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β3-adrenergic agonists mimic eustress response and reduce leptin-mediated proliferation in a GBM cell line
CELL BIOLOGY AND SIGNALING

CB-01. PTEN PHOSPHORYLATION BY FIBROBLAST GROWTH FACTOR RECEPTORS AND SRC MEDIATES RESISTANCE TO EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITORS IN GliOMA
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CB-02. Glioblastoma Tumor Microenvironment Drives Selection of Cell Subpopulations with Distinct RTK Amplification Genotypes and Drug Sensitivities
Nicholas Szerlip1, Alicia Pedraza1, Jason Huse1, Tom Mikkelson1, and Cameron Brennan1; 1Memorial Sloan Kettering Cancer Center, New York, NY; 2Henry Ford Hospital, Detroit, MI

CB-03. SUR1-REGULATED NONSELECTIVE CATION CHANNEL REPRESENTS A POSSIBLE NEW THERAPEUTIC TARGET FOR GLIOMAS
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INTRODUCTION: Glioblastomas (GBMs) represent a broad and heterogeneous entity. Finding targeted tumor-specific treatments is a challenge. One such target may be a novel channel formed by a protein complex consisting of SUR1 regulatory subunits and TRPM4 pore-forming subunits. We hypothesize that this complex may serve as a novel, naturally occurring target for pharmacological therapy that may be of benefit in GBM.

METHODS: METHODS: Using next generation sequencing, we identified a SUR1/ TRPM4 channel that is constitutively expressed in tumor tissue. In vitro luciferase assays confirmed that the human ABC8 channel promoter is specifically stimulated by HIF-1alpha. DISCUSSION: This is the first report of the presence of the SUR1/TRPM4 channel complex in GBM. This unique receptor complex represents a viable therapeutically target that is constitutively expressed in tumor tissue. We continue to elucidate the role of this complex in possible treatment paradigms, to exploit the cell death cascade controlled by this channel. Our data suggest that this complex may serve as a novel, naturally occurring target for pharmacological therapy that may be of benefit in GBM.

INTRODUCTION: The majority of glioblastomas (GBMs) harbor amplification/mutation of a receptor tyrosine kinase (RTK), although clinical trials of RTK inhibitors have shown inconsistent responses. One possible mechanism of resistance is activation of multiple RTKs in a tumor. While co-activation has been documented at the protein level in GBMs, its significance in maintaining cell populations is unknown. We investigated whether co-activation of different RTKs can occur independently in GBM tumor cell subpopulations, the mechanism of clonal evolution, and the functional significance of genotype heterogeneity for pharmacologic treatment.

METHODS/RESULTS: ACGH profiles of 460 TCGA GBMs and 150 tumors from MSKCC were examined for focal amplifications spanning EGFR, PDGFRα, and MET. Multicolor FISH was performed for EGFR, PDGFRα, and c-Met for 24 coamplified cases. For two cases, derived tumor sphere lines were expanded for study. Coamplification of 2 or more RTKs was observed in 24 specimens. Remarkably, 43% of GBMs with focal PDGFRα amplification also contained either EGFR or MET amplifications. FISH-demonstrated amplicons were primarily in distinct tumor cell subpopulations, interspersed rather than regionally segregated. Expanded cell lines from EGFR/PDGFRα coamplified tumors maintained distinct RTK-driven subpopulations that were subject to selection under EGFR or PDGFRα ligand-stimulation in vitro. Simultaneous inhibition of both EGFR and PDGFRα by gefitinib and imatinib was necessary to suppress PI3 kinase pathway activation. Analysis of whole exome and whole genome sequencing of coamplified TCGA samples and cell line subpopulations supports a common, clonal origin with late divergence of RTK-amplified clades, although sequencing depth is limited. CONCLUSION: This is the first documentation of multiple subpopulations with distinct, high-level RTK amplifications in GBM. These results show certain GBMs commonly harbor subpopulations with other RTK amplifications requiring drugs targeting each subpopulation for effective treatment. This data should be considered for current trials of RTK inhibitors, particularly those targeting PDGFRα.
assay. The transient ALD and Chk1 activation noted at early time points (>12 hours post-TMZ exposure) in all cells was consistent with base excision repair of TMZ-induced N7-guanine and 3-methyl adenine DNA adducts, while the persistent ALD and Chk1 activation noted at late time points (<24 hours post-TMZ exposure) in MGMT-deficient cells was the result of undefined, MMR-independent processing of TMZ-induced O6MG lesions. These results redefine the series of events that activate the DNA damage response following TMZ exposure and show that pChk1 is a biomarker of TMZ-induced DNA lesions (in some cases O6MG lesions), but not of their cytotoxic sequelae.

