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Effects of β3-adrenergic receptor agonist on gene expression of leptin in glioblastoma

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CB-01. THE EFFECT OF WT1 ON TUMORGENICITY, CELL PROLIFERATION, AND APOTOPSIS IN GLIOBLASTOMA
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Glioblastoma (GBM), one of the most frequently occurring malignancies in the central nervous system, still has a very poor prognosis. To improve the prognosis of GBM patients, various attempts have been made. Immunotherapy targeting Wilms’ tumor 1 (WT1) has proved to be effective in GBM (Ishimoto et al., 2008). However, the functional roles of WT1 in GBM have not been intensively studied. In this study, we aim to examine the functional roles of WT1 in GBM. We established WT1 shRNA knocked down GBM cell lines (U87 and U251) and examined the functional roles in vitro and in vivo. In cell-proliferation assays, we plated 3 × 10^5 cells in 12-well plates. On day 4 the numbers of the proliferated cells were (39 ± 7.8) × 10^4, (19 ± 3.5) × 10^4, (17 ± 6.5) × 10^4, and (3.4 ± 0.5) × 10^4 cells in the U87 control shRNA, U87 WT1 shRNA, U251 control shRNA, and U251 WT1 shRNA, respectively (P < 0.05). Furthermore, an Annexin V apoptosis assay showed the numbers of the apoptotic cells per 30 times magnified microscope field were 2.5 ± 0.6, 15.3 ± 5.7, 3.0 ± 1.7, and 9.0 ± 1.0 cells in the U87 control shRNA, U87 WT1 shRNA, U251 control shRNA, and U251 WT1 shRNA, respectively (P < 0.05). In the in vivo experiment, U87 control shRNA and U87 WT1 shRNA cells were intracranially injected into newborn pups of immunodeficient mice. At day 30, all of the mice transplanted with U87MG control shRNA developed GBM, whereas none of the mice transplanted with U87MG WT1 shRNA developed GBM. These results suggest that WT1 is involved in GBM cell proliferation, apoptosis, and tumor formation.

CB-02. RESULTS OF IMMUNOHISTOCHEMICAL STAINING OF CELL-CYCLE REGULATORS—THE PREDICTION OF RECURRENCE-FREE SURVIVAL OF ANTERIOR PITUITARY ADENOMA
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To investigate the possible prognostic values of several cell-cycle regulators for the prediction of anterior pituitary adenoma (APA) recurrence after surgical resection, we immunohistochemically analyzed tumor samples obtained by surgical resection. The study population consisted of 156 patients with APA diagnosed from January 2000 to December 2009 at the Department of Neurosurgery at Samsung Changwon Hospital in Korea. By retrospectively reviewing medical records, we examined clinical manifestations associated with excess adenohypophysial hormone and biochemical study findings for serum adenohypophysial hormone. We performed the Cox regression model on the basis of p16, RB protein, and cyclin D1. The results suggest that p16, RB protein, and cyclin D1 are associated with longer recurrence-free survival in APA.

CB-03. FIBULIN-3 PROMOTES GLIOMA GROWTH AND RESISTANCE THROUGH A NOVEL PARACRINE REGULATION OF NOTCH SIGNALING
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Malignant gliomas are highly invasive and chemoresistant brain tumors with extremely poor prognosis. Glioma invasion is strongly associated with the resistance of these tumors to therapy, but the mechanisms that underlie this association are poorly understood. Targeting soluble factors triggering invasion and resistance could substantially affect the difficult-to-reach, infiltrative glioma cells that are a major source of recurrence. Fibulin-3, a matrix protein absent in normal brain tissue but upregulated in gliomas, promotes tumor invasion by unknown mechanisms. We show here that fibulin-3 is a novel solvable activator of Notch signaling that antagonizes DLL3, an autocrine inhibitor of Notch, and promotes tumor-cell survival and invasion in a Notch-dependent manner. Using a strain of inducible knock-in mice, we demonstrated that controlled downregulation of fibulin-3 reduces Notch signaling and leads to increased apoptosis, reduced self-renewal of glioblastoma-initiating cells, and impaired growth and dispersion of intracranial tumors. Finally, we show that the in vivo expression of fibulin-3 in gliomas impairs Notch signaling. Our findings provide a novel mechanism of a noncanonical, solvable activator of Notch in a cancer model and demonstrate how Notch signaling can be reduced by targeting tumor-specific accessible molecules in the tumor microenvironment.

CB-04. NF-κB INDUCED IL-6 ENSURES STAT3 ACTIVATION AND TUMOR AGGRESSIVENESS IN GLIOBLASTOMA
Braden C. McFarland, Suk W. Hong, Rajani Rajbhandari, George B. Twitty, Jr., G. Kenneth Gray, Hao Yu, Catherine P. Langford, G. Vance Gillespie, E tty N. Benveniste, and Susan E. Nussell; University of Alabama at Birmingham, Birmingham, AL, USA

Glioblastoma (GBM) is the most aggressive, neurologically destructive, and deadliest tumor of the CNS. Two signaling proteins, NF-κB and STAT3, have been linked to the mesenchymal genetic subtype, the most malignant subtype of GBM. Our lab has previously shown that both NF-κB and STAT3 are overly abundant and active in GBM and that both signaling pathways activate numerous pro-survival genes. In addition, the NF-κB and STAT3 pathways contribute to cellular communication within the tumor environment, activating additional non-tumor cells, particularly immune cells, that then suppress immune defense mechanisms and allow continued growth of the tumor. In this study, we have evaluated the activity of NF-κB and STAT3 to participate in a vicious cycle of cross-talk, which ultimately ensures that GBM tumors survive, proliferate, invade healthy tissue, and resist therapeutic efforts. In vitro, we demonstrated that TNF-α-induced activation of NF-κB is sufficient to induce interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) expression, activate STAT3, and elevate STAT3 target gene expression in cultured human and mouse glioma cell lines. Furthermore, TNF-α-induced STAT3 activation, as well as IL-6, LIF, and SOCS-3 expression are dependent on NF-κB p65 and require signaling through the JAK/STAT3 complex. In vivo, we demonstrated that TNF-α-induced STAT3 activation occurs in other glioma sources, including human GBM xenograft tumors. Using pharmacological inhibition and shRNA techniques targeting NF-κB and STAT3, we are currently evaluating the in vivo expression of NF-κB and STAT3 with specific emphasis on GBM size and mortality.

CB-05. GLIOBLASTOMA CELLS EXPRESSING EGFRVIII ARE MORE SENSITIVE TO CK2 INHIBITION
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Conclusively, our findings indicate that p16, RB protein, and cyclin D1 are associated with longer recurrence-free survival in APA.

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Glioblastoma (GBM) is the most common and fatal primary brain tumor in humans. Since no cure exists for GBM, it is essential to develop new and better therapies to treat this disease. Previous research has shown that co-cogenic kinasease 2 (CK2) may be a promising therapeutic target for GBMs. CK2 has enhanced expression or activity in a wide variety of cancers, including GBM, and it was demonstrated that inhibitors to CK2 regressed tumor growth in GBM xenograft mouse models. Through our own work, we demonstrated that GBM patients with a high expression of CK2 had a much worse prognosis than did patients with low levels. Currently, the mechanisms enabling enhanced expression or activity of CK2 are still unknown. Our studies have demonstrated that a deletion mutation of the epithelial growth factor receptor (EGFRvIII) is involved in CK2-dependent tumorigenesis in GBM cell lines. We generated GBM cell lines (U87-MG, U138) that stably express EGFRvIII and showed that these cells are more sensitive to depletion of CK2. Initial studies showed that siRNAs specific to a particular CKN subunit (CK2alpha) decreased GBM cell growth approximately by 50% in the control GBM cells. Interestingly, expression of EGFRvIII sensitized the GBM cells to growth arrest since cell growth was reduced 14%-20% with reduced expression of CK2alpha. In addition, inhibition of CK2alpha activity using commercially available inhibitors (TBR, TBR2) also reduced GBM cell growth (50%-66%), but we observed a more dramatic reduction (25%-50%) in EGFRvIII-overexpressing cells. We have also conducted some preliminary studies demonstrating that CK2 alpha kinase activity in vivo is enhanced by EGFRvIII expression. Our data suggest that EGFRvIII may play an important role in GBM tumorigenesis by regulating CK2 activity, and a combination treatment targeting both EGFRvIII and CK2alpha might be more efficacious than targeting each one individually.

CB-06. A NOVEL METHOD OF SONIC HEDGEHOG SIGNALING ACTIVATION IN THE CHEMORESISTANCE OF GliOBLASTOMA: A LIGAND-INDEPENDENT MECHANISM
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Glioblastoma (GBM) is the most common and lethal adult primary tumor of the central nervous system (CNS). GBM treatment with concomitant surgical resection and radiotherapy, GBM 3-year survival is approximately 3%. A better understanding of the cellular pathways in tumor proliferation and survival is required to develop novel targeted treatments. Previously, the Sonic Hedgehog (SHH) pathway has been shown to be dysregulated in chemoresistant GBM. Pharmacological inhibition of SHH signaling with cyclopamine enhances GBM sensitivity to temozolomide (TMZ). The SHH receptor PTCH1 tonically represses signaling, and repression is removed by ligand binding followed by receptor-mediated endocytosis. Here we show SHH signaling in GBM is ligand-independent and is initiated by post-transcriptional regulation of PTCH1. MicroRNA (miR), small non-coding RNA molecules, regulate a number of cell processes such as development and oncogenesis. As far as we are aware, there is no report on miRNA regulation of PTCH1. Knockdown of Dicer, a Type III RNase, which is required for miR synthesis, increased the cellular toxicity to TMZ, thus revealing the importance of miR in GBM chemoresistance. We have identified and characterized PTCH1 as a functional target of miR-768-3p. Overexpression of miR-768-3p in GBM cells results in downstream pathway activation and resistance to TMZ treatment. We also explored a number of miR-9/PTCH1-dependent mechanisms of chemoresistance and uncovered downstream upregulation of the ATP-binding cassette drug efflux transporters, MDR1 and ABCG2. Taken together, our data indicate a role for miR-9 and PTCH1 in the acquisition of chemoresistance in GBMs and suggest that miR-9 may serve as a novel therapeutic target.

CB-07. MECHANISMS OF CHEMORESISTANCE IN CB-08. NFI-DEFICIENT GLIOBLASTOMAS ACQUIRE MEK INHIBITOR RESISTANCE THROUGH RECEPTOR TYROSINE KINASE OR PI3K/MTOR UPREGULATION
Vivian Rodriguez-Cruz1, Jessel J. Munoz2, and Pranela Rameshwar; New Jersey Medical School, Newark, NJ, USA

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor. Dysregulation of microRNA (miRNA) expression occurs in GBM and may drive tumor growth and recurrence. We have identified miR-768-3p as one of the most downregulated miRNAs using a microarray analysis of GBM stem cells. We verified miRNA expression by real-time PCR in the microarray samples and further validated that this miRNA is downregulated in human GBM as compared with normal brain tissue. Indeed, in seven of twelve GBM specimens, 768-3p was underexpressed. Given the biological relevance of miR-768-3p is totally unknown. Tarascon and colleagues suggested that KRAS and PAK1 as potential target mRNAs. KRAS and PAK1 are involved in the PI3K/mTOR pathway effectors phospho-AKT, phospho-S6, and phospho-4EBP1 and sensitization to PI-103, a dual PI3K/mTOR inhibitor. Together these data suggest that NF1-deficient GBM derive tumor growth and recur. We have identified miR-768-3p as one of the most downregulated miRNAs using a microarray analysis of GBM stem cells. We verified miRNA expression by real-time PCR in the microarray samples and further validated that this miRNA is downregulated in human GBM as compared with normal brain tissue. Indeed, in seven of twelve GBM specimens, 768-3p was underexpressed. Given the biological relevance of miR-768-3p is totally unknown. Tarascon and colleagues suggested that KRAS and PAK1 as potential target mRNAs. KRAS and PAK1 interact in the same cell-migration pathway and both proteins have been proposed to contribute to GBM growth and invasion. miR-3 and immunoblot analysis of KRAS and PAK1 in 768-3p-treated glioma cells indicated that both targets were likely regulated by miR-768-3p in isolated CD133-expressing cells from U87 and T98G GBM cell lines and confirmed their dose-dependent chemoresistance to temozolomide (TMZ).

The mechanisms that underlie this chemoresistance are currently unknown. TMZ is an O6-methylguanyl alkylating agent and TMZ may take up a variety of pathways. We explored cell-cycle regulation in CD133-expressing GBM and have confirmed no difference in the cycling status of these cells. ATP-binding drug efflux pumps can bind to compounds such as TMZ and transport them out of the cell. The ATP-binding cassette transporters (ABCB1) and BCRP (ABCG2) are two such transporters known to be upregulated in GBM. Gene expression analysis confirms increased expression of both MDR1 and BCRP in CD133 GBM cells. Inhibition of MDR1 and BCRP (using cyclosporin A (CyA) and suramin) increased cell death when treated with TMZ. Knockdown by siRNA of both transporters confirmed their importance in GBM chemoresistance. Taken together, our data indicate that CD133 GBM resistance to TMZ is not caused by cell-cycle detontation but rather by MDR1/ABC2 upregulation.

