostasis. However, prolonged and unresolved ER stress will ultimately lead to autophagy and apoptosis. The ER stress response is also induced by exposure of GBM cells to the synthetic cannabinoid agonist HU-210 in vitro and in vivo, a known ER stressor.

Inhibition of translation initiation impedes cell growth and proliferation. Translation initiation is a highly regulated step in protein synthesis. The endoplasmic reticulum (ER) is an organelle critically involved in protein folding and lipid and steroid biosynthesis as well as intracellular Ca2+ storage in eukaryotic cells. Various physiological or pathological stimuli cause disruption to these physiological functions of the ER, namely ER stress, and unfolded protein response (UPR) is subsequently activated to restore the ER homeostasis. However, prolonged and unresolved ER stress will ultimately lead to autophagy and apoptosis.

CB-71. NUCLEAR PTEN-INDUCED AUTOPHAGY IS MEDIATED THROUGH INDUCTION OF ER STRESS IN GLIOBLASTOMA CELLS

CB-72. A REGULATORY ROLE FOR TAZ IN PRONEURAL TO MESENCHYMAL DIFFERENTIATION IN Glioblastoma

CB-73. THE HELICASE PROTEIN DHX29 IS REQUIRED FOR PROLIFERATION OF MALIGNANT GLIOMAS

CB-74. NOVEL FUNCTION OF P14ARF IN HUMAN GLIOGENESIS: REGULATION OF TFPI AND GLIOMA-MEDIATED COAGULATION

The malignant progression of many tumors, including malignant gliomas, involves the loss of the p14ARF tumor suppressor gene. This genetic alteration occurs with the transition to high-grade glioma and precedes the associated pathological features of intravascular thrombosis, the formation of hypoxic regions and dramatically increased neovascularization. The mechanisms underlying and possibly connecting these biological features are poorly understood. The tissue factor pathway inhibitor-2 (TFPI2) is an extracellular serine protease inhibitor that prevents initial blood coagulation reactions. Given the high frequency of ARF gene deletions and the decreased expression of TFPI2 with astrocytoma progression, ARF loss might be one of the genetic events that dysregulates TFPI2 and promotes plasma clotting and vascular thrombosis/hypoxia within gliomas. To examine novel functions of ARF, we expressed the ARF gene in ARF-deficient glioma cells and examined the expression level of TFPI2. Pharmacological inhibitors, RNA interference, and CHIP assays were used to examine ARF effects on TFPI2 expression and plasma clotting triggered by gliomas. Our findings show that ARF up-regulates TFPI2 at the transcriptional level and significantly reduces the ability of glioma cells to promote plasma clotting as demonstrated by the increase in coagulation time of plasma in contact with ARF-induced versus ARF-uninduced glioma cells. ARF increases ATP and phosphatase 2A levels and induces JNK activation and subsequently the binding of the AP-1 transcription factor to its specific sites located on the TFPI2 gene promoter. Furthermore, this new ARF’s signaling axis is p53-independent. In summary, these data present the first evidence that p14ARF-mediated genetic control of plasma coagulation, initiated by glioma cells through AP1-mediated activation of TFPI2 expression, and suggest that therapies directed toward restoring ARF activity, TFPI2 expression, or TFPI2 activity could interrupt the intrathrombotic multinuclear cascade that may initiate hypoxia-driven malignant progression.