CB-05. HUMAN BRAIN-ENRICHED MICRO RNA-125B (MIRNA-125B) INDUCES BOTH PROLIFERATION AND SENECEENCE OF HUMAN ASTROGLIAL (HAG) CELLS IN PRIMARY CULTURE

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microRNAs (miRNAs) represent a class of 22 nucleotide, noncoding, single-stranded RNAs that regulate the stability, translation, and expression of their target messenger RNAs (mRNAs). Of the approximately 100 miRNAs known in human cells, only about 80 miRNAs are highly expressed in human brain cells. Levels of a brain-abundant miRNA-125b, transcribed from multiple genes at chr 11q24 and chr 21q21, are significantly upregulated in cultured human gloma and glioblastoma cell lines and in interleukin-6 (IL-6)-stressed normal astroglial (HAG) cells; the latter is a treatment known to trigger HAG cell proliferation. Herein we report that miRNA-125b added to cultured HAG cells induces astrogliosis and increases markers for senescence, including nuclear atrophy and increased cytoplasmic/nuclear ratios. Anti-miRNA-125b (AM-125b) abrogated with increased efficacy of radiation and chemotherapy, particularly of eHsp90 signaling potently suppressed basal AKT activity and overrode all known cellular tyrosine kinases. Strikingly, we found that blockade of eHsp90 in mediating signaling pathways in GBM, we have undertaken a knowledge is lacking concerning its broader signaling effects and downstream pathologic outcomes.

CB-08. EXTRACELLULAR HSP90 IS A CENTRAL MEDIATOR OF ONCOCGENIC SIGNALING NETWORKS IN GLOBLASTOMA

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Glioblastoma multiforme (GBM) represents one of the most highly aggressive brain tumors due to the propensity of tumor cells to invade and migrate into surrounding normal brain tissue. It is precisely this infiltrative behavior of GBM that renders the disease largely incurable. An additional clinical challenge is the notorious genetic and molecular heterogeneity associated with this disease. As such, a main component of treatment failure is elicited by the activation of multiple receptor kinases (RTKs), which facilitates signaling redundancy and compensation upon specific therapeutic RTK targeting. In key signaling mediators, the ability of multiple growth factors to stimulate this non-redundant, recurrent glioblastoma expression. CNA showed deletion to be the major mechanism and are associated with higher MIB-1 and MVD indices than did their adult counterparts. There was no statistical difference between the MB-1 or MB-2 samples and normal control brain specimens. MB-1 (proliferation index) and microvessel density (MVD) were also analyzed on these samples. Cotransfer by analysis (CNA) using TaqMan copy number assay for the 5 mismatches showed no protein expression for any of the molecules. The grade II and III pediatric samples showed higher MB-1 and MB-2 indices than did their adult counterparts. There was no statistical difference between the MB-1 or MB-2 samples and normal control brain specimens. MB-1 (proliferation index) and microvessel density (MVD) were also analyzed on these samples. Cotransfer by analysis (CNA) using TaqMan copy number assay for the 5 mismatches showed no protein expression for any of the molecules.

The role of hypoxia-inducible factors (HIF) is well established in gliomas. Protein expression of 5 major molecules of the HIF pathway (VEGF, CA9, GLUT-1, HIF-1, and HIF-2) was evaluated by immunohistochemistry on human non-small cell lung cancer, glioblastoma, and metastasis samples. These results suggest that miRNA-125b contributes to the promotion and premature aging of HAG cells, and that anti-miRNA (AM; antagonor) strategies may be clinically useful in treating astroglial cell proliferative disease. Support: Translational Research Initiative [LSUHSC-NO] and an Alzheimer Association IRG Award [W1L]

CB-07. EVALUATION OF THE HYPOXIA PATHWAY IN PEDIATRIC AND ADULT EPENDYMOMAS

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This study for the first time intends to comprehensively evaluate key molecules of the HIF pathway in the oncogenesis of these tumors. Protein expression of 5 major molecules of the HIF pathway (VEGF, CA9, GLUT-1, HIF-1, and HIF-2) was evaluated by immunohistochemistry (IHC) on 34 paraffin-embedded pediatric and 17 adult ependymoma samples and normal control brain specimens. MB-1 (proliferation index) and microvessel density (MVD) were also analyzed on these samples. Cotransfer by analysis (CNA) using TaqMan copy number assay for the 5 mismatches showed no protein expression for any of the molecules. The grade II and III pediatric ependymoma samples showed higher MB-1 and MVD indices than did their adult counterparts. There was no statistical difference between the MB-1 or MB-2 samples and normal control brain specimens. MB-1 (proliferation index) and microvessel density (MVD) were also analyzed on these samples. Cotransfer by analysis (CNA) using TaqMan copy number assay for the 5 mismatches showed no protein expression for any of the molecules.