CB-08. NFI-DEFICIENT GLIOBLASTOMAS ACQUIRE MEK INHIBITOR RESISTANCE THROUGH RECEPTOR TYROSINE KINASE OR PI3K/MTOR UPREGULATION
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The Ras pathway is aberrantly activated in most glioblastomas (GBM) and signals downstream through RAF/MEK/ERK and the PI3K pathway to promote growth and survival. In the mesenchymal GBM subtypes, Ras activation is associated with mutations and/or deletions of NFI, a negative regulator of Ras signaling. We previously identified a subset of NFI-deficient GBM that was especially sensitive to the single agent MEK inhibitor PD0325901 or AZD6244, suggesting that NFI-deficient GBMs may be ideal candidates for MEK inhibitor-based trials. However, prolonged exposure to targeted therapies ultimately leads to tumor relapse. Understanding the mechanisms of acquired resistance can help identify novel therapies and may also help us better understand the mechanisms of intrinsic resistance. To investigate the mechanisms of acquired resistance in NFI-deficient GBM, we chronically treated MEK inhibitor-sensitive LN229 and U373 GBM cells with 1 μM PD0325901 for 4.8 weeks, isolated MEK inhibitor-resistant clones, and conducted some preliminary studies demonstrating that these cells are more sensitive to MEK inhibition. MEK inhibitor-resistant clones demonstrated increased levels of the receptor tyrosine kinases (RTKs) phospho-EGFR and phospho-PDGFR, which were associated with increased levels of the RAF/MEK/ERK effectors phospho-ERK and cyclin D1 and with sensitization to combination treatment with erlotinib and imatinib (RTK inhibitors of EGFR and PDGFR, respectively). By contrast, U373 MEK inhibitor-resistant clones did not demonstrate upregulation of RTK signaling but instead demonstrated increased levels of the PI3K/mTOR pathway effectors phospho-AKT, phospho-S6, and phospho-4EBP1 and sensitization to PI-103, a dual PI3K/mTOR inhibitor. Together these data suggest that NFI-deficient GBM acquire MEK inhibitor resistance through multiple mechanisms, including upregulation of RTK signaling that leads to reactivation of the target pathway or through activation of alternative pathways, such as PI3K, which was as-
CB-10. NOVEL NF-KAPPA-B DECOY OLIGONUCLEOTIDE DERIVED FROM MGMT ENHANCER INDUCES AN ANTINEOPLASTIC EFFECT IN HUMAN CANCER CELLS
Iris Lavon, Daniel Zrihan, Miri Refael, Ariel Bier, Tamar Canello, and Tali Siegal; Hadassah University Medical Center, Jerusalem, Israel

INTRODUCTION: Alkylating agents show efficacy in the treatment of various tumors including gliomas. High cellular levels of O(6)-methylguanine (MGMT) affect the cytotoxicity of alkylating chemotherapeutics because it confers the efficient repair of DNA alklation; thus, MGMT is an ideal pharmacological target. Two pseudosubstrates, O6-benzylguanine (O6-BG) and O6-(4-bromothymyl)guanine, that aimed to reduce MGMT activity had low efficacy because the combined treatment induced a profound hematotoxicity and the MGMT levels rapidly recovered. Accordingly, the aim of the present study was to develop a chemo-potentiation treatment that will effectively and specifically reduce MGMT expression in tumors without an increase in toxicity in normal tissues.

RESULTS: We previously described two NF-kappaB binding sites within the MGMT promoter and proved the pivotal role of kappaB in MGMT expression. We assumed that interference with the binding of NF-kappaB to MGMT enhancer will attenuate MGMT expression and consequently will reverse MGMT-induced chemoresistance. For that purpose, we designed locked nucleic acid modified decoy oligonucleotides (LMDONs) corresponding to the specific sequence of MGMT-kappaB1. We demonstrated in the HEK293T cell line that MGMT-kb1 LMDONs interfere with the binding of NF-kappaB to the MGMT enhancer. The treatment effect of these LMDONs was studied in vitro in three human cancer cell lines. MGMT-kb1-LMDON concentrations of 2 nM for T98G or 1 nM for MCF-7 and ACHN lines significantly augmented (P < 0.05) temozolomide-induced cell killing by 2, 4, 8, and 2.1 times, respectively. Furthermore, MGMT-kb1 LMDON monotherapy in concentrations of 4 nM for T98G or 1 nM for MCF-7 and ACHN induced 70%, 79%, and 68% cell killing. CONCLUSIONS: Our results suggest that MGMT-kb1-LMDON may provide a novel strategy for treating human cancers, and it should be evaluated as a monotherapy or in combination with alkylating agents.

CB-11. TGFα IS THE MAJOR TARGET GENE OF THE CONSISTENTLY UNDEREXPRESSED LARGE CLUSTER OF 42 MICRO-RNAS LOCATED ON CHROMOSOME 14q32.31 IN GliOMAS
Daniel Zrihan, Avital Granit, Tali Siegal, and Iris Lavon; Hadassah Hebrew University Medical Center, Jerusalem, Israel

BACKGROUND: We previously demonstrated that in gliomas a cluster of 42 microRNAs (miRNAs) from chromosome 14q32.31 are consistently underexpressed. We hypothesized that this region operates as a tumor suppressor and that dysregulation of the related miRNAs may lead to abnormal expression of their targets, resulting in tumorigenesis. AIM: We aimed to identify the target genes of the miRNAs from the 14q32.31 region and their related oncogenic functions. METHODS: Four bioinformatic algorithms (PITA, TargetScan, PicTar, and miRanda) were used to identify the potential target genes of miRNAs from this cluster. Three miRNAs from three different regions on 14q32.31 (miR-323-3p, miR-433, and miR-369-3p) were introduced into mouse and human glioma cell lines, and we evaluated their effect on tumor proliferation and migration.

RESULTS: TGFα was selected for further analysis out of the identified candidate because 71% of the miRNAs from this cluster were predicted to target this oncogene. Minor overexpression (approximately 10-fold) of the 2 miRNAs 323-3p and 369-3p significantly reduced the proliferation and migration rate of the human and mouse glioma cell lines, whereas miR-433 had no obvious effect. Overexpression of miR-323-3p and miR-369-3p in the U87 cell line decreased TGFr expression by 70% and 50%, respectively, whereas overexpression of miR-433 had no effect. The ability of these miRNAs to modulate TGFα protein expression was validated by Western blot analysis. CONCLUSIONS: Our study indicates that the large miRNA cluster in chromosome 14q32.31 may function as a tumor-suppressor gene through mechanisms that inhibit tumor cell growth and migration. Our results also suggest that this miRNA cluster suppresses tumors via post-transcriptional regulation of TGFα, as 71% of the underexpressed miRNA from this region targeted the TGFα oncogene. These findings support previous observations that overexpression of TGFα in gliomas augments features of tumor proliferation and migration.

CB-12. PREDICTED SIGNALING PATHWAYS INVOLVED IN THE PATHOGENESIS OF MENINGIOMAS AND EGFL6 AS A POTENTIAL NOVEL SERUM BIOMARKER FOR BENIGN MENINGIOMAS
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PURPOSE: In this study, we investigated the possible signaling pathways involved in the tumorigenesis of benign and anaplastic meningiomas. We also found evidence that EGFL6 serves as a potential novel serum biomarker for benign meningiomas. EXPERIMENTAL DESIGN: Differential gene-expression profiles between meningiomas and brain arachnoidal tissue were established through microarray analysis. KEGG pathway analysis was performed to identify potential signaling pathways that may be involved in the pathogenesis of meningiomas. Quantitative real-time PCR (qRT-PCR) was performed to validate the differentially expressed genes in the KEGG pathways. EGFL6 mRNA levels were determined in brain arachnoidal tissue, meningiomas, and other tumors by qRT-PCR. Serum EGFL6 levels were measured via ELISA and compared to the plates and patients with various tumors. RESULTS: Fibroblastic meningiomas exhibited upregulated PI3K/Akt and TGFα signaling pathways and accelerated the G1/S progression cell cycle. Focal adhesion and ECM-receptor interaction pathways were activated in anaplastic meningiomas. The INI1 gene, an INI1 binding partner in meningiomas and ovarian cancers but found in low levels in all other tumors. We also found high levels of EGFL6 protein in serum samples of patients with benign meningiomas (675 pg/mL) and ovarian cancers (617 pg/mL). Healthy people and patients with all other tumors, however, had very low levels of serum EGFL6. CONCLUSIONS: Our results suggest that activation of PI3K/Akt and integrin-mediated signaling pathways are involved in the pathogenesis of benign and anaplastic meningiomas, respectively. These findings also suggest that EGFL6 might serve as a novel serum biomarker for benign meningiomas.

CB-013. MITOSIS INTERFERENCE OF CANCER CELLS BY NOVOTTF-100A DECREASES CELLULAR VIABILITY
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Cancer cell death from conventional cytotoxic chemotherapy and radiation is a result of triggering the cell-cycle checkpoint, with p53 playing a prominent role in orchestrating this effect. Unlike traditional approaches, the NovoTTF-100A device, which has been approved for the treatment of recurrent glioblastomas, kills tumor cells by a novel mechanism that interferes with their proper progression through mitosis. Our research reveals that the tumor-treating field (TTField) affects processes proximal to the metaphase-to-anaphase transition, leading to cell-cycle arrest in anaphase and eventually to cell death. Tissue culture cells exposed to the TTField during mitosis exhibit a variety of outcomes, including cell arrest in anaphase, cytokinesis defects with cytokinetic furrow regression, and slippage out of mitosis without division. Time-lapse movies of cells stained with DRAQ5 revealed membrane blebbing and oscillation, together with failed segregation of sister chromatids from the metaphase plate, both of which were initiated near the time of metaphase-to-anaphase transition. Immunofluorescence of cells after short-term TTField treatment during mitosis showed disordered separation of chromosomes from metaphase plates accompanied by laggard chromosomes, dispersion of chromosomes from the metaphase plate, and chromosome decondensation in the absence of cytokinesis, and daughter cells with asymmetric chromosomal segregation. Flow cytometry also revealed the presence of cells with abnormal DNA content. In contrast to the p53 mutant cell line MDA-MB-231, U87 cells expressing the p53-expressing counterpart p53-expressing U87 cells exhibited Annexin V binding in both TTField exposure during mitosis, suggesting that TTField-induced cellular damage is able to trigger p53-dependent cell death in cells that escape killing during mitosis. These data show that the TTField causes cellular damage during mitosis that validates and raises the hypothesis that the differential response seen in patients are likely rooted in the underlying genetic alterations of the tumors. This study was supported in part by NovoCare, Inc. and A Reason To Ride research fund.
CB-14. VHL AND PPARα ARE RESPONSIBLE FOR MIR-21-REGULATED EGFR/AKT SIGNALING IN THREE “PTEN” DIFFERENT EXPRESSION STATUS GLIOMA CELL LINES
Kaiilang Zhang, Luyue Chen, Junxia Zhang, Zhendong Shi, Lei Han, Peiyu Pu, and Chunsheng Kang; Tianjin Medical University General Hospital, Tianjin, China

The epidermal growth factor receptor (EGFR) is frequently amplified, overexpressed, or mutated in human glioblastomas. MicroRNA-21 (miR-21), shown to target several tumor suppressor genes, functions as an oncogene. In a previous study, we demonstrated that miR-21 controls EGFR/AKT signaling independent of PTEN status, though PTEN is a direct target of miR-21. In the present study, we demonstrated that VHL and PPARα are direct targets of miR-21 and that miR-21 regulates EGFR/AKT signaling partly through the VHL/b-catenin and PPARα/AP-1 pathway. The lentivirus-delivered miR-21 inhibitor impairs the in vitro colony forming and invasion in three PTEN different expression status glioma cell lines. We used a luciferase activity assay to identify VHL and PPARα as two direct targets of miR-21. A Top/Fop luciferase activity experiment showed that miR-21 could regulate EGFR/AKT signaling through the VHL/b-catenin pathway. In miR-21-downregulated glioma cell lines, PPARα siRNA could restore the expression of EGFR and AKT. Our findings suggest that miR-21 regulates EGFR/AKT signaling by targeting VHL and PPARα.

CB-15. GINSENSIDE Rg3-INDUCED APOPTOSIS IN THE HUMAN GLOBLASTOMA U87MG CELLS THROUGH THE MEK SIGNALING PATHWAY AND REACTIVE OXYGEN SPECIES
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OBJECTIVE: Ginsenoside might be an effective substance for cancer prevention. The major active components in red ginseng represent diverse kinds of ginsenosides, including ginsenoside Rg3, Rg5, and Rk1, and each of these ginsenosides has different pharmacological activities. Among them, ginsenoside Rg3, a kind of chemical extracted from Korean red ginseng (Panaxginseng C.A. Meyer), has been reported to control anti-cancer activities through inhibiting angiogenesis and the proliferation of various kinds of cancer cells. However, the effect of ginsenoside Rg3 and its molecular mechanism in glioblastoma multiforme (GBM) is still unclear. In the present study, we investigated the effect of ginsenoside Rg3 on a human GBM cell line and its regulating signaling molecular mechanisms.