CB-75. MECHANISM OF CROSSTALK BETWEEN THE NF-KAPPA-B AND STAT3 SIGNALING PATHWAYS IN GLIOMAS

The malignant progression of many tumors, including malignant gliomas, involves the loss of the p14ARF tumor suppressor gene. This genetic alteration occurs with the transition to high-grade glioma and precedes the associated pathological features of intravascular thrombosis, the formation of hypoxic regions and dramatically increased neovascularization. The mechanisms underlying and possibly connecting these biological features are poorly understood. The tissue factor pathway inhibitor-2 (TFPI2) is an extracellular serine protease inhibitor that prevents initial blood coagulation reactions. Given the high frequency of ARF gene deletions and the decreased expression of TFPI2 with astrocytoma progression, ARF loss might be one of the genetic events that dysregulates TFPI2 and promotes plasma clotting and vascular thrombosis/hypoxia within gliomas. To examine novel functions of ARF, we expressed the ARF gene in ARF-deficient glioma cells and examined the expression level of TFPI2. Pharmacological inhibitors, RNA interference, and CHIP assays were used to examine ARF effects on TFPI2 expression and plasma clotting triggered by gliomas. Our findings show that ARF up-regulates TFPI2 at the transcriptional level and significantly reduces the ability of glioma cells to promote plasma clotting as demonstrated by the increase in coagulation time of plasma in contact with ARF-induced versus ARF-uninduced glioma cells. ARF increases ATP and phosphatase 2A levels and induces JNK activation and subsequently the binding of the AP-1 transcription factor to its specific sites located on the TFPI2 gene promoter. Furthermore, this new ARF’s signaling axis is p53-independent. In summary, these data present the first evidence that p14ARF-mediated genetic control of plasma coagulation, initiated by glioma cells through AP1-mediated activation of TFPI2 expression, and suggest that therapies directed toward restoring ARF activity, TFPI2 expression, or TFPI2 activity could interrupt the intrathrombotic multinuclear cascade that may initiate hypoxia-driven malignant progression.
presence of TNF-alpha, which activates NF-kappa-B. We found that NF-kappa-B was activated and correlated with enhanced IL-6 mRNA and protein expression, followed by STAT3 activation and the expression of STAT3-regulated genes. Using cell lines that inducibly express PMA, we found that Ral signaling is mediated by RalA activation. We then selected DAOY as a model cell line for further evaluation of the outcome of inhibiting Ral expression. Using a lentivirus expressing anti-RalA shRNA, we successfully inhibited Ral expression as compared to a negative control virus. Upon treatment, we observed a greater than 65% reduction in proliferation by day three post-infection. We concluded that high levels of active RalA are needed for cell survival in the DAOY cell line and may represent a new therapeutic target for medulloblastoma. Our future work will focus on the evaluation of the effects of inhibition of Ral signaling on the invasiveness and in vivo tumorogenicity of medulloblastoma cells.

CB-78. THE ING4 TUMOR SUPPRESSOR INDIRECTLY SUPPRESSES STAT3 SIGNALING BY REDUCING IL-6 EXPRESSION
Susan E. Nuzzo, Suk W. Hong, George B. Twitty, Jr., Braden C. McFarland, and Etty N. Benveniste; University of Alabama at Birmingham

ING4 is a tumor suppressor that is absent or mutated in gliomas. Previously, we showed that ING4 attenuates the transcriptional activity of NF-kappa-B. STAT3 is a transcription factor activated by IL-6. Both NF-kappa-B and STAT3 regulate the expression of genes that promote cell proliferation, apoptotic resistance, and angiogenesis. In gliomas, both NF-kappa-B and STAT3 are constitutively activated and may contribute to the activity of STAT3. Therefore, the loss of ING4 expression in gliomas may explain why both the NF-kappa-B and STAT3 signaling pathways are constitutively activated.

CB-80. DIVERSITY OF ENGRAFTMENT BEHAVIORS AMONG CD133-EXPRESSING PATIENT-DERIVED BRAIN TUMOR CELL LINES
Christine Brown and Michael Barish; Beckman Res Inst City of Hope

INTRODUCTION: Brain tumors can be thought of as heterogeneous tissues developing within a complex brain environment. We are working to achieve understanding of tumor initiation, progression, and dissemination using populations of well-characterized patient-derived brain tumor cell lines. METHODS: We derived pools of patient-derived glioma cells, lentivirus-transduced them to express fluorescent tracking proteins, and implanted them in immunodeficient mice. Patterns of engraftment were assessed at different time points post-implantation by wide-field and confocal microscopy of serial cryostat sections. RESULTS: Drawing on a library of characterized patient-derived glioma lines, we focused on two lines, PBT003 and PBT008, both of which form tumor spheres expressing the putative tumor stem cell marker CD133 in culture and show multipotential differentiation into mesenchymal and neural lineages. Using these lines, we were able to identify the cell type responsible for the diversity of in vivo behaviors by examining cell lines that exhibit very different patterns of engraftment. By 2 months post-implantation, PBT003 cells formed a tumor mass surrounded by peripheral disseminating cells. In contrast, PBT008 cells were found scattered through cortex but had not initiated tumor foci. We are working to understand the underlying biology of this diversity of in vivo behaviors. CONCLUSION: Despite similarities of marker expression and in vitro growth and differentiation patterns, presumed populations of tumor-initiating cells can display wide variation in their capacities to form tumors in vivo. Understanding these differences will enhance our understanding of glioma dissemination and secondary focus initiation.