The role of hypoxia-inducible factors (HIF) is well established in gliomas. To date, its role in the development of ependymomas remains ill-defined. This study for the first time intends to comprehensively evaluate key molecules of the HIF pathway in the oncogenesis of these tumors. Protein expression of 5 major molecules of the HIF pathway (VEGF, CA9, GLUT-1, HIF-1, and HIF-2) was evaluated by immunohistochemistry (IHC) on 34 paraffin-embedded pediatric and 17 adult ependymoma samples and normal control brain specimens. MB-1 (proliferation index) and microvessel density (MVD) were also analyzed on these samples. Cotransfer by analysis (CNA) using TaqMan copy number assay for the 5 mismatches showed no protein expression for any of the molecules. The grade II and III pediatric ependymoma samples showed higher MB-1 and MVD indices than did their adult counterparts. There was no statistical difference between the MB-1 or MB-2 samples and normal control brain specimens. MB-1 (proliferation index) and microvessel density (MVD) were also analyzed on these samples. Cotransfer by analysis (CNA) using TaqMan copy number assay for the 5 mismatches showed no protein expression for any of the molecules.
CB-09. CORTICOSTEROIDS IMPAIR GLIAL PROGENITOR CELL SURVIVAL PATHWAYS IN THE CENTRAL NERVOUS SYSTEM
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Corticosteroids, such as dexamethasone and methylprednisolone, are well known for their powerful immunosuppressive, anti-inflammatory, and anti-edematous effects, and are commonly used in the treatment of autoimmune-inflammatory diseases and brain cancer. Despite their widespread use in clinical practice, little is known about their effects on glial progenitor cells and myelin integrity in the adult brain. The aim of the present study was to determine the effects of commonly used corticosteroids on self-renewal and differentiation of oligodendrocyte precursor cells (OPCs). Primary cultures of purified OPCs were exposed to different concentrations of corticosteroids. Cell proliferation and differentiation were determined by BrDU-incorporation and immunohistochemical analysis both in mass cultures and in clonal cultures. Corticosteroid treatment was associated with a dose-dependent impairment of cell survival of OPCs and postmitotic oligodendrocytes. Clonal studies revealed that corticosteroids were shifting the balance between self-renewal and differentiation towards progenitor cell differentiation. In vitro studies were predictive of in vivo studies, showing impairment of glial progenitor proliferation in subcorical white matter tracts of mice systematically exposed to methylprednisolone. Corticosteroid-associated impairment of progenitor cell proliferation was in part reversed by the powerful antioxidant N-acetylcysteine, suggesting a redox-dependent shift of corticosteroid efficacy on neural progenitor cells for endogenous repair mechanisms, corticosteroid-associated impairment of oligodendrocyte precursor cell function could negatively influence myelination and repair processes in the adult central nervous system.

CB-10. UNDERMINING SPARC-INDUCED PROSURVIVAL SIGNALING THROUGH HSP27 IS A MORE EFFECTIVE THERAPEUTIC STRATEGY THAN PROMOTING SPARC-INDUCED, TEMOZOLOMIDE (TMZ)-ASSOCIATED PRODEATH SIGNALING IN GLIOMA CELLS
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SPARC promotes invasion through hPS27 upregulation and promotes survival via Akt activation, suggesting it is a therapeutic target. Because hPS27 also upregulates pAKT1, we proposed that suppressing hPS27 could inhibit SPARC-induced survival. Alternatively, published data suggest SPARC is a chemosensitizer. Therefore, we assessed the effects of SPARC expression ± TMZ or SPARC inhibition and downstream HSP27 inhibition to assess its role as a therapeutic agent versus target. 1) Control and SPARC-expressing U87 cells or LN443 cells treated with control, SPARC, HSP27, or AKT siRNAs ± TMZ were subjected to western blot analyses. Quantitation was performed (ImageJ [n ≥ 3]). Two-fold changes were considered statistically significant. 2) The cells were also subjected to cell genomic assays to assess survival (n ≥ 2). Results indicate: 1) SPARC promotes pro-survival (HSP27, AKT) and prodeath (caspase 8, cleaved caspase 3) signal, and SPARC and control cells survive similarly. 2) TMZ activates survival (HSP27, AKT) and prodeath (caspase 8, cleaved caspase 3) signaling. Therefore, suppressing SPARC signaling may be more effective than promoting SPARC-induced, TMZ-associated prodeath signaling.