MATERIALS AND METHODS: U87MG cells (a human GBM cell line) were treated with ginsenoside Rg3, and we measured cell viability using an MTT [3-(4,5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide] assay and a trypan blue exclusion assay. The apoptotic characteristics were detected by TUNEL [Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling] assay, flow cytometry analysis, and Western blotting. The molecular mechanisms were evaluated through various pre-treated inhibitors (MAPKs inhibitors, general caspase inhibitors, and antioxidants).

RESULTS: Ginsenoside Rg3 inhibited U87MG cell viability in a dose- and time-dependent manner. The apoptotic effect was determined based on the positive stained cells by TUNEL assay, immunocytochemistry, and flow cytometry. Ginsenoside Rg3-induced apoptosis was associated with the MEK signaling pathway and reactive oxygen species. CONCLUSION: These results might suggest that ginsenoside Rg3 induces apoptosis in U87MG cells and that its effect is controlled by the MEK signaling pathway and reactive oxygen species. Therefore, ginsenoside Rg3 could be an innovative chemotherapeutic agent against malignant glioma.

CB-16. STRESS-REGULATED EXPRESSION OF MIR-451 CONTROLS THE PROLIFERATION/MIGRATION DICHOTOMY OF GLOBLASTOMA IN VITRO AND RADIOSENSITIVITY IN RESPONSE TO RADIATION AND CHEMOTHERAPY
Daisuke Ogawa, Jakub Godlewski, Agnieszka Bronisz, and E. Antonio Chiocca; The Ohio State University, Columbus, OH, USA

In rapidly growing tumors such as glioblastoma (GBM), where glucose availability may fluctuate, cells must engage adaptive strategies to survive periods of metabolic stress. We previously identified a pathway mediated by microRNA 451 (miR-451) that not only allows rapid proliferation under favorable conditions (high glucose) but also is essential for survival and cell migration under conditions of glucose starvation through modulating the activity of the LKB1/AKT pathway. Based on our in silico analysis, we selected two candidates for promoting/maintaining glioma cell viability: Oct1 and PPARγ. By a siRNA approach, we found that Oct1 (known to increase anaerobic glucose metabolism, thus promoting tumor glucose-dependency) promotes miR-451 expression, whereas PPARγ, whose agonists are known anti-neoplastic compounds, inhibited miR-451 expression. We have also demonstrated negative feedback between miR-451 and EGFR signaling. Our results show that although miR-451 is essential for tumor progression, it can also render tumor cells less capable of survival under conditions of stress. In fact, when treated with temozolomide or radiation, tumors overexpressing miR-451 exhibit higher migration. We also show that compound C (an AMPK inhibitor), which induces miR-451 expression, sensitizes glioma cells to radiation and chemotherapy. These results suggest the usefulness of miR-451 for boosting therapy effectiveness. Intriguingly, oncogene kinase EphB2, which has pro-angiogenic and proliferating actions in glioma, is a putative target of miR-451. We have shown that miR-451 decreases the EphB2 mRNA level, suggesting that EphB2 is another pathway that explains the observed phenotype. Finally, we also tested the relationship between miR-451 and glucose in vivo. We have published a hyperglycemia model in rats and performed qPCR on brain extracts. miR-451 expression was significantly elevated in hyperglycemic than in normoglycemic brain tissue. Furthermore, global miR expression analysis revealed that miR-451 was 1 of only 12 significantly upregulated miRNAs. Our preliminary findings clearly show the importance of miR-451 in glioma biology.

CB-17. IDENTIFICATION OF GENES INVOLVED IN BRAIN METASTASIS OF THE MOST COMMON CANCERS
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Approximately 40% of patients with systemic cancer will develop central nervous system (CNS) metastases. In adults, metastases to the brain are most commonly from primary tumors of the lung, breast, skin (melanoma), and gastrointestinal tract. Current systemic treatments have failed to prevent the development of CNS metastasis of these malignancies. CNS involvement is almost always fatal, even in the cases in which the primary tumor is under control. Therefore, preventing the development of CNS metastasis would be a great step forward in the treatment of patients with potentially disseminating or disseminated tumors. So far, no therapeutic agents preventing tumor cells from colonizing the brain are available. Our study aims to identify gene expression patterns of various primary tumors involved in the formation of cerebral metastasis and to determine if similar genes are operative. To this end, we compared the gene expression profiles of primary tumors from patients suffering from a particular cancer type and cerebral metastases with the profiles of primary tumors in comparable stages from patients who did not have brain metastases. To investigate whether the identified genes in the primary tumors are still active in the metastases, we also compared the gene expression profiles of cerebral metastases from various primary tumors. This strategy yielded several differentially expressed genes. To validate them, we silenced the genes in the cells line and tested their effect in an in vitro blood brain barrier (BBB) model.

CB-18. MULTI-CYCLE MULTIPLEXED IN SITU IMMUNOFLUORESCENCE ANALYSIS OF 18 BIOMARKERS ON A SINGLE FFPE GLOBLASTOMA TISSUE SECTION
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Glioblastoma multiforme (GBM) is a highly heterogeneous disease at both the cellular and the molecular level. A better understanding and characterization of this inherent heterogeneity could improve diagnostics for determining prognosis and therapy response. We have developed a multi-cycle multiplexed in vitro AT assay that allows in situ analysis of multiple proteins on a single FFPE section. Software tools have been developed to quantify and visualize each protein in the context of the overall tissue morphology. In the current study, we show an example of sequential staining of 18 biomarkers on a single section of tissue metastases constructed from 100 GBM patients. Markers included EGFR, PTEN, Glut1, signaling molecules in PI3K/AKT and TGF-beta pathways, and biomarkers for vessels. The expression pattern of each marker was analyzed, and significant heterogeneity was observed. Some profile markers had a high expression (eg, EGFR), whereas others had a continuous pattern (eg, PTEN). Co-localization of these markers was visualized and demonstrated on single cell/subcellular levels. Further analysis will determine associations with clinical outcome. In summary, we demonstrate the powerful technology that allows multiple biomarkers to be analyzed on single GBM tissue sections, which forms complete characterization of...
CB-19. ENDOGENOUS DOWNREGULATION OF DRUG RESISTANCE GENE MGMT BY MICRORNA
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O6-methylguanine-DNA methyltransferase (MGMT) is related to the resistance of aggressive glioblastoma (GBM) to DNA alkylating agent temozolomide (TMZ) therapy. In this study, we searched for microRNAs that modulate MGMT expression and give better sensitivity to TMZ. Out of tens of candidates generated by several binding site prediction programs, we selected 6 microRNAs that have a complementary seed region with 3'-UTR of MGMT. One out of 6 microRNAs downregulated more than 40% MGMT mRNA and protein expression levels in both the T98G glioma cell line and the primary GBM cell line (GBM30). MicroRNA-transfected T98G cells showed increased sensitivity to TMZ as did U87MG, a cell line that does not express MGMT, and the T98G cells transfected with siRNA of MGMT. MNSide mice with microRNA-expressing GBM30 transplants showed better survival with injection of TMZ in vivo. Thus, this endogenous mechanism of suppressing the drug-resistant gene MGMT will increase chemosensitivity to TMZ. Our results suggest a direct interaction between microRNA and MGMT and describe the function of that microRNA in drug resistance. Our findings suggest a new target to enhance the efficacy of chemotherapy.

CB-20. HYPOXIA INDUCES CELL PROLIFERATION, EXPRESSION OF ANGIOGENIC FACTORS, AND PRIMITIVE NEURONAL MARKERS IN NORMAL GLIAL CELLS
Lana Leis, Ivana Manni, and Per Øyvind Enger; University of Bergen, Bergen, Norway

Cancer-associated fibroblasts (CAFs) have been shown to foster tumor growth in several solid cancers. In the brain, the stroma is mainly composed of glial cells such as astrocytes and oligodendrocytes whose role in brain tumor-stroma interactions is poorly characterized. We previously identified a unique gene-expression profile of tumor-associated glial cells (TAGs) by fluorescence activated cell sorting (FACS) of glial cells from GFP/Nod Scid mice engrafted with human glioblastoma biopsies. Although little is known about the mechanisms mediating these expression changes, hypoxia is a prominent feature of most malignant brain tumors. Thus, we investigated how hypoxia affected the phenotype of glial cells from normal mouse brains. Briefly, mouse brain tissue was enzymatically digested to obtain single-cell suspensions, and then glial cells were isolated by FACS with simultaneous removal of immune cells (CD11b+ and endothelial cells (CD31+). These cells were then seeded at a density of 50 000 cells/well in a 48-well plate and incubated under either normoxic (21% oxygen) or hypoxic (0.5% oxygen) conditions for 7 days. Notably, glial cells upregulated Sox2, HIF-2a, Nestin, and Vimentin in hypoxic not normoxic conditions. In addition glial cells also upregulated angiogenic factors such as VEGF, Angiopoietin 2, and FGF2. Furthermore, cell-cycle analysis revealed a significantly higher fraction of cycling cells in hypoxia than in normoxia. These changes strongly resembled the changes observed in tumor-associated glial cells, suggesting that hypoxia is a major contributor to the phenotype of tumor-associated glial cells. Ongoing work aims at further characterizing the TAG population to reveal its cell composition and to address the role of various subpopulations of glial host cells.

CB-21. β-CATENIN SIGNALING INITIATES ASTROCYTE ACTIVATION AND CONTRIBUTES TO THE PATHOGENESIS OF GLIOMAS
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Astrocytes, the most abundant cells in the central nervous system (CNS), respond to a variety of stress and pathologic conditions through a process known as astrocyte activation. Dysregulation of the molecular pathways involved in this process is thought to lead to an aggressive cellular state associated with neoplasia. We investigated whether a comparable correlation exists between the response of astrocytes to injury and the malignant phenotype of gliomas. We found that loss of contact inhibition plays a critical role in the initiation and regulation of reactive astrocytes during wound healing. Specifically, interruption and reversal of axon guidance cues at the cell membrane lead to nuclear translocation of β-catenin and a subset of characteristic changes associated with cell activation. We further identified similar expression changes and dysregulated signaling pathways in gliomas and glioblastoma-derived stem cells. β-catenin signaling is critical for cell proliferation since inhibition of β-catenin diminished activation of both normal astrocytes and the malignant phenotype of gliomas. These findings provide a unique mechanism of astrocyte activation and shed light on the pathogenic mechanism of glioma, which may help to develop diagnostic and therapeutic targets.

CB-22. FEASIBILITY OF AN INTRAMOLECULAR COMPLEMENTATION STRATEGY FOR SPVT-REPORTER GENE IMAGING OF DRUGGABLE PROTEIN MISFOLDING IN BRAIN CANCER
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INTRODUCTION: Misfolded proteins may lead to brain cancer through loss-of-function phenotype for VHL and NF2, through dominant-negative expression of p53, or through cooperation or activation of Src family kinases. We preliminary demonstrate a new generalizable application of a protein-fragment complementation assay based on a split bioluminescence reporter (synthetic humanized Remila luciferase [hRluc]) for potential future imaging of protein misfolding. We have hypothesized that the normal conformational changes in protein folding, which result in close approach of amino and carboxy termini, can be used to drive intramolecular complementation of correctly oriented chimeric split imaging reporters in a strategy to pre-clinically image and quantify effects of new drugs on protein misfolding in brain cancer animal models. METHODS AND RESULTS: We used split hRluc to indirectly record the folding of 3 test proteins: the mutant (sr39) HSV1 thymidine kinase (HSV1-TK), firefly luciferase, and enhanced green fluorescent protein (EGFP) and enhanced green fluorescent protein (EGFP) in mammalian cells. We first engineered a mutant EGFP (meEGFP) that deliberately misfolds and, then we constructed the following expression vectors: NhRluc—sr39TK—ChRluc, ChRluc—sr39TK—NhRluc, NhRluc—EGFP—ChRluc, NhRluc—meEGFP—ChRluc, and NhRluc—Fluc—ChRluc. We transiently transfected 293T cells and assayed them for in vitro complemented hRluc and, separately, folded protein activities using luminometry, or TK uptake assay of cell lysates, or fluorescence microscopy of EGFP labeled cells. Co-transfected NhRluc—TK and TK—ChRluc were used as positive controls. Activity of hRluc in RLU/microgram protein/minute was NhRluc—TK plus TK—ChRluc (82.9 ± 8.8), ChRluc—sr39TK—NhRluc (1.7 ± 0.1), NhRluc—meEGFP—ChRluc (1.6 ± 0.1), NhRluc—sr39TK—ChRluc (1.2), NhRluc—EGFP—ChRluc (90.3 ± 5.8), and NhRluc—Fluc—ChRluc (14.9 ± 2.0). CONCLUSIONS: Our preliminary in vitro and cell culture experiments to detect protein folding are encouraging. Further studies are necessary to optimize this strategy prior to animal imaging. The future ability to detect, locate, and quantify protein misfolding in vivo and to develop new targeted therapies will have important implications for a wide variety of research endeavors in drug discovery and molecular neuro- oncology.