CB-76. RASGRP3 INTERACTS WITH THE ACTIN-RELATED PROTEIN ARP3 AND REGULATES THE MIGRATION OF GLIOMA CELLS
Hae Kyung Lee, Susan Finniss, Cunli Xiang, Simona Cazacu, and Chaya Brodie; Henry Ford Hospital

Gliomas, the most frequent primary brain tumor, are characterized by increased cell growth in the surrounding normal brain tissue. Signaling pathways coupled to DAG production are highly active in glioma cells, mainly downstream of the growth factor receptors EGFR and PDGFR. Similarly, Ras proteins are activated in gliomas and play a role in their malignant phenotype. Although gliomas express high Ras activity, Ras-activating mutations have not been identified in these tumors. RasGRP represents a new family of GEFs that belong to the DAG/phorbol ester receptor family and that act as Ras activators by promoting the acquisition of GTP to maintain the active GTP-bound form. We found that RasGRP3 regulates glioma cell migration and invasion and that it regulates the active GTP-bound form. We found that RasGRP3 regulates glioma cell migration and invasion and that it activates the Ras and Rap1 proteins in these cells. In addition, RasGRP3 activates the Erk and AKT pathways, and AKT is partially involved in the effect of RasGRP3 on glioma cell migration.

Together, these data indicate that RasGRP3 is an important regulator of glioma cell migration and that act as Ras activators by promoting the acquisition of GTP to maintain the active GTP-bound form. We found that RasGRP3 regulates glioma cell migration and invasion and that it activates the Ras and Rap1 proteins in these cells. In addition, RasGRP3 activates the Erk and AKT pathways, and AKT is partially involved in the effect of RasGRP3 on glioma cell migration. Together, these data indicate that RasGRP3 is an important regulator of glioma cell migration and that it activates the Ras and Rap1 proteins in these cells. In addition, RasGRP3 activates the Erk and AKT pathways, and AKT is partially involved in the effect of RasGRP3 on glioma cell migration.

CB-77. RAL SIGNALING IN MEDULLOBLASTOMA: BIOLOGICAL AND THERAPEUTIC OUTCOMES
Kevin F. Ginn1, Amanda Wise2, and Faris Farassati2; 1Children’s Mercy Hospital and Clinics; 2University of Kansas Medical Center

Medulloblastoma is one of the most common malignant central nervous system tumors in children. Treatment is often associated with untoward long-term effects, and new targeted therapies are needed for this devastating tumor. Ras, one of the most important proto-oncogenes involved in human cancers, has been shown to be involved in the development of neurological malignancies. We studied Ras in DAOY (Ras-like) activation as a novel downstream effector of Ras, with a goal of targeting this pathway using cell lines characterized by specific marker expression, followed by STAT3 activation and the expression of STAT3-regulated genes. Using cell lines that inducibly express PMA, we found that Ral signaling is mediated by RalA activation. We then selected DAOY as a model cell line for further evaluation of the outcome of inhibiting Ral expression. Using a lentivirus expressing anti-RalA shRNA, we successfully inhibited Ral expression as compared to a negative control virus. Upon treatment, we observed a greater than 65% reduction in proliferation by day three post-infection. We concluded that high levels of active RalA are needed for cell survival in the DAOY cell line and may represent a new therapeutic target for medulloblastoma. Our future work will focus on the evaluation of the effects of inhibition of Ral signaling on the invasiveness and in vivo tumorogenicity of medulloblastoma cells.

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CB-81. VARIABILITY OF RESPONSE TO TEMOZOLOMIDE TREATMENT IN ORTHOTOPIC GBM NEUROSPHERE XENOGRAFTS REFLECTS PARENTAL TUMOR MOLECULAR PROPERTIES

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BACKGROUND & OBJECTIVE: Temozolomide (TMZ), a cytoxic DNA-alkylating agent, is employed in the standard of care of glioblastomas. The methylation status of the promoter of the DNA repair protein O6-methylguanine DNA-methyltransferase (MGMT) is a biomarker for response to TMZ. Because this line of treatment remains palliative and has only a modest effect on survival, the search for molecular vulnerabilities that can be targeted in a combinational approach is warranted. We investigated response to TMZ using glioblastoma multiforme (GBM) preclinical models from patient-derived neurosphere cultures in search of candidate pathways associated with TMZ resistance. EXPERIMENTAL APPROACHES: Neurosphere cultures enriched in cancer stem cells were obtained from resected tumors with different MGMT promoter methylation and TP53 status. GBM neurosphere cells expressing luciferase were implanted intracranially in nude mice. Tumor growth was monitored by noninvasive in vivo imaging using the Xenogen IVIS System (Caliper Life Sciences). One 5-day cycle of TMZ was administered intragastrically to two groups of mice. One group received treatment before tumor growth was detectable and the other after a significant increase in bioluminescence was observed. Six days of TMZ control mice were vehicle alone. Response to treatment was monitored by bioluminescence, overall survival, and molecular alterations. RESULTS: TMZ monotherapy significantly increased the survival of xenografts from GBMs positive for MGMT promoter methylation, while no effect on survival was observed for the xenografts obtained from GBMs with unmethylated status. There was no statistical difference between the early and late treatment schedules. Molecular subtype specific upregulation of genes associated with DNA repair and mesenchymal lineage was observed in tumors in the untreated control xenografts was observed. CONCLUSIONS: GBM models using neurospheres have recapitulated the TMZ sensitivity expected on the basis of MGMT promoter methylation of parental tumors, constituting an appropriate model to investigate tumor subtype-specific pathway activation in response to therapy.