CB-11. DECREASED MIR-218 EXPRESSION PROMOTES TUMOR CELL SURVIVAL PATHWAYS IN Glioblastomas
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Glioblastoma multiforme (GBMs) are primary malignant brain tumors with a median survival of approximately 12-14 months. Recently, it has been reported that numerous microRNAs are differentially expressed in GBMs when compared with adjacent non-neoplastic brain tissue. Among these, miR-218 is expressed at significantly lower levels in GBMs compared to adjacent normal neurons. Our functional analyses indicated that miR-218 silencing increases sensitivity to cell death and tumor cell invasion. Since miR-218 overexpression sensitizes tumor cells to apoptotic stimuli. Temozolomide (TMZ), a widely used chemotherapeutic agent for human GBM patients, was utilized in an orthotopic xenograft brain tumor model to test the effect of miR-218 silencing with control (U87-NS) or miR-218 silenced human glioblastoma cells (U87-218) developed intracranial orthotopic tumors without a significant difference in survival rate (~20 days), TMZ delivered to the U87-NS group increased survival up to an average of 45 days. However, the combinational use of miR-218 and TMZ significantly increased survival, and 50% of animals failed to develop tumors even after 288 days of treatment. ECOP, PLCy1, and NAC-1 were identified as direct miR-218 targets and experimentally validated. Repression of ECOP and PLCy1 in GBM cells resulted in increased sensitivity to cell death, suggesting these genes as contributors to miR-218 effects on cell survival. Cell death mediated by increased miR-218 expression was not restored with PLCy1 over-expression alone; however, reduced cell invasion was completely rescued with PLCy1. Since PLCy1 modulates PKC and NF-kappaB further work is warranted to that low miR-218 patients. In conclusion, miR-218 inhibitors may be a new therapeutic strategy for GBMs and other human cancers.

CB-12. A SURVEY OF HUMAN CYTOMEGALOVIRUS GENOMIC LOCUS PRESENT IN Glioblastoma Multiforme TISSUE SAMPLES
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Human cytomegalovirus (HCMV) causes CNS disorders, birth defects, and immunological complications and has been recently associated with glioblastoma multiforme (GBM) based on the detection of selected viral DNA segments, miRNAs, and protein antigens in tumor tissues. GBM is the most common form of malignant brain tumor and is highly refractive to various treatment options, resulting in a high rate of recurrence and mortality. A greater insight into the mechanism of tumorigenesis and identification of specific oncogenic agents or oncomodulatory factors is necessary to develop better therapeutic alternatives. HCMV is a good oncogenic candidate for glioma genesis as many viral gene products are capable of controlling protooncogenic cellular processes such as differentiation, proliferation, inflammation, migration, and angiogenesis in addition to conferring resistance to chemotherpay. However, it is not known if specific HCMV gene products are expressed in GBMs. In fact, no reported studies have examined whether the entire 235 kilobase double-stranded DNA viral genome or only select viral genes are retained in these tumors. In order to specifically identity regions of the HCMV genome maintained in GBMs, we generated primers for 15 loci spaced at regular intervals along the viral genome and identified that miR-218 overexpression inhibits protooncogenic cellular processes such as differentiation, proliferation, inflammation, migration, and angiogenesis in addition to conferring resistance to chemotherpay. However, it is not known if specific HCMV gene products are expressed in GBMs. Therefore, we analyzed a set of 12 surgical tissue samples as well as HCMV DNA. We report that altogether the incidence of HCMV DNA in GBM is higher than previously reported and the viral DNA copy number was not correlated with tumor grade.

CB-13. GENOMIC ANALYSIS OF CYTOMEGALOVIRUS IN MALIGNANT GLIOMAS
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Human cytomegalovirus (HCMV) causes CNS disorders, birth defects, and immunological complications and has been recently associated with glioblastoma multiforme (GBM) based on the detection of selected viral DNA segments, miRNAs, and protein antigens in tumor tissues. GBM is the most common form of malignant brain tumor and is highly refractive to various treatment options, resulting in a high rate of recurrence and mortality. A greater insight into the mechanism of tumorigenesis and identification of specific oncogenic agents or oncomodulatory factors is necessary to develop better therapeutic alternatives. HCMV is a good oncogenic candidate for glioma genesis as many viral gene products are capable of controlling protooncogenic cellular processes such as differentiation, proliferation, inflammation, migration, and angiogenesis in addition to conferring resistance to chemotherpay. However, it is not known if specific HCMV gene products are expressed in GBMs. In fact, no reported studies have examined whether the entire 235 kilobase double-stranded DNA viral genome or only select viral genes are retained in these tumors. In order to specifically identify regions of the HCMV genome maintained in GBMs, we generated primers for 15 loci spaced at regular intervals along the viral genome and standardized the assay to detect viral DNA in tumor tissues by PCR. We then obtained 75 GBM biopsy samples and 30 controls comprising of both non-tumor and nonglioma tumor tissues. DNA extracted from these tissues has been examined for the presence of viral DNA by PCR. Additionally, we analyzed a set of 12 surgical tissue samples as well as HCMV DNA. We report that altogether the incidence of HCMV DNA in GBM is higher than previously reported and the viral DNA copy number was not correlated with tumor grade.
grade. These viral DNA loads are low relative to DNA levels found in productive infections and suggest that CMV is not replicating in the tumors. Viral genes associated with all phases of CMV replication were expressed in the tumors, but expression levels were extremely low compared to the levels in cells or biopsies. These observations suggest that atypical infections occur in tumors. The viral UL83 gene, encoding pp65, was sequenced to determine whether a single viral sequence is present in a GBM and pressures exist to retain CMV viral sequence information.