CB-23. THE DEVELOPMENTAL LINEAGE MARKER PAX2 IS EXPRESSED IN CNS HEMANGIOBLASTOMAS IN VON HIPPEL-LINDAU DISEASE
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INTRODUCTION: Von Hippel-Lindau (VHL) disease is a neoplasia syndrome resulting from a germline mutation in the VHL gene, followed by a “second hit” in the wild-type allele. Affected individuals may develop hemangioblastomas (HBs) in the cerebellum, brainstem, spinal cord, and retina, as well as tumors of the inner ear and kidney. HBs, the most common manifestation of VHL disease, are benign tumors classified as “of unknown histogenesis.” Expression of HB and expression of PAX2, a developmental marker (eg, SCL or Notch) indicates that HBs arise from pluripotent embryonic cells. Paired box protein 2 (PAX2) is a transcription factor essential for proper lineage commitment during the development of the hindbrain, spinal cord, eye, inner ear, and kidneys, all of which contain or are derived from HNs. These findings provide a unique mechanism of astrocyte activation and shed light on the pathogenic mechanism of glioma, which may help to develop diagnostic and therapeutic targets.
GLIOBLASTOMA

CB-25. UNRAVELLING ACQUIRED RESISTANCE IN GLIOBLASTOMA

Together, these findings suggest that, in meningiomas, deregulation of the Hippo signaling pathway comprises a kinase cascade that culminates with the phosphorylation and inhibition of Yes-associated protein 1 (YAP1). Phospho-YAP1 is retained in the cytoplasm, but in the absence of Hippo signaling, YAP1 translocates to the nucleus, associates with co-activators TEAD1-4, and functions as a transcriptional factor promoting the expression of key target genes. Loss of the NF2 tumor-suppressor gene is the most common genetic alteration in meningiomas, and the NF2 gene product Merlin acts upstream of the Hippo signaling pathway.

We also tested specimens for MLH1 promoter methylation and the regulation variability. Despite undeniable progress in the care and treatment of primary brain tumors, most patients diagnosed with glioblastoma (GBM), prognosis is poor, and few survive more than 3 years. GBM is highly refractory to the standard treatment involving maximal surgical resection followed by treatment with the alkylating agent temozolomide (TMZ) combined with radiotherapy (RT). This study aimed at identifying the most suitable reference genes identified by interfering with histone deacetylases.

CB-26. ACQUIRED RESISTANCE TO ERLOTINIB (TARCEVA®) IN AN EGFR-AMPLIFIED GLIOBLASTOMA MODEL: A CRITICAL ROLE FOR EGFRVIII AND HISTONE ACETYLATION

CB-24. YES-ASSOCIATED PROTEIN 1 AND ITS ONCOGENIC FUNCTION IN MENINGIOMAS

The deregulation of the Hippo pathway has been implicated in several human cancers and seems to alter the balance of cell proliferation and apoptosis, promoting tumorigenesis. The core components of this signaling pathway comprise a kinase cascade that culminates with the phosphorylation and inhibition of Yes-associated protein 1 (YAP1). Phospho-YAP1 is retained in the cytoplasm, but in the absence of Hippo signaling, YAP1 translocates to the nucleus, associated with co-activators TEAD1-4, and functions as a transcriptional factor promoting the expression of key target genes. Loss of the NF2 tumor-suppressor gene is the most common genetic alteration in meningiomas, and the NF2 gene product Merlin acts upstream of the Hippo pathway. In this study, we show that primary meningioma tumors have high nuclear expression of YAP1. In meningioma cells, Merlin expression is associated with phosphorylation of YAP1. Using a siRNA transient knockdown of YAP1 in NF2 mutant meningioma cells, we show that suppressing YAP1 impaired cell proliferation and migration. Conversely, YAP1 overexpression strongly augmented cell proliferation and anchorage-independent growth and restricted cisplatin-induced apoptosis. In addition, expression of YAP1 in nontransformed arachnoidal cells led to the development of tumors in nude mice. Together, these findings suggest that, in meningiomas, deregulation of the Hippo pathway is largely observed in primary tumors and that YAP1 functions as an oncogene that promotes meningioma tumorigenesis.

CB-27. ACTB and SDHA ARE SUITABLE ENDODGENOUS REFERENCE GENES UNDER DIFFERENT TREATMENT REGIMENS FOR GENE EXPRESSION STUDIES IN ANGIOCYSTOMAS USING QUANTITATIVE REAL-TIME REVERSE-TRANSCRIPTION-POLYMERICASE CHAIN REACTION

Quantitative real-time reverse-transcription PCR (qPCR) is frequently used as a research tool in experimental oncology. This qPCR analysis was performed to test a panel of the six most suitable reference genes identified by other studies as representing different physiological pathways (ACTB, GAPDH, POLR2A, RPL13A, SDHA, and TBP) in all common glioma groups: diffuse astrocytoma WHO grade II, anaplastic astrocytoma WHO grade III, and secondary glioblastoma WHO grade IV with and without chemotherapy. Only ACTB, GAPDH, and RPL13A are expressed at higher levels than SDHA, POLR2A, and TBP. The candidate genes were not differentially expressed between primary and secondary glioblastomas. Furthermore, they remained stable before and after radiotherapy and/or chemotherapy. Only ACTB, GAPDH, and RPL13A showed significantly different expression levels between astrocytoma grade II and primary glioblastoma; however, these groups are unlikely to be compared. Therefore, they are adequate references for glioma gene expression studies. Using NeuroCyto EM Compare different glioma entities, we found that SDHA and ACTB are the most stable reference genes. Our data revealed the lowest intragroup variation in SDHA and the highest in RPL13A. In summary, ACTB and SDHA exhibited the best stability values and showed the lowest intergroup expression variability.
CB-28. MAST CELLS ARE TUMOR-DERIVED IN VON HIPPEL-LINDAU DISEASE CNS HEMANGIOBLASTOMAS
Nancy A. Edwards, Russell R. Lonser, and Marsha J. Merrill; National Institutes of Health, Bethesda, MD, USA

INTRODUCTION: Von Hippel-Lindau (VHL) disease is a tumor syn-
drome resulting from an inherited mutation in the VHL gene followed by a
mutation in the other allele. Central nervous system (CNS) hemangioblas-
tomas (HBs) of the cerebellum, brainstem, and spinal cord are a major in-
festation of this disease. HBs are benign tumors comprising two predomi-
ant cell types: tumor stromal cells and vascular cells. Several reports have also noted abundant mast cells in HBs, although the significance and
source of these cells remain unknown. HBs likely arise from pluripotent
embryonic hemangioblasts, and some of these tumor cells have the capacity
to differentiate in vitro into erythroid, granulocytic, and endothelial pro-
genitor cells. We hypothesized that mast cells in HBs are derived from
VHL-deficient tumor cells. METHODS: HB surgical specimens were
frozen, sectioned, and stained for either mast cell tryptase or inhibin to visu-
alize mast and tumor stromal cells, respectively. Each cell type exhibited a
distinct morphology, allowing selective microdissection. Lymphocytes were
harvested from the peripheral blood from the same patient. For each
patient, DNA was extracted from the 3 cell types (tumor, mast, and lympho-
cyte) and analyzed for loss of heterozygosity (LOH) by polymerase chain re-
action (PCR) using primers that flank the VHL gene. RESULTS: In all four
VHL-deficient tumors, mast cells and stromal cells exhibited an im-
paired capacity to proliferate, migrate, and invade. These data suggest that
VHL-deficient HB stromal cells are pluripotent embryonic cells and that
some of these tumor cells have the capacity to differentiate along multiple
hematopoietic lineages in vivo.

CB-29. DUAL ROLE OF MRK IN GLIOBLASTOMA INVASION AND RADIO-RESISTANCE
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Glioblastoma multiforme (GBM), the most aggressive tumor of the central
nervous system, has poor prognosis both because of diffuse infiltration of
tumor cells in the normal parenchyma and resistance to radiation treatment
and chemotherapy. We found that MRK, a stress-activated MAP3K protein
kinase, is overexpressed in GBM compared with normal brain tissue, evident
in microarray expression data and confirmed by immunohistochemical anal-
ysis. We have also shown that knockdown of MRK in GBM cell lines
was associated with a 43% reduction in proliferation of PD- cells. In addition,
knockdown of MRK in GBM cells decreased migration and invasion abilities in both of the cell lines. The tumor suppressor gene PTEN was
demonstrated to have a synergistic effect, extending survival by more than 35
days. In conclusion, these data previously showed that genomic amplification of PDE1C in approximately 90%
high grade astrocytoma (HGA) cultures using array CGH analysis. These
data suggested that PDE1C could be involved in the development of HGA
as a novel therapeutic target. In the current study, in a panel of 15 short-term HGA cultures (with known PDE1C
copy numbers), PDE1C expression levels were determined by QPCR and
correlated with aCGH data. In 6 of these cultures, PDE1C was depleted using
RNAi. PDE1C expressing (PD+) and nonexpressing (PD−) cells were em-
ployed both to study intracellular levels of cAMP and cGMP and to assess
their capacity to proliferate, migrate, and invade. PDE1C-specific RNAi re-
sulted in almost 90% depletion of PDE1C mRNA levels. We detected ap-
proximately a 43% reduction in proliferation of PD− cells. In addition,
PD+ cells showed less migratory and invasive capacity [almost 45% reduc-
tion]. We noted that these effects were associated with an accumulation of intracellular cAMP and cGMP levels. Comparison of whole genome expression analysis (Affymetrics HG-U133 plus2) between PD+ and PD− cells was
carried out for 2 cultures (IN1472 and IN1760). A fold change in expression
of 2 or more and a P value of less than 0.05 were considered significant.
Differentially expressed genes that were common between IN1472 PD +
and IN1760 PD +/PD− cells included APOE, HSD11B1, MMP1, and ST6GLNAC5, alongside other genes that affected similar cellular path-
ways. These genes are involved in processes affecting metabolism, prolifer-
ation, migration, and invasion of cells. Therefore, PDE1C has the potential
to be a novel therapeutic target in HGA.

CB-30. DEPLETION OF CA2 + /CAM-STIMULATED PHOSPHODIESTERASE 1C (PDE1C) ATTENUATES
PROLIFERATION, MIGRATION, AND INVASION OF ADULT HIGH-GRADE ASTROCYTOMA CELLS
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Cyclic nucleotides (cAMP and cGMP) are critical intracellular second
messengers involved in the transduction of a diverse array of stimuli. The ca-
tabolism of cyclic nucleotides is mediated by phosphodiesterases (PDEs).
PDE1C has a putative role in mediating radioresistance and invasion. We pre-
viously showed that genomic amplification of PDE1C in approximately 90%
high grade astrocytoma (HGA) cultures using array CGH analysis. These
data suggested that PDE1C could be involved in the development of HGA
as a novel therapeutic target. In the current study, in a panel of 15 short-term HGA cultures (with known PDE1C
copy numbers), PDE1C expression levels were determined by QPCR and
correlated with aCGH data. In 6 of these cultures, PDE1C was depleted using
RNAi. PDE1C-expressing (PD+) and nonexpressing (PD−) cells were em-
ployed both to study intracellular levels of cAMP and cGMP and to assess
their capacity to proliferate, migrate, and invade. PDE1C-specific RNAi re-
sulted in almost 90% depletion of PDE1C mRNA levels. We detected ap-
proximately a 43% reduction in proliferation of PD− cells. In addition,
PD+ cells showed less migratory and invasive capacity [almost 45% reduc-
tion]. We noted that these effects were associated with an accumulation of intracellular cAMP and cGMP levels. Comparison of whole genome expression analysis (Affymetrics HG-U133 plus2) between PD+ and PD− cells was
carried out for 2 cultures (IN1472 and IN1760). A fold change in expression
of 2 or more and a P value of less than 0.05 were considered significant.
Differentially expressed genes that were common between IN1472 PD +
and IN1760 PD +/PD− cells included APOE, HSD11B1, MMP1, and ST6GLNAC5, alongside other genes that affected similar cellular path-
ways. These genes are involved in processes affecting metabolism, prolifer-
ation, migration, and invasion of cells. Therefore, PDE1C has the potential
to be a novel therapeutic target in HGA.