CB-82. EFFECTS OF DYSREGULATED HGF/CMET SIGNALING ON CEREBELLAR DEVELOPMENT AND MEDULLOBLASTOMA PATHOGENESIS

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Medulloblastoma (MB) is a primitive neuroectodermal tumor of the cerebellum. HGF/cMET signaling plays a role in cerebellar development, and the overactivation of this pathway has been implicated in several human malignancies. Our genome-wide epigenetic screens on human MB cell lines and primary tumor specimens have identified SPINT2/HAI-2, an HGF/cMET signaling inhibitor, as a novel tumor suppressor gene that is frequently silenced by promoter hypermethylation in MB. Furthermore, the aberrant activation of the HGF receptor cMET tyrosine kinase has been associated with the pathogenesis of MB. To determine whether mutation contributes to aberrant HGF/cMET signaling in MB, we will perform a mutational analysis of MB cell lines and primary tumors for mutations in the cMET gene. In the large cohort of primary MB cell lines, this can provide direct genetic evidence implicating HGF/cMET signaling in MB tumorigenesis. Furthermore, to assess the role of aberrant HGF/cMET signaling on cerebellar development and MB pathogenesis, we will construct a transgenic mouse overexpressing a mutant, constitutively active form of cMET specifically in the granule precursor cells of the developing cerebellum, which are the suspected cells of origin for MB. Mice will be examined at various ages to characterize the effects of upregulated cMET activity on cerebellar development and be observed for evidence of tumor formation. This work will help provide greater insight into the involvement of HGF/cMET signaling in the genesis of MB and will provide a valuable preclinical model for testing the existing and novel HGF/cMET targeted antimtumor agents.

CB-83. CURCUMIN AND TRAIL INDUCE APOPTOSIS IN GLIOMA CELLS AND GLIOMA STEM CELLS VIA DOWNREGULATION OF NOVEL PKC ISOFORMS AND INHIBITION OF AUTOPHAGY

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TRAIL induces apoptosis in cancer but not in normal cells and is therefore considered a promising antimtumor agent. Some cancer cells, however, are resistant to the apoptotic effect of TRAIL. We examined the effect of the natural compound curcumin on the resistance of glioma and glioma stem cells (GSCs) to TRAIL. Some of the glioma cell lines and primary glioma cultures demonstrated sensitivity to TRAIL, whereas all the glioma stem cells to TRAIL. Curcumin induced autophagy in glioma cells. However, a combined treatment with curcumin and TRAIL abolished the autophagy induced by curcumin and induced apoptosis in all the resistant cell lines. Treatment with curcumin and PKC-delta play a role in the resistance of glioma cells to TRAIL. Curcumin or TRAIL alone did not induce significant changes in the expression or cleavage of PKC-epsilon and PKC-delta in the TRAIL-resistant cells. In contrast, combined treatment agents induced some accumulation of the catalytic fragment of both PKC isoforms and significantly decreased their expression. Overexpression of PKC-epsilon and PKC-delta partially protected the cells from the apoptotic effect of curcumin plus TRAIL. The caspase-resistant mutant PKC-epsilon D383A rendered the cells more resistant to the combined treatment, whereas the caspase-resistant mutant of PKC-delta exerted a smaller protective effect, suggesting an opposite role of the cleavage of PKC-epsilon and PKC-delta in this effect. Treatment of the cells with curcumin and TRAIL also decreased the expression of Akt in a PKC-epsilon-dependent manner. In summary, curcumin sensitized glioma cells and GSCs to TRAIL by decreasing the expression of PKC-delta and PKC-epsilon and by downregulating Akt downstream of PKC-epsilon. The combined treatment of TRAIL and curcumin abolished the induction of autophagy by curcumin and induced cell apoptosis. Combining curcumin and TRAIL may be useful therapeutically in the treatment of gliomas and the eradication of glioma stem cells.