CB-14. THERAPEUTIC POTENTIAL OF AZD1480 FOR THE TREATMENT OF HUMAN GliOBLASTOMA BRITANNIA ENTERPRISES, Birmingham, AL; 2AstraZeneca R&D Boston, Waltham, MA

Cancer patients; those diagnosed with glioblastoma multiforme (GBM) are no exception. Different types of stress invariably impact what has been referred to as the “cancer stress cycle.” In this study, we have evaluated the effects of AZD1480, a pharmacological inhibitor of JAK1 and JAK2, on GBM xenograft tumors, JAK2 andSTAT-3 phosphorylation in both human and murine glioma cells and leads to a decrease in cell proliferation. Furthermore, we utilized human xenograft GBM samples as models for the study of JAK/STAT3 signaling in vivo. In these xenograft tumors, JAK2 and STAT3 are constitutively active, but levels vary among tumors, which is consistent with the heterogeneity of GBMs. AZD1480 inhibits constitutive and stimulus-induced phosphorylation of JAK2 and STAT3 in these GBM xenograft tumors in vitro downstream gene expression and inhibits cell proliferation. Furthermore, AZD1480 suppresses STAT3 activation in the stem cell population in GBM tumors. In vivo, AZD1480 inhibits GBM tumor growth, increases tumor necrosis, and decreases tumor vascularity, indicating that AZD1480 will need to be considered in future studies. Overall, our data indicate that pharmacological inhibition of the JAK/STAT3 pathway by AZD1480 should be considered for study in the treatment of patients with GBM tumors.

CB-15. beta3-ADRENERGIC AGONISTS MIMIC EUSTRESS RESPONSE AND REDUCE LEPTIN-MEDIATED Proliferation in a GBM CELL LINE.

A beta3-adrenergic agonist may be beneficial for anxiety and depression, further improving the quality of life for brain tumor patients.

CB-16. STRESS-REGULATED EXPRESSION OF MIR-451 CHANGES MIGRATORY AND PROLIFERATIVE PROPERTIES OF GLIOMA CELLS IN VITRO AND IN VIVO: IMPLICATIONS FOR THERAPEUTIC RADIOTHERAPY AND CHEMOTHERAPY RESPONSE

GBMs are characterized by high levels of leptin and its receptor are expressed at much higher levels than in normal glial cells and provides a potential autocrine signaling pathway. In this study, we confirm that 200 ng/mL of leptin-conditioned media increases xenograft tumor growth and that leptin receptor agonism or linked beta3-adrenergic agonists may counteract leptin’s effects. To the contrary, adding 300 pg/mL of epinephrine to leptin-conditioned media blocked leptin-mediated proliferation. Treatment with analogs of beta3-adrenergic receptor antagonist resulted in expression and release in adipocytes. We have hypothesized that a beta3-adrenergic agonist would counteract leptin’s effects on T98G cell proliferation. Use of the beta3-adrenergic agonist BRL 37344 did not only counteract leptin’s effects but also significantly reduced T98G cell proliferation and chemotherapy (temozolomide). We also discuss the role of upstream stress-responsive transcription factors having their binding sites in close proximity to miR-451 genomic locus. To address how miR-451 regulates glioma cell invasiveness in vivo, we performed series of experiments co-injecting invasive (+/-) or non-invasive cells into nude mice intracranially. We demonstrate that miR-451 expressing cells have severe limited invasive phenotype, which requires sufficient glucose and facilitates rapid cell growth. Cancer cells ensure an adequate glucose supply through increased angiogenesis and migration; but in rapidly growing tumors such as GBM, where glucose availability may fluctuate, cells must engage adaptive strategies to survive. We perform a series of experiments to elucidate the role of microRNA (miR-451) that controls glioma cell proliferation, migration, and invasion, and responsiveness to glucose deprivation through modulating activity of the LKB1/AMPK signaling axis. This allows cells to survive metabolic stress and to seek out favorable growth conditions. In the current study, we show that expression of miR-451 is regulated by stress, as we were able to demonstrate profound effect of glucose withdrawal, radiotherapy, and chemotheraphy in response to fluctuating glucose levels in glioblastoma microenvironment. In response to fluctuating glucose levels in the glioblastoma microenvironment, it is essential for high growth and dissemination and is revealing novel targets for therapeutic intervention.
for periods of time showed disordered separation of chromosomes from metaphase plates accompanied by lagging chromosomes, dispersion of chromosomes from the metaphase plate, chromosomes decondensation in the cytoplasm, and daughter nuclei asymmetrically segregated, with derangement of cells increasing with the time of exposure. These data suggest that TTF/Exposure resulted in perturbation of processes following entry into anaphase and interfered with processes necessary for orderly exit from mitosis. Furthermore, cells exposed to TTF/Exposure showed no detectable p53 induction, indicating that cell death is achieved by a p53-independent mechanism. NovoTTF-100A appears to affect cell death by interference with the mitotic apparatus that differs from currently used spindle poisons and therefore may have synergism when combined with conventional cancer treatments. (Supported in part by a grant from NovoCure, Inc. and A Reason To Ride research fund.)