CB-31. RADIATION-INDUCED HIF-1α INDUCES RADIORESISTANCE AND ENHANCES THE MIGRATION
ABILITIES IN GLIOBLASTOMA CELLS THROUGH THE PTEN-SRC-HIF-1α SIGNALING AXIS
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PURPOSE/OBJECTIVE: Glioblastoma is one of the most radioresistant
tumors, and hypoxic regions are commonly observed in it. Hypoxia induc-
fable factor-1α (HIF-1α), a molecular marker of hypoxia, is expressed in gli-
oblastoma and is associated with poor survival. Recent studies have shown that HIF-1α is induced by irradiation even under normoxic conditions, al-
though the mechanism and the role are still unclear. We investigated the as-
sociation between radiation-induced HIF-1α and its role in glioblastoma cells.
MATERIALS/METHODS: We used a shRNA transfection approach to
knock down HIF-1α expression in two established cell lines (U87 and
LN18). A clonogenic survival assay and an MTS assay were used to evaluate
cell survival and radiosensitivity after irradiation. The migration and inva-
sion ability were evaluated by a wound-healing assay and a matrigel invasion
chamber assay. The protein expressions were analyzed with Western blots.
RESULTS: HIF-1α was increased in irradiated LN18 and U87 and
knocked down by shRNA. Knockdown of HIF-1α decreased the radiosresis-
tance significantly in LN18 but only slightly in U87. Knockdown of HIF-1α
also decreased migration and invasion abilities in both of the cell lines.
Knockdown of HIF-1α increased the expression of PTEN in U87 (wild-
specy PTEN) but not in U87 (mutant PTEN). The phospho-Tyr (416) was less
observed in HIF-1α knockdown LN18 than in normal LN18. CONCLUSIONS: These results suggest that radiation-induced HIF-1α is as-
associated with radiosensitivity and radiation-induced migration and invasion
abilities in glioblastoma cells. The degree of the reduction of radiosensitivity was different between the two cell lines. The tumor suppressor gene PTEN and oncogene Src might be associated with this mechanism.

CB-32. DETECTION OF “ONCOMETABOLITE” 2-HYDROXYGLUTARATE BY MAGNETIC RESONANCE
ANALYSIS AS A BIOMARKER OF IDH1/2 MUTATIONS IN GLIOMA
Juliva Kalinina1, Anne Carroll1, Liya Wang1, Qui Yang1, Danny E. Manchenco1, Xiaoqiong Wu1, Frank Liu1, Jun Ahn1, Hao He1, Hu Mao1, and Erwin G. Van Meir1; 1Emory University School of Medicine, Atlanta, GA, USA; 2Emory University, Atlanta, GA, USA

PURPOSE: We aimed to develop a magnetic resonance (MR)-based methodology for detecting 2-Hydroxyglutarate (2HG) as a biomarker for isocitrate dehydrogenase (IDH1/2) mutations in human gliomas. EXPERIMENTAL DESIGN: We used a study set of 65 human glioma PD- vs. representative of the different histologies and grades. In 10

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non-tumoral brain samples. The mutation status of hotspot codons in IDH1/2 genes was determined by sequencing and immunohistochemistry. Metabolic profiles of tissue samples were analyzed by solid-state high-resolution NMR (HS-HR-NMR). The purified 2H2O sample was initially used to identify the 2H2O-specific spin-system and proton resonances using one- (1D) and two-dimensional (2D) correlation spectroscopic (COSY) methods. Then, using the 2H2O-specific cross-peak pattern, the sample was isolated from the 2H2O-H2O mixture. The 1HMR spectrum of each tissue sample was identified and subsequently quantified using the signal integrals in 1D spectra. RESULTS: Somatic mutations in IDH1/2 were detected in 37 (57%) of the 65 tumors analyzed, all of which showed elevated levels of 2H2O (1-11 mM) by NMR. The levels of 2H2O in the 28 mutant-negative tumors were at (n = 1) or below (n = 27) the quantification limit (0.1 mM) but were not detectable in all 10 non-tumoral controls. The sensitivity and specificity of detecting 2H2O in IDH1/2 mutation-bearing samples by the 2D COSY reached 96% and 95.2%, respectively. The overall accuracy of identifying IDH1/2 mutations by NMR using 2H2O as a biomarker was 97.8%. CONCLUSIONS: This study demonstrates the feasibility of using MR detection of 2H2O for identifying IDH1/2 mutation-positive brain tumors and opens up the possibility of developing analogous noninvasive MR-based imaging and spectroscopy for detecting 2H2O in direct brain tumors.

CB-33. EPHA2/EPHRINA1 SYSTEM IN CONJUNCTION WITH TKS5 REGULATES INVADOPODIA FORMATION IN GBM CELLS

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EphA2 receptors are overexpressed in glioblastoma (GBM) cells but not in normal brain tissue. EphA1 binds to and downregulates EphA2 receptors, delaying cell migration and invasion. Invadopodia are electron-dense, actin-rich protrusions that bud from to and proteolytically degrade extracellular matrix (ECM) proteins. In this study, we explored the role of EphA2/ ephrinA1 complexes in invadopodia formation and matrix degradation. We also investigated the activation of Tks5, a substrate of the protooncogene Src and an adaptor protein, which is overexpressed in GBM and is linked to patient survival. Invadopodia formation and matrix degradation were assessed by an in situ gelatin zymography assay combined with fluorescence microscopy. GBM cells form invadopodia-like structures that degrade the ECM; the Snb-19 cell line exhibits the greatest degradation activity. Expression of the EphA2 receptor was examined by immunoblot analysis using parental, vector control, and ephrinA1-expressing U-251 cells, and A-172, G48a, and Snb-19 GBM cells. Src-transformed NIH3T3 (Src3T3) cells were used as a positive control. Unexpectedly, immunoblot analysis showed low levels of EphA2 among the cells that most degraded the ECM, such as Snb-19 cells, and high levels among cells that least degraded the ECM, such as G48a cells. Accordingly, diminishing levels of EphA2, either by knockdown of its ligand and activator, or by genetic down-regulation of EphA2, demonstrated that EphA2 is inversely correlated with matrix degradation and EphA2 expression. This inverse correlation is also seen between EphA2 and Tks5, suggesting independence of these two signaling pathways. These findings have potentially important implications concerning the role of the EphA2/ ephrinA1 complex and Tks5 in brain tumor migration and invasion.

CB-35. ECTOTIC OVEREXPRESSION OF MIIR15 COMPROMISES THE INVASIVE ABILITY OF MALIGNANT GLIOMAS BY MODULATING NEDD9

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Recent results define microRNA 145 (miR145) as a tumor suppressor implicated in proliferation, invasion, and stemness of cancer stem cells (Zheng et al., 2011). We confirm that miR145 is strongly downregulated in glioblastoma (GB) specimens and in corresponding GB stem-like cells (GSCs) compared with normal brain tissue (P < 0.001). We also observed that microRNA-145 (miR-145), a low-grade glioma (LGG) marker, is overexpressed in high-grade GSCs (P < 0.03). After comparing the results of a microarray analysis performed on GSCs overexpressing miR145 (miRover-GSCs) with Empty-GSCs, we identified relevant differences in gene-expression profiles, including genes involved in proliferation, invasion, and stemness. We focused on HIF1α (GLI/NEDD9), a scaffold protein involved in invasion and survival of cancer cells (O'Neil, 2007). We confirmed by RT-PCR a significant down-modulation of NEDD9 expression in miRover-GSCs (P = 0.0001). Moreover, NEDD9 is upregulated in GB and in GSCs compared with LGGs (P = 0.015). About $30\%$ of GSCs expressed higher levels of NEDD9 than normal brain tissue (2.3 ± 0.8), and progression-free survival (PFS) of these patients was shorter than that of patients with a lower expression of NEDD9 (0.8 ± 0.3; median PFS 41 vs 82 months, respectively, P = 0.04). We also observed that miR145 overexpression in GSCs affects tumor development after intracranial injection in nude mice. Mice injected with miRover-GSCs survived significantly longer than mice injected with Empty-GSCs (P = 0.03). Notably, histological characterization and quantification showed that NEDD9 is significantly downregulated in gliomas from miRover-GSCs compared with controls (P = 4.9 ± E-07). In conclusion, our results show an important involvement of miR145 in regulating GB invasion and a potential role of NEDD9 as a biomarker for glioma progression.

CB-36. THE EFFECTS OF CD44 SILENCING ON CYTOSKELETAL PROTEINS: A POSSIBLE MECHANISM OF CD44’S INVOLVEMENT IN GLIOMA CELL INVASION

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The complex machinery of cellular invasion involves key players such as cell-adhesion molecules (CAMs), the extracellular matrix (ECM), and the cytoskeleton. Components of the CD44-ephrinA1 system in invadopodia function and promotes invasion through its interaction with hyaluronic acid (HA) and other ECM components. To further identify potential mechanisms involved in CD44’s promotion of glioma invasion, we examined the expression of several cytoskeletal-associated proteins. Here, we report the possible association of CD44 with the cytoskeleton dynamics in glioblastoma (GBM) cells. Snb-19, a grade IV GBM, was used to investigate the effects of CD44 silencing on F-actin, Vimentin, GFAP, and microtubules through immunocytochemistry (ICC), flow cytometry, and Western blotting. Variation in morphology and distribution of CD44 and the cytoskeleton-linked proteins were demonstrated by ICC. CD44 was well distributed on the surface of wild-type Snb-19 cells, with higher expression at the peripheral edges, suggesting an essential link between CD44 and the cytoskeleton. siRNA CD44-treated cells showed rather faint staining and rounded cells devoid of any processes. Disorganized ruffles of F-actin were particularly detected in knockdown cells. The consequent decline in the above-mentioned protein levels following siRNA CD44 treatment was observed by Western blotting. Significant decreases in the expression levels in knockdown cells was confirmed by flow cytometry. There was a marked decline in the expression levels of CD44 from 98.44 % to 74.13%, of F-actin from 99.77% to 70.35%, of microtubules from 99.50% to 76.54%, of GFAP from 92.28% to 72.84%, and of vimentin from 97.32% to 68.61% in Snb-19 wild-type compared with siRNA CD44 silenced cells (P < 0.05). We previously demonstrated that CD44 influences glioma cell invasion. The results from the present study provide information on glioma cell morphology and cytoskeleton-associated proteins that are more differentially expressed when CD44 is silenced. Current studies are underway to identify signaling pathways associated with CD44 and glioma cell invasion.

CB-37. TEMOZOLOMIDE ACTIVATES UPSTREAM REGULATORY KINASES OF NA+K+-2CL–COTRANSPORTER ISOFORM 1 IN GLIOBASTOMA CANCER CELLS

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Na+-K+-2Cl– cotransporter isoform 1 (NKCC1) plays a role in glioma migration. We recently reported that NKCC1 is the primary cell volume regulatory protein to maintain K+, Cl–, and volume homeostasis against temozolomide (TMZ)-induced apoptotic volume decrease and to promote survival of glioma cells (GCs). However, it is not well understood how NKCC1 is regulated in GCs after TMZ treatment. With no-lysine kinases (WNK1) and oxidative stress-responsive kinase-1 (OSR1) are likely links between WNK1 and NKCC1 to regulate volume homeostasis. We recently reported that NKCC1 is the primary cell volume regulatory protein to maintain K+, Cl–, and volume homeostasis against temozolomide (TMZ)-induced apoptotic volume decrease and to promote survival of glioma cells (GCs). However, it is not well understood how NKCC1 is regulated in GCs after TMZ treatment. With no-lysine kinases (WNK1) and oxidative stress-responsive kinase-1 (OSR1) are likely links between WNK1 and NKCC1 to regulate volume homeostasis. Therefore, we further investigated the role of NKCC1-mediated signaling transduction pathways in regulation of NKCC1 in response to TMZ treatment. Exposure of GCs to TMZ triggered a concurrent activation of NKCC1, SPAK, OSR1, and NKCC1 detected with an increased protein...
CB-38. UNEXPECTED NUCLEAR SUBLOCALIZATION OF THE TYROSINE KINASE RECEPTOR TIE2 INDUCES RADIORESISTANCE OF MALIGNANT GLIOMAS
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Striking radioresistance of malignant gliomas accounts for the inevitable recurrence and the subsequent poor prognosis associated with this disease. The development of new therapeutic strategies requires identifying the key molecular pathways that regulate the resistant phenotype of malignant gliomas. Previous work from our laboratory showed that the tyrosine kinase receptor Tie2 is expressed in brain tumor stem cells. Current knowledge supports a significant role of this stem cell population in the recurrence and relapse of malignant gliomas after radiotherapy. Here, we have shown that irradiation (IR) induces trafficking of a tyrosine kinase receptor Tie2 into the nucleus, where it hyperphosphorylates and localizes as a full-length protein into the nucleus. Nuclear Tie2 binds H2AX, one of the key DNA repair proteins, as part of the DNA repair foci, as assessed by co-immunoprecipitation and confocal microscopy. Of clinical significance, the presence of Tie2 in the DNA-repair foci, as assessed by co-immunoprecipitation and Western blot analysis showed that upon IR, Tie2 is hyperphosphorylated in brain tumor stem cells. This observation was surprising, but it has important implications for the understanding of the ligand-receptor dynamics and for the future production of novel EphA1-based, EphA2-targeted therapeutics.