CB-18. SHP-2/PTPN11 MEDIATES GLIOGENESIS DRIVEN BY PDGFR AND INK4A/ARF ABERRATIONS IN MICE AND HUMANS

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Human gliomas account for the most common and malignant tumors in the central nervous system. Despite intensive treatments, including maximal surgical resection combined with radiotherapy and concurrent or adjuvant chemotherapies, median survival time of patients with high-grade glioblastoma multiforme (GBM) remains 14-16 months after diagnosis. Recently, coordinated genomic analyses of a large cohort of clinical GBM specimens have subclassified malignant glioblastomas into 4 clinical relevant subtypes based on their signature genetic lesions. PDGFRα overexpression is concomitant with a loss of CDKN2A locus (encoding P16INK4A and P14ARF) in a large number of tumors within one subtype of glioblastomas. Here, we report that activation of PDGFRα promotes tumorigenicity to Ink4a/Arf-deficient mouse astrocytes and human glioma cells in vivo. Restoration of p16INK4a but not p19ARF suppresses PDGFRα-driven gliomagenesis. Mechanistically, abrogation of signaling modules in PDGFRα-activated glioma that lost capacity to bind to SHP-2 or PI3K significantly diminished PDGFRα-driven gliomagenesis. Furthermore, inhibition of SHP-2 by shRNAs or pharmacologic inhibitors disrupted the interaction of PI3K with PDGFRα, suppressed downstream AKT/mTOR activation, and impaired tumorigenesis of Ink4a/Arf-null cells, whereas expression of an activated PI3K mutant rescued the effect of SHP-2 inhibition on tumorigenicity. In clinical glioblastoma specimens, PDGFRα and PDGF-A are co-expressed and such co-expression is linked with activation of SHP-2/AKT/mTOR-signaling. Together, our data suggest that in glioblastomas with Ink4a/Arf deficiency, overexpression of PDGFRα promotes tumorigenesis through the PI3K/AKT/mTOR-mediated pathway regulated by SHP-2 activity. These findings functionally validate the genomic analysis of glioblastomas and identify SHP-2 as a potential target for treatments of glioblastomas with PDGFRα overexpression.

CB-19. FUNCTIONAL CHARACTERIZATION OF MICRONRNAS IN PDGFR-DRIVEN GliOMAs

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MicroRNAs (miRNAs) are a class of small, noncoding RNAs that regulate gene expression on a posttranscriptional level by binding loosely complementary sequences in target mRNAs. Each miRNA likely represses numerous mRNA targets, and this promiscuity speaks to the ability of individual miRNAs to mediate complex biological phenotypes. Recent comprehensive genomic analyses have emphasized the importance of receptor tyrosine kinases (RTKs) and their downstream signaling cascades in the process of gliomagenesis. Among these, the platelet-derived growth factor (PDGF) pathway plays a crucial role in the initiation and maintenance of both glioma and glioblastoma. Improved understanding of how PDGF signaling mediates its oncogenic effects and the mechanisms for its regulation would be of obvious benefit to the development of effective targeted therapies. Based on an analysis of mRNA involvement in the phenotypic expression of gliomagenesis, we have identified a group of miRNAs whose expression levels are responsive to PDGFR pathway activation in vitro and have recapitulated these findings in human glioblastomas, particularly those driven by aberrant PDGFR signaling. Similarly, using data from The Cancer Genome Atlas (TCGA), we found that these miRNAs are differentially expressed in glioblastomas and, when overexpressed, drive gliomagenesis in vitro. Furthermore, cells exposed to TIEF showed no detectable p53 induction, indicating that cell death is achieved by a p53-independent mechanism. NovoTTF-100A appears to affect cell death by interference with the mitotic apparatus that differs from currently used spindle poisons and therefore may have synergism when combined with conventional cancer treatments. (Supported in part by a grant from NovoCure, Inc. and A Reason To Ride research fund.)

CB-20. CXCR4 ACTIVATION DEFINES A NEW SUBGROUP OF SONIC HEDGEHOG DRIVEN MEDIOLLOBLASTOMAS

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Medulloblastoma is the most common malignant brain tumor of childhood. Despite aggressive multimodal therapy, overall survival rates remain less than 70%, and survivors often suffer from severe neurologic, cognitive, and development side effects. The development of new, improved therapies may be facilitated by molecular subgrouping of disease in which the primary drivers of malignant growth are distinguished and the cell(s) of origin are identified. Recent gene expression profiling has revealed 4 distinct subtypes characterized by different gene expression signatures including the WNT, sonic hedgehog (SHH), MYCN, or an as yet unidentified signaling pathway. In the current study, we defined a new molecular subgroup of medulloblastoma characterized by co-activation of the SHH and CXCR4 pathways. Numerous reports involving more than 20 cancer types have suggested that CXCR4 expression has prognostic significance and that its activation can regulate cancer cell migration, proliferation, and survival. In using as vitro in vivo experimental models, we demonstrated a critical functional interaction between the SHH and CXCR4 pathways and report for the first time that both cell surface localization and Galphai-induced signaling through CXCR4 can be regulated by SHH activation. Human gene array analyses as well as genetically engineered mouse models of medulloblastoma indicated that the interaction between these pathways results in increased Cyclin D1 expression and enhanced tumor growth. Furthermore, we showed that this mechanistic interaction renders SHH-driven medulloblastomas, including those with activating mutations of Smoothened, sensitive to CXCR4 antagonism in vivo. Taken together, these data reveal a novel mechanism whereby the SHH and CXCR4 pathways augment each other’s function, advance medulloblastoma molecular subgrouping, and mandate evaluating combined SHH and CXCR4 antagonism in medulloblastoma where clinical trials with individual SHH antagonists have been limited by resistance related to Smoothened mutation.
INVASION, AND CELL SURVIVAL IN MALIGNANT GLIOMAS
progression.
into the surrounding brain tissue. This finding highlights the importance of
gene Olig2. Together, these results suggest that brevican secreted by
PDGFR signaling in these cells as well as expression of the proliferative
apoptosis in vitro and in vivo, while knockdown caused the opposite
effect. Because Notch signaling is a predominant prosurvival pathway in
gliomas and fibulin-3 bears homology with the Notch ligands of the Delta
family, we investigated a possible interaction of fibulin-3 with this
Notch signaling. In summary, our results reveal a potentially novel
mechanism by which a tumor-associated matrix molecule can activate
Notch signaling and modulate this major protumor pathway in gliomas.
These results highlight fibulin-3 as a tumor-specific, highly-accessible
target with therapeutic potential.

CB-22. BREVICAN, A PRO-INVASIVE MATRIX PROTEIN, ACTIVATES PDGFR SIGNALING AND STIMULATES OLIGODENDROCYTE PRECURSOR RECRUITMENT IN THE GLIOMA MICROENVIRONMENT
Hosung Sim, Paula A. Agudelo-Garcia, Bin Hu, and Mariano S. Viapiano; The Ohio State University, Columbus, OH

Malignant gliomas are virtually impossible to treat with conventional therapies because of the tumor's resistance to chemotherapy, radiation, and targeted therapies. Glioma invasion through upregulation of the small heat-shock protein and caspase-3-specific inhibitor alpha-B-crystallin. In addition, nuclear Bcl2L12 physically interacts with the p53 tumor suppressor and robustly represses p53 transactivation activities. To therapeutically suppress Bcl2L12’s diverse and potent glioma-promoting effects, robust cellular uptake, biocompatible intratumoral delivery upon systemic i.v. and local administration, reduced off-target effects, and diminished activation of innate immune responses compared to conventional lipoplex-delivered RNAi. In particular, we show that RNA-Au-NPs exhibited highly effective in knocking down Bcl2L12 than conventional RNAi-based methods and do not require auxiliary transfection agents. MB1 studies using Gd(III)-conjugated RNA-Au-NPs confirmed that these nanoparticles penetrate into the brain tumors highly effectively without the need for convection-based enhanced delivery. Finally, RNA-Au-NPs exhibited highly effective uptake into various primary and transformed glial cell lineages, in particular glioma stem cells, and provoked robust and persistent knockdown of Bcl2L12, which resulted in sensitization of cells toward apoptosis. Thus, silencing Bcl2L12 signaling by nano-RNAi represents a novel therapeutic approach to restrain GBM pathogenesis.

CB-23. FIBULIN-3 REGULATES NOTCH SIGNALING, TUMOR INVASION, AND CELL SURVIVAL IN MALIGNANT GLIOMAS
Bin Hu, Paula A. Agudelo-Garcia, Joshua Saldivar, Hosung Sim, Claire Dolan, Maria Mora, Gerard Nuors, Susan Cole, and Mariano S. Viapiano; The Ohio State University, Columbus, OH