CB-39. EFFECTS OF β3-ADRENERGIC RECEPTOR AGONIST ON GENE EXPRESSION OF LEPTIN IN GLIOBLASTOMA
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In the 25 years since temozolomide entered phase I clinical trials, few new primary or adjuvant therapies have been developed for the treatment of glioblastoma multiforme (GBM) tumors. Our laboratory has been exploring novel methods for the treatment of GBMs. Recent studies indicate that the expression of the hormone leptin and its receptor (OB-R) increases in gliomas and positively correlates with the malignancy of the tumor. Interestingly, β3-adrenergic receptor agonists are known to decrease leptin expression in adipocytes but have not been examined in GBM cells. We hypothesized that β3-adrenergic agonists downregulate the expression of leptin and its receptor. In our studies, the relative expression of leptin, OB-R, and β3-adrenergic receptor were measured in the GBM cell lines T98G and LN229. The results showed that upon IR, Tie2 is hyperphosphorylated and localized as a full-length protein into the nucleus. Nuclear Tie2 binds H2AX, one of the key DNA repair proteins, as part of the DNA repair foci, as assessed by co-immunoprecipitation and confocal microscopy. Of clinical significance, the presence of Tie2 in the DNA-repair foci, as assessed by co-immunoprecipitation and Western blot analysis showed that upon IR, Tie2 is hyperphosphorylated in brain tumor stem cells. This phenomenon seems to be ligand-dependent, as increased levels of Angiopoietin1 (Ang1) are observed after ionizing radiation. In addition, blocking the Ang1/Tie2 interaction by using siRNA against Tie2 or soluble Tie2 as a decoy receptor results in radiosensitization of the malignant glioma cells. Similarly, the use of a Tie2 chemical inhibitor reverts their radioresistance. Collectively, our data present solid evidence of the trafficking of a tyrosine kinase receptor Tie2 into the nucleus, where it exerts a new role in the DNA repair mechanism and in the radioresistance of brain tumor stem cells. These results encourage the design of Tie2-targeting combinational therapies, such as Tie2 inhibitors and radiotherapy, for patients with glioblastomas.

CB-40. GLYCOSYLATION IS CRITICAL FOR EPHRIN A1 PRODUCTION AND EPHA2 ACTIVATION
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INTRODUCTION: EphrinA1, a member of the ephrin family of ligands that interacts with the Eph-related tyrosine kinase receptors, specifically binds to EphA2 receptor and is highly overexpressed in glioblastoma (GBM) but not in normal adult brain tissue. This binding induces a receptor-mediated internalization and downregulation and subsequently suppresses tumors, making EphrinA1 an attractive target for the development of new anti-glioma therapeutics. In the crystal structure, EphrinA1, in complex with EphA2, was shown to be glycosylated on Asparagine 76; however, no data available confirm the presence or explains the importance of this glycosylation. We have thus initiated a study to investigate the role of glycosylation in EphrinA1 biological activity. METHODS: 6xHis-tag ephrinA1 and EphrinA1-Fc were produced in S99 insect cells using the baculovirus expression system. Proteins were purified by protein G and Ni-NTA affinity chromatography, using FPLC. EphrinA1 activity was evaluated in U-251 MG GBM cells by examining cell morphology and EphA2 receptor downregulation. Enzymatic deglycosylation with PNGaseF and ENDO H was performed as recommended by the manufacturer. RESULTS: Enzymatic deglycosylation with PNGaseF and ENDO H decreased the molecular weight of EphrinA1 as detected by SDS-PAGE. Western blot analysis confirmed the cleavage by PNGaseF (EphrinA1 + 1) conditioned media. Unglycosylated mutants of EphrinA1 (EphrinA1-N26A/N26Q/N26D) overexpressed in U-251 cells were almost completely retained by cells and minimally released into the medium. Immunofluorescence studies using confocal microscopy and subcellular fractionation followed by Western blot analysis revealed that EphrinA1 was transported to lysosomes in EphrinA1 overexpressing cells. CONCLUSIONS: Glycosylation is essential for proper production of EphrinA1 and its release from cells and is possibly involved in EphA2 receptor activation.

CB-41. GALECIN-3 SELECTIVELY KILLS BRAIN TUMOR CELLS THROUGH N-GLYCAN DEPENDENT BINDING OF β1 INTEGRIN AND INDUCTION OF APOPTOSIS
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Galecin-3 (Gal3), the only chimeric family member of the galectin family, consists of a collagen-like-N-terminal region and a C-terminal carbohydrate recognition domain (CRD), which has binding affinity for galactose and N-acetylgalactosamine. Gal3 is secreted into a Golgi-independent pathway. We found that extracellular Gal3 can specifically induce apoptosis in a variety of tumor cell types including malignant glioma cells. We hypothesized that Gal3-mediated tumor cell killing was mediated by the interaction (CRD), which has binding affinity for galactose and N-acetylgalactosamine. In our studies, we have identified the critical role of glycosylation in the binding of Gal3 to the EphA2 receptor that is highly overexpressed in glioblastoma tumors, making EphrinA1 an attractive target for the development of new anti-glioma therapeutics. In the crystal structure, EphrinA1, in complex with EphA2, was shown to be glycosylated on Asparagine 76; however, no data available confirm the presence or explains the importance of this glycosylation. We have thus initiated a study to investigate the role of glycosylation in EphrinA1 biological activity. METHODS: 6xHis-tag ephrinA1 and EphrinA1-Fc were produced in S99 insect cells using the baculovirus expression system. Proteins were purified by protein G and Ni-NTA affinity chromatography, using FPLC. EphrinA1 activity was evaluated in U-251 MG GBM cells by examining cell morphology and EphA2 receptor downregulation. Enzymatic deglycosylation with PNGaseF and ENDO H was performed as recommended by the manufacturer. RESULTS: Enzymatic deglycosylation with PNGaseF and ENDO H decreased the molecular weight of EphrinA1 as detected by SDS-PAGE. Western blot analysis confirmed the cleavage by PNGaseF (EphrinA1 + 1) conditioned media. Unglycosylated mutants of EphrinA1 (EphrinA1-N26A/N26Q/N26D) overexpressed in U-251 cells were almost completely retained by cells and minimally released into the medium. Immunofluorescence studies using confocal microscopy and subcellular fractionation followed by Western blot analysis revealed that EphrinA1 was transported to lysosomes in EphrinA1 overexpressing cells. CONCLUSIONS: Glycosylation is essential for proper production of EphrinA1 and its release from cells and is possibly involved in EphA2 receptor activation.

This observation was surprising, but it has important implications for the understanding of the ligand-receptor dynamics and for the future production of novel EphrinA1-based, EphA2-targeted therapeutics.
CB-42. CLINICAL SIGNIFICANCE OF KALLIKREIN 6 IN GLOBLASTOMA MULTIFORME
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Kallikrein 6 (KLK6) is potentially highly relevant to GBM, since it is upregulated at sites of CNS tumors and was recently linked to astrocytic gliomas (Scarsbrick et al., 2012). To determine the clinical significance of KLK6 to GBM, we investigated the association between patient survival and levels of KLK6-immunoreactivity (IR) in 60 grade IV astrocytoma patient tumor samples. We evaluated the trend of elevated KLK6 expression in the response of GBM cell lines (U251, SF767) to cytotoxic agents. A range of KLK6 expression levels was observed across grade IV tumors; we found that higher levels indicated a poor prognosis of patient survival (univariable analysis, \( P = 0.02 \)). GBM patients with KLK6-IR scores below 10 had a median survival of 480 days and patients with scores at 10 or above had a median survival of 276 days (HR = 2.36, 95% CI = 1.19-4.68, unvariable analysis \( P = 0.01 \)). The prognostic significance of KLK6-IR remained after adjusting for gender and Eastern Cooperative Oncology Group performance scores. Our results suggest a mechanism by which elevated KLK6 reduces patient survival: KLK6 promotes resistance of GBM cell lines to cell death induced by staurosporine, cisplatin, or to radiation or temozolomide alone, or in combination. The ability of KLK6 to elicit pro-survival effects warrants further development at least in part on activation of the thrombin receptor and that targets β-catenin for degradation. β-Catenin is a key signaling molecule that regulates development, proliferation, and differentiation. Dysregulation of this pathway has been implicated in the oncogenesis of multiple malignancies (Nelson et al., 2004). We hypothesized that in the absence of pVHL, Jade-1 is downregulated, and β-catenin is upregulated in HBs.

METHODS: Frozen and formalin-fixed paraffin-embedded surgical HB specimens from VHL patients were used for immunohistochemical, immunofluorescence, and molecular analyses of KLK6, Jade-1 and β-catenin.

RESULTS: By immunohistochemistry, Jade-1 was not observed in HB stromal cells (VHL-negative) but was observed in larger blood vessels. Western blot analysis was minimally detected, of p75NTR by siRNA knockdown of p75NTR (Johnston et al., 2007). In contrast, siRNA knockdown of p75NTR decreases glioma invasion (Johnston et al., 2007). More recently, we have showed that p75NTR is also involved in invasion of BTICs in vivo (Wang et al., 2008). These results are particularly stimulating in light of the fact that p75NTR is also highly overexpressed in aggressive, malignant melanoma tumors (Berger et al., 2012), and neurotrophin binding to p75NTR on cultured melanoma cells increases their invasiveness in vitro (Herrmann et al., 1993) and promotes their survival (Marchetti et al., 2004). p75NTR is a multifunctional protein that regulates neurite outgrowth, myelin formation, and cell survival and death in vivo (Barker, 2004), and can induce differentiation of neuronal and non-neuronal cells (Cattaneo and McKay, 1990; Moscatelli et al., 2009). Here, we demonstrate that p75NTR and its ligand NGF are produced in BTICs isolated from glioblastomas. Downregulation of p75NTR expression by siRNA decreased proliferation of BTICs and their differentiation into astrocytes. Further, specific activation of p75NTR by NGF induced receptor proteolysis and increased proliferation and differentiation of BTICs. Our data suggest targeting p75NTR as a unique therapeutic strategy for gliomas. Future research is aimed at investigating the mechanisms involved in p75NTR-mediated glioma proliferation and invasion.

CB-45. P75 NEUROTROPHIN RECEPTOR IS REQUIRED FOR GLIOMA INVASION AND PROLIFERATION
Katappa S. Kenchappa1, J Gerardo Valadez1, Michael K. Cooper1, Bruce D. Carroll2, and Peter A. Forsyth1; 1H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; 2Vanderbilt University Medical School, Nashville, TN, USA

More than 70% of all brain tumors are malignant gliomas. Although how gliomas arise is not certain, isolation and characterization of CD133+ brain tumor initiating cells (BTICs) from primary tumors suggest that gliomas arise from stem cell-like neoplastic clones. Previous studies from our laboratory have demonstrated that the p75 neurotrophin receptor (p75NTR) is overexpressed in gliomas, that p75NTR is also involved in invasion of BTICs in vivo (Wang et al., 2008). These results are particularly stimulating in light of the fact that p75NTR is also highly overexpressed in aggressive, malignant melanoma tumors (Berger et al., 2012), and neurotrophin binding to p75NTR on cultured melanoma cells increases their invasiveness in vitro (Herrmann et al., 1993) and promotes their survival (Marchetti et al., 2004). p75NTR is a multifunctional protein that regulates neurite outgrowth, myelin formation, and cell survival and death in vivo (Barker, 2004), and can induce differentiation of neuronal and non-neuronal cells (Cattaneo and McKay, 1990; Moscatelli et al., 2009). Here, we demonstrate that p75NTR and its ligand NGF are produced in BTICs isolated from glioblastomas. Downregulation of p75NTR expression by siRNA decreased proliferation of BTICs and their differentiation into astrocytes. Further, specific activation of p75NTR by NGF induced receptor proteolysis and increased proliferation and differentiation of BTICs. Our data suggest targeting p75NTR as a unique therapeutic strategy for gliomas. Future research is aimed at investigating the mechanisms involved in p75NTR-mediated glioma proliferation and invasion.
CB-47. NEUROTROPHINS PROMOTE THE GROWTH OF GLIOMA-INITIATING CELLS VIA TRK RECEPTORS
Samuel Lown1, Rajappa Kenchappa2, and Peter Forsythe1. 1University of Calgary, Calgary, AB, Canada; 2H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Glioblastoma multiforme (GBM) is a highly invasive disease that is refractory to current treatments. Previous work by this lab has identified and characterized the role of the neurotrophin receptor p75NTR in promoting neurotrophin-dependent glioma cell invasion. Neurotrophins are a group of growth factors that signal through the p75NTR receptor (TNFR family) and Trk receptors (tyrosine kinases) to regulate diverse functions such as neuronal survival and cell motility. Here, we have further investigated the role of p75NTR and Trk receptors in mediating the response of patient-derived glioma cultures (commonly referred to as brain tumor initiating cells [BTICs]) to neurotrophins. Neurotrophin receptor and ligand expression was analyzed by a panel of BTICs by RT-PCR, Western blotting, and FACS. Growth assays were performed to measure the response of BTIC cultures to different neurotrophin ligands, and signaling pathways downstream of tyrosine kinase receptors were examined. Function of neurotrophin receptors was investigated by chemical inhibition and RNAi. BTIC cultures express a diverse repertoire of neurotrophin receptors, and the expression of specific Trk receptors (TrkA, TrkB, and TrkC) correlates with the response of each BTIC line to specific neurotrophin ligands (NGF, BDNF, and NT3). In particular, BDNF and NT3 promote the growth of BTICs and stimulate MAPK and PI3K pathway activity. Inhibiting Trk receptor function by pharmacological therapeutics or by lentiviral-mediated shRNA knockdown renders BTICs unresponsive to neurotrophins in terms of growth promotion and MAPK stimulation, whereas inhibition of p75NTR has no effect on these phenotypes. In addition, growth stimulation by neurotrophins can compensate for inhibition of EGF-stimulated growth by erlotinib.

CB-48. NOTCH LIGANDS, LATERAL INHIBITION, AND STEM- LIKE GlioBLASTOMA CELLS
Kah JIng Lim, Eli E. Bar, and Charles G. Eberhart; Johns Hopkins University, Baltimore, MD, USA

Cancer stem cells are thought to be critical for long-term growth and therapeutically resistant to glioblastoma (GBM). The Notch pathway helps to maintain this poorly differentiated subpopulation, but the mechanisms of Notch induction are still poorly understood. The “lateral inhibition” model, a central concept in Notch signaling, explains how Notch signaling can drive two initially identical progenitor cells to adopt different fates. Jagged or Delta-like ligands initiate this process by binding to Notch receptors on adjacent cells, causing both induction of Notch signaling in the neighboring cell and suppression of pathway activity in the ligand-expressing cell. Although Notch is known to be a critical player in stem-like GBM cells, and ligand expression and juxtacrine signaling have been demonstrated in these tumors, it is not yet known whether lateral inhibition occurs. To test this, we engineered a model system in which isogenic cells expressing low and high levels of the Jagged1 ligand are co-cultured; we then performed flow-based separation and determined the Notch pathway activity in each respective population. In the GBM neurosphere lines HSRR-GBM1 and 040821, the cell culture induced expression of Notch targets Hes5 and Hey1 in Jagged1-low “signal-receiving” cells but inhibited expression of these targets in Jagged1-overexpressing “signal sending” isogenic cells. The ligand intracellular domain (ICD) appears to play a role in downregulating Notch signaling in these “signal sending” cells. In support of this observation, expression of Icd1 or Igf1R in glioblastoma cells significantly reduced Notch target transcript levels and decreased GBM clonogenic growth potential. Furthermore, we found that ligand ICD localizes to the nucleus, suggesting that the observed effects may be mediated through transcriptional regulation. Our data suggest conservation of a developmental mechanism regulating Notch activation in cancer and a novel role for the ligand ICD that could help treat Notch-dependent tumors.

CB-49. DNA HYPERMETHYLATION AND 1P LOSS SILENCE NHE-1 IN OLIGODENDROGLOMIA
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Oligodendroglioma is characterized by mutations of IDH1 and CIC, 1p/19q loss, and slow growth. We found that NHE-1 on 1p is silenced in oligodendrogliomas secondary to IDH-associated hypermethylation and 1p allelic loss. Silencing lowers intracellular pH and attenuates acid load recovery in oligodendroglioma cells. Others have shown that rapid tumor pH recovery is not possible without NHE-1-mediated neutralization of the acidosis generated by the Warburg glycolytic shift. Our findings show for the first time that the pH regulator NHE-1 can be silenced in a human cancer and also suggest that pH deregulation may contribute to the distinctive biology of human oligodendroglioma.

CB-50. HETEROGENEITY OF RESISTANCE TO EPITHELIAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS IN OLIGODENDROGLIOMA: PATHWAY-DEPENDENT AND -INDEPENDENT MECHANISMS
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Epithelial growth factor receptor (EGFR)-targeted therapies, such as the tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib, have had limited success for the treatment of glioblastoma (GBM). In an effort to model and characterize mechanisms of resistance to EGFR TKIs in GBM, we examined the effect of EGFR TKI-sensitive ink4a/arf-/- astrocytes overexpressing the constitutively active mutant ΔEGRF (or EGFRVIII) were seeded in soft agar in the presence of gefitinib or erlotinib. Following initial response to the drug as measured by inhibition of colony formation, the dose was escalated and TKI-resistant colonies were isolated to generate clonal cell lines. TKI-resistance was verified in vitro and in vivo using an intracranial model. In TKI-resistant cell lines, gefitinib inhibited ΔEGRF phosphorylation, indicating the ability of the drug to effectively inhibit kinase activity. Interestingly, gefitinib suppressed Akt phosphorylation in parental cells and 4 out of 7 resistant cell lines. The remaining 3 lines presented reduced PTEN expression concomitant with elevated and sustained PI3K/Akt pathway signaling. Furthermore, TKI-resistance was reversible upon drug removal in cells having no change in PTEN expression and was irreversible in those cells with decreased PTEN. There were also common features amongst the resistant cell lines: an inability to undergo PARP cleavage in the presence of gefitinib and a dramatic elevation of autophagy. TKI re-sensitization was accompanied by significant reduction in autophagy, whereas cells with irreversible TKI-resistance maintained high levels of autophagy. Importantly, this model system has allowed for the isolation of resistant clones that are phenotypically distinct from one another, thereby demonstrating a potential for intratumoral resistance heterogeneity. These findings also suggest that reversibility of TKI-resistance following drug removal may depend on the resistance mechanism, which has potential clinical implications. Moreover, the existence of common features involving resistance to apoptosis and autophagy suggests that Notch may confer a potential therapeutic opportunity that may apply despite apparent differences in molecular mechanisms.
CB-52. BLOCKADE OF NDRG4 INDUCES P53 AND BAX MEDIATED APOPTOSIS IN HUMAN MENINGIOMA CELL LINES
Ramakrishna Peasadarao Konamurthy, Dennis Hwang, ThotaI, and Jerry Jasmin; Washington University, St. Louis, MO, USA

Meningiomas are the most commonly occurring intracranial tumors and account for approximately 20-30% of central nervous system tumors. Patients whose tumors recur after surgery and radiation therapy have limited therapeutic options. It has also been reported that radiation triggers DNA repair, cell survival, and cell proliferation, and reduces apoptosis via the induction of cellular protective mechanisms. We have recently identified the overexpression of N-Myc downregulated gene 4, NDRG4, in aggressive meningiomas. We have demonstrated that NDRG4 inactivation synergizes with cell viability, migration, and invasion properties. In the present study we investigated the role of NDRG4 in cell proliferation and mitochondrial-mediated apoptosis. We utilized shRNA-containing lentiviral plasmids to downregulate NDRG4 mRNA and protein expression in two high-grade meningioma cell lines, 10MM-Lee and CH137MN. In follow up to our prior studies on the effects of NDRG4 gene silencing in meningioma, we performed DNA ladderization and Annexin V/Propidium Iodide flow cytometry assays to demonstrate that the predominant form of cell death is apoptosis. We show that apoptosis caused by transduction of lentiviral shNDRG4 involves Bcl-2, Bcl-XL inhibition, cytochrome c release from mitochondria, and caspase-9 and caspase-3 activation followed by PARP I cleavage. After treated cells with LVshNDRG4, we detected upregulation of p53 and Bax, downregulation of Bcl-2, and inhibition of mitochondrial apoptotic molecules survivin and XIAP. Flow cytometry analysis revealed changes in mitochondrial membrane potential, which was observed by JC-1 membrane permeabilization as a differential mitochondrial mass, and the percentage of mitochondrial Δψm damage was measured using a lipophilic, cationic, fluorescent probe (DiOC6). Subcellular distribution of Bax and cytochrome c indicated mitochondrial-mediated apoptosis following depletion of NDRG4. In conclusion, this study demonstrates that NDRG4 gene silencing induces Bax activation and subsequently initiates mitochondrial-mediated apoptosis. This therapeutic strategy may have promise in the management of meningiomas.

CB-53. AN IN VIVO LOSS OF FUNCTION SCREEN FOR GENES INVOLVED IN EPIDERMAL GROWTH FACTOR RECEPTOR VIII-INDEPENDENT GLIOMA GROWTH
Tiffany E. Taylor1, Jill Wykosky1, Anna C. Schuerr1, William C. Hahn2, Webster K. Cavene1, and Frank B. Furnari1; Ludwig Institute for Cancer Research, La Jolla, CA, USA; 1Dana-Farber Cancer Institute, Boston, MA, USA

Amplification and mutation of the epidermal growth factor receptor (EGFR) gene are common genetic hallmarks of glioblastoma (GBM). The most common mutation is an in-frame deletion of exons 2-7 of EGFR, resulting in a constitutively active variant, EGFRvIII, and ΔEGFR-specific tyrosine kinase inhibitors (TKIs) are currently in clinical trials for the treatment of GBM; however, their efficacy has been limited owing to both upfront and acquired drug resistance. Using a genetic model of tetracycline-regulated ΔEGFR expression, we previously found that while this model is essential for the maintenance of glioma growth in vivo, similar to a clinical situation of acquired resistance, some tumors eventually regained aggressive growth after a significant period of stasis. Surprisingly, these breakthrough tumors persisted despite sustained suppression of ΔEGFR, indicating that other gene expression profiles of ΔEGFR-dependent cell lines. Using microarray technology, we confirmed that 84% (16/19) of the genes revealed in the microarray analyses are indeed upregulated and potentially reflect a descriptive genetic signature of acquired ΔEGFR independence. To test this hypothesis, we established a non-invasive loss-of-function in vivo screen to identify which of these genes alone or in combination are most essential to overcome dependence on ΔEGFR signaling. Genetic screens in mouse models have been shown to be highly effective in identifying cooperating cancer genes. Given that the genetic profiles generated for each individual ΔEGFR-independent cell line are not identical, results from these studies will shed light on genetic mechanisms of resistance to EGFR inhibition and may reveal novel putative targets for therapeutic development.

CB-54. ANALYSIS OF THE EPITHELIAL GROWTH FACTOR RECEPTOR VIII-INDUCTED TRANSCRIPTOME IN RAT GLIOMAS AND HUMAN GLIOBLASTOMAS
Gurpreet S. Kapoor2, Luke Macyszyn1, Yingtao Bi2, Heather Fetting1, Harish Poptani1, Rangan Iyer-Raghavan1, RamaVana V. Dhabal1, and Donald O’Rourke1; 1Pediatric Brain Tumor Foundation Institute. We found that both NKR1 and NKR31A1 are expressed in all 8 pediatric GBM xenografts. To determine whether NKR1 plays a role in the growth of pediatric GBM, we examined NKR1 expression and function in a pediatric GBM cell line (D2368 MG), which was established from a pediatric GBM. Real-time quantitative PCR analysis of RNA isolated from D2368 MG cells indicates that these cells mainly express the truncated form of NKR1. Stimulating these cells with SP causes little or no increase in Akt phosphorylation while simultaneously using Erk1/2 phosphorylation. On the other hand, blocking NKR1 with L703, 606 (an NKR1 antagonist) decreases the phosphorylation of both Akt and Erk1/2. Incubation of D2368 MG cells with L703,606 for 6 days reduces the cell number by over 60%. Taken together, our study provides the first evidence that NKR1 may play a role in the growth of pediatric GBM and represents a novel target to inhibit these tumors.

CB-55. DECADRON TREATMENT INHIBITS GLIOMA CELL PROLIFERATION IN VIVO
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Glioblastoma multiforme (GBM) is the most common and malignant primary brain neoplasm. Standard therapy for GBM is surgical resection followed by radiation therapy (RT) and temozolomide. Additionally, the corticosteroid dexamethasone (Dex) is frequently used for symptomatic improvement prior to surgical resection and, in many cases, for maintenance through post-operative radiation therapy. Several studies have investigated the role of Dex in promoting glioma growth in various glioma models, but the results have been inconsistent. The purpose of this study was to investigate the effect of Dex on the PDGF-driven RCAS oncogene in a rat glioma model and human GBMs but also characterize potential targets to inhibit EGFRvIII-mediated transforming phenotypes in malignant gliomas.
genes downregulated by Dex predicts a significantly longer survival compared with tumors with lower levels. In summary, our data suggest that Dex inhibits tumor cell proliferation, potentially through tumor microenvironment interactions. TCGA analysis suggests that downregulation of our Dex-regulated gene set may have an adverse effect on survival. It is possible that if Dex decreases tumor cell proliferation, it may also decrease the efficacy of antineoplastic therapy that is most toxic to proliferating cells. This study underscores the importance of defining the mechanism of how Dex affects tumor and stromal cells in glioma.

CB-56. SIGNALING NETWORK-BASED ANALYSES OF SONIC HEDGEHOG PATHWAY COMPONENTS: PREDICTIONS AND POSSIBILITIES Andru Malhotra1, Chad Potts1, Africa Fernandez-Lopez2, and Anna M. Kenney3; 1Vanderbilt University, Nashville, TN, USA; 2Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Medulloblastomas show considerable dependence on the Sonic Hedgehog (Shh) pathway. Since cellular functions result from coordinated networks involving protein-protein networks, it is imperative to explore different events cooperating with Shh entities in order to develop novel targets. We identified new targets, namely with EGFR activity in medulloblastoma therapy. YAP, a component of the Hippo pathway, has been previously established as a downstream target of the Shh pathway (Fernandez et al., 2011). We developed several interesting gene targets featured in the networks using YAP overexpression in CGNPs. A concurrent upregulation of IG2 and H19, both imprinted genes, was observed. Possible links between the Shh/Hippo pathways and IGF2 signaling also featured in the networks developed in our study. Important links to lipid metabolism, a process highly active in medulloblastomas and essential for the proliferation of CGNPs, were also predicted. The study includes results of signaling interactions between the Shh/Hippo pathways and entities from other relevant pathways that together play a role in medulloblastoma development and sustenance.

CB-57. PHOSPHORYLATION OF DOCK180Y722 BY SRC FAMILY KINASES MEDIATES EGFRVIII-DRIVEN Glioblastoma tumorigenesis Shiyuan Cheng, Hatzibong Feng, Bo Hu, Michael J. Zarzynka3, Yanlin Lu4, Susan Kreer5, Terrence G. Johns1, Ronald L. Hamilton1, Kristuna Vuori6, Ryo Nishikawa7, Jann N. Sarkaria1, Tim Fenton7, Tao Cheng1, Frank B. Furnari8, and Webster K. Cavenee9; 1Northwestern University, Chicago, IL, USA; 2University of Pittsburgh Medical Center, Pittsburgh, PA, USA; 3University of Pittsburgh, Pittsburgh, PA, USA; 4Cell Signaling Technology, Danvers, MA, USA; 5Monash Institute of Medical Research, Victoria, Australia; 6Sanford-Burnham Medical Research Institute, La Jolla, CA, USA; 7Saitama Medical University, Saitama, Japan; 8Mayo Clinic, Rochester, MN, USA; 9Memorial Sloan Kettering Cancer Research and the University of California-San Diego, La Jolla, CA

Glioblastoma, the most common primary malignant cancer of the brain, is characterized by rapid tumor growth and infiltration of tumor cells throughout the brain. These traits cause glioblastoma to be highly resistant to current therapies, resulting in poor prognosis. While aberrant oncogenic signaling driven by signature genetic alterations such as epithelial growth factor receptor (EGFR) gene amplification and mutation, plays a major role in glioblastoma pathogenesis, the responsible downstream mechanisms remain less clear. Here, we report that EGFRVIII (also known as ΔEGFR and de2-7EGFR), a constitutively active EGFR mutant that is frequently co-overexpressed with EGFR in human glioblastoma, promotes tumorigenesis. These SFKs, Src, Fyn, and Lyn, induce phosphorylation of Dock180, a guanine nucleotide exchange factor for Rac1. EGFRVIII induces phosphorylation of Dock180 at tyrosine residue 722 (Dock180Y722) and stimulates Rac1-signaling, glioblastoma cell survival, and migration. Consistent with this causal relationship, siRNA knockdown of Dock180 or expression of a Dock180Y722F mutant inhibits each of these EGFRVIII-stimulated activities. The SFKs, Src, Fyn, and Lyn, induce phosphorylation of Dock180Y722, and inhibiting these SFKs by pharmacological therapeutics or shRNA depletion markedly attenuates EGFRVIII-induced phosphorylation of Dock180Y722, Rac1 activity, and glioblastoma cell migration. Lastly, phosphorylated Dock180Y722 is co-expressed with EGFRVIII and phosphorylated SrcY418 in clinical specimens; this co-expression correlates with an extremely poor survival in glioblastoma patients. These results suggest that targeting the SFK-pDock180Y722-Rac1 signaling pathway may offer a novel therapeutic strategy for glioblastomas with EGFRVIII overexpression.

CB-58. EPITHELIAL MESENCHYMAL TRANSITION AS A THERAPEUTIC TARGET IN GLIOMA Andrei M. Mikheev, svetlana A. Mikheeva, John R. Silber, Philip J. Horner, and Robert Rostomily; University of Washington, Seattle, WA, USA

Epithelial mesenchymal transition (EMT) is felt to drive carcinoma metastasis through activating cell invasion, intravasation, and promotion of stem cell phenotypes. We previously showed that TWIST1, a central driver of EMT-related invasion in carcinoma, is upregulated in malignant gliomas and promotes human glioma cell invasion in vivo and glioblastoma (GBM) stem cell growth and self-renewal in vitro. Combined with the emerging recognition of the importance of mesenchymal phenotypes in glioma, these observations support our hypothesis that targeting EMT-associated genes like TWIST1 may have a unique therapeutic benefit for glioma patients. To address this possibility, we studied the effect of inhibiting TWIST1 and its putative target gene Periostin (POSTN), also a known regulator of carcinoma EMT, in human GBM stem cell xenograft models. Using stable expression of gene-specific shRNAs, we found that 80% knockdown of TWIST1 in the GBM8 stem cell line and 90% knockdown of POSTN in GBM4 stem cells resulted in nearly a complete eradication of tumor cells, whereas a 50% knockdown resulted in prolongation of median survival from 75 days to 100 days. Unlike GBM8, POSTN is expressed and regulated by TWIST1 in GBM4. Therefore, we tested the effect of POSTN knockdown alone and found that a 90% knockdown of POSTN in GBM4 dramatically phenocopied the effect of TWIST1 inhibition in GBM8. TWIST1 and POSTN inhibition also corresponded with cell line-specific decreases in stem cell marker expression (CD133) and functional indicators of stem cell activity (self-renewal and sphere formation). These results indicated that inhibition of EMT-associated genes like TWIST1 and POSTN does reduce glioma growth, possibly through effects on stem cell function. We conclude that EMT is not only a useful framework for conceptualizing glioma biology but also a potentially productive resource for identifying new molecular targets for GBM invasion and stem cell function.

CB-59. BCL-2 FAMILY MEMBER MCL-1 REGULATES HYPOXIA-INDUCED CELl DEATH THROUGH BLOCKING BH3-ONLY MEMBER BNIP3 FUNCTION IN MALIGNANT GLIOMA CELLS Elizabeth S. Henson1, Michelle Brown2, David D. Eisenstat3, and Spencer B. Gibson3; 1University of Minnesota, Minneapolis, MN, USA; 2Manitoba Institute of Cell Biology, Winnipeg, MB, Canada; 3University of Alberta, Edmonton, AB, Canada

Brain tumors that have areas of low oxygen (hypoxia) are resistant to chemotherapy. We investigated proteins found in malignant glioma cells that regulate hypoxia-induced cell death. We have shown that the Bcl-2 family member Mcl-1 protects cells from hypoxia-induced cell death, whereas BNIP3 (pro-cell death) regulates hypoxia-induced cell death. However, even though BNIP3 expression leads to cell death, it is associated with a poor prognosis for patients with solid tumors, and we investigated this dichotomy by determining whether the function of BNIP3 is lost in tumor cells. We have shown that normal mouse astrocytes are more resistant to hypoxia-induced cell death when compared with knockout mouse astrocytes that lack BNIP3 expression. Furthermore, treatment with epidermal growth factor (EGF) increased Mcl-1 expression and blocked both hypoxia and BNIP3-induced cell death. Furthermore, treatment with epidermal growth factor (EGF) increased Mcl-1 expression and blocked both hypoxia and BNIP3-induced cell death. Knockdown of Mcl-1 expression blocked EGF protection against BNIP3 and hypoxia-induced cell death. Expression of mutated EGF receptor also blocked Mcl-1 expression, even under hypoxic conditions. EGF/EGF-like signaling in tumor xenografts grown in SCID mice showed increased Mcl-1 and BNIP3 expression, correlating with hypoxia regions in these tumors. Thus, Mcl-1 expression can be regulated by hypoxia and BNIP3 expression. Furthermore, treatment with epidermal growth factor (EGF) increased Mcl-1 expression and blocked both hypoxia and BNIP3-induced cell death. Knockdown of Mcl-1 expression blocked EGF protection against BNIP3 and hypoxia-induced cell death. Expression of mutated EGF receptor also blocked Mcl-1 expression, even under hypoxic conditions. EGF/EGF-like signaling in tumor xenografts grown in SCID mice showed increased Mcl-1 and BNIP3 expression, correlating with hypoxia regions in these tumors. Thus, Mcl-1 expression can be regulated by hypoxia and BNIP3 expression.
Recently, several groups have demonstrated the presence of cytomegalovirus (CMV) protein and DNA in a large majority of glioblastomas (GBMs), though the role of CMV in tumors remains controversial. Although CMV is capable of activating several oncogenic pathways, it has never been shown to be a transforming virus. Because of this, we hypothesized that CMV infection can modify the rate of gliomagenesis. To test this hypothesis, we perinatally infected Mut3 (GFAP-cre; Nf1loxP/+; Trp53+/-) mice that develop spontaneous gliomas at an adult age with mouse CMV (MCMV). After viral inoculation, mice exhibited infection throughout the body, including the brain. MCMV-infected Mut3 mice succumbed to glioma significantly earlier than Mock-infected Mut3 mice. To validate our results, we used an additional syngeneic model of intracranial mouse GBM. Wild type mice were perinatally MCMV- or Mock-infected and injected intracranially with mouse GBM cells as adults. MCMV-infected mice with intracranial tumors died significantly earlier than Mock-infected mice. Analysis of pre-tumorigenic Mut3 mice revealed a significant increase of pSTAT3 in the subventricular zone, a hypothesized origin of GBMs. Additionally, MCMV-infected mice with intracranial syngeneic GBMs expressed higher pSTAT3 in tumors. Since pSTAT3 mediates proliferation in GBMs, we measured PCNA, a marker of proliferation, in our tumors. MCMV-infected intracranial tumors contained a significantly higher number of PCNA-positive cells, suggesting a higher rate of proliferation. To investigate CMV in human tumors, we infected patient-derived GBM neurospheres in vitro with human CMV (HCMV). Infection activated STAT3 and significantly increased proliferation. Additionally, HCMV-infected neurospheres injected into flanks of nude mice grew faster than Mock-infected tumors. Addition of Stattic, a STAT3 inhibitor, abrogated proliferation of HCMV-infected cells, showing that the proliferation advantage garnered by HCMV infection occurs via STAT3 activation. Taken together, our data suggest that CMV in gliomas accelerate GBM progression by STAT3 signaling and increase proliferation.

CB-61. ENHANCING ONCOLYTIC HSV-1 THERAPY BY HDAC6 INHIBITION IN GLIOBLASTOMA
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Novel strategies, including oncolytic viral (OV) therapy using conditionally replicating viruses, have shown promise in various neurological tumors, but some glioma cells resistant/evade the lytic effect of the OV. We have found that, right after infection and entry into a glioma cell, incoming oncolytic HSV (oHSV) are transported via microtubules (MTs) to autophagosomes and lysosomes, inducing an innate antiviral signal within the cancer cell. This appears to be a host defense pathway, and inhibition of one particular HDAC (HDAC6) suppresses this host defense pathways, thus improving oHSV1 replication in glioma. To prove that HDAC6 mediates antiviral effects, we have employed genetic approaches, including shRNA knockdown and overexpression of HDAC6. Since HDAC6 deacetylates alpha-tubulin and alters MT dynamics, we attempted oHSV infection in cells overexpressing acetyl-mimic and -deficient forms of tubulin. Our experimental results show that inhibition of HDAC6 suppresses the lysosomal degradation of incoming oHSV during infection. In addition to MT-mediated defensive pathway, we found that HDAC6 also modulated interferon-stimulated genes (ISGs), patterns, and expression upon oHSV infection, suggesting a novel role for HDAC6 in antiviral immune responses. Using biochemical approaches, we discovered that HDAC6 physically interacts with one of the ISGs after IFN-treatment. The roles of HDAC6 in antiviral response and post-entry oHSV1 trafficking are highly novel, and manipulating HDAC6 activity could improve oHSV1 replication in vitro and in vivo in order to increase therapeutic efficacy in GBM.

CB-62. REVERSAL OF MIRNA-125B-TRIGGERED HUMAN GLIAL CELL PROLIFERATION USING ANTI-MIRNA (AM; ANTAGOMIR) STRATEGIES
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A conserved class of soluble, 22 nucleotide, single-stranded non-coding ribonucleic acids known as microRNAs (miRNAs) bind to the 3′-untranslated region (3′-UTR) of their target messenger RNAs (mRNAs), thereby regulating specific gene expression. The total number of known miRNAs is currently about 2000; however, human brain cells are specifically enriched in a significantly smaller subclass of about 40 miRNAs that include a high abundance miRNA-125b. Levels of human miRNA-125b, transcribed from multiple genes at chr 11q24 and chr 21q21, are significantly upregulated in glioma and glioblastoma tissues, in cultured human glioma and glioblastoma cell lines, and in interleukin-6 (IL-6)-stressed normal human astroglial (HAG) cells. IL-6 treatment triggers HAG cell proliferation. Upregulated miRNAs downregulate their mRNA targets and hence downregulate selective gene expression. Here we report that miRNA-125b, when added to cultured HAG cells, induces astrogliosis and increases markers for senescence, including nuclear atrophy and increased cytoplasmic-to-nuclear ratios. Anti-miRNA-125b (AM-125b) strategies robustly inhibit these triggered effects. We further demonstrate a strong positive correlation between miRNA-125b abundance in biopsied human glioma and glioblastoma tissues and the astroglial cell markers glial fibrillary acidic protein (GFAP) and vimentin. Taken together, these results suggest that miRNA-125b contributes to the proliferation and accelerated senescence of HAG cells and that anti-miRNA-125b (AM-125b; antagonim-125b) based strategies may be clinically useful in treating cellular proliferative diseases that involve astroglial cells. This study was supported in part by the Translational Research Initiative (LSUHSC-NO), an Alzheimer Association IIRG Award, an NIH NIA AG18031, and an NIH NIA AG038834.