Glioblastomas are the most common primary brain tumors and have extremely poor prognosis owing to their highly invasive nature. Glioma cells secrete a variety of ECM proteins that are absent in their glial counterparts and are critical molecules that favor invasion. We recently identified fibulin-3 as an ECM protein highly expressed in gliomas but absent in normal brain and rarely expressed in other solid tumors. We demonstrated that fibulin-3 regulates the expression of metalloproteases and is sufficient to promote tumor cell invasion through brain parenchyma. In the present work, we describe a novel role of fibulin-3 regulating the sensitivity of glioma cells to apoptotic treatments. Using gain- and loss-of-function approaches, we observed that fibulin-3 overexpression reduced glioma cell apoptosis in vitro and in vivo, while knockdown caused the opposite effect. Because Notch signaling is a predominant prosurvival pathway in gliomas and fibulin-3 bears homology with the Notch ligands of the Delta family, we investigated a possible interaction of fibulin-3 with this pathway. We observed that fibulin-3 induced Notch cleavage and activated a Notch-dependent reporter in cultured glioma cells. Moreover, overexpression or knockdown of this protein regulated the expression of downstream Notch effectors, such as Hes-1 and Hes-5, both in vitro and in vivo. Furthermore, analysis of fibulin-3 and Hes-1 in clinical samples showed a strong correlation between expression of both proteins and tumor grade. Finally, we demonstrated that the pro-invasive effect of fibulin-3 can be abolished by silencing of pro-invasive Notch1 or Notch2 or Notch1 siRNA. In summary, our results reveal a potentially novel mechanism by which a tumor-associated matrix molecule can activate Notch signaling and modulate this major protumor pathway in gliomas. These results highlight fibulin-3 as a tumor-specific, highly-accessible target with therapeutic potential.
CB-27. MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF miR-137 IN OLIGODENDROGLIOMAL TUMORS
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MicroRNAs are short noncoding RNAs that function as key regulators of diverse cellular processes through negative control on gene expression at the posttranscriptional level. A recent study reported (Silber et al., BMC Med 2008;6:14) that miR-137 expression was diminished in anaplastic astrocytoma and glioblastoma. The aims of this study were to investigate whether miR-137 was involved in oligodendrogliomal tumors and to elucidate the biological functions of miR-137 in gliomagenesis. Quantitative RT-PCR analysis revealed that miR-137 was significantly downregulated in 17 of 20 (85%) oligodendroglialomas and 16 of 16 (100%) oligoastrocytomas examined compared to normal brain tissues (p < 0.05). Ectopic expression of miR-137 inhibited cellular proliferation and induced apoptosis in oligodendroglioma and glioblastoma cells. To identify miR-137 targets, a computational approach was employed for target prediction. One of the candidate genes, CSE1L (chromosome segregation 1-like), was found to be downregulated at the protein but not at the mRNA level upon forced miR-137 overexpression. Luciferase reporter assay showed that miR-137 could interact with the putative miR-137 binding site in the 3′ untranslated region of CSE1L but not with the predicted binding site. These results suggest that CSE1L is a target of miR-137. Immunohistochemical analysis further demonstrated that CSE1L was overexpressed in oligodendrogliomas. Moreover, knockdown of CSE1L by RNA interference led to reduced proliferation and induced apoptosis in glioma cells. These effects were similar to those observed after miR-137 overexpression, but to a lesser extent, suggesting that miR-137 may mediate its effects partly through CSE1L. CSE1L has been implicated in cellular proliferation and apoptosis and is involved in modulating expression of a subset of p53 target genes. In conclusion, our results demonstrate that miR-137 deregulation is common in oligodendrogliomas and suggest that the miR-137/CSE1L axis may serve as a potential therapeutic target for treatment of oligodendrogliomas.

CB-28. THE SMALL GTPASE RHOG MEDIATES THE INVASIVENESS AND SURVIVAL PROPERTIES OF GliOBlastoma CELLS
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The invasion of glioblastoma cells into regions of the normal brain is a critical factor that limits current therapies for malignant astrocytoma. We examined the role of RhG in the invasive behavior of glioblastoma cells. We found that siRNA-mediated depletion of RhoG strongly inhibits invasion of glioblastoma cells through brain slices ex vivo. In addition, depletion of RhG strongly decreases colony formation, demonstrating a role for RhoG in glioblastoma cell survival. Importantly, we found that RhoG is activated by HGF and EGF, 2 factors that are clinically relevant drivers of glioblastoma malignant behavior. In addition, depletion of RhoG strongly inhibits activation of the Rac1 GTPase by both HGF and EGF. We also observed that in addition to controlling cell invasion, RhoG regulates glioblastoma cell migration and the formation of lamellipodia and invadopodia, all of which are functions that have been shown to be Rac1-dependent. However, unlike Rac1, depletion of RhoG does not significantly inhibit cell proliferation, suggesting that RhoG regulates a subset of Rac1-controlled functions. Importantly, functions of Rac1 in normal cells appear to be rather restricted, and RhoG knockout mice display no significant phenotypes. Thus, our results suggest that targeting RhoG-mediated signaling presents a novel avenue to limit the malignant behavior of glioblastoma tumors.

CB-29. MOLECULAR BASES OF RESISTANCE TO TARGET THERAPIES IN PATIENTS WITH PRIMARY AND RECURRENT MENINGIOMA
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INTRODUCTION: Meningiomas are common primary brain tumors that are minimally treated by surgical resection. However, atypical and anaplastic meningiomas have higher rates of recurrence. Frequently, multiple recurrences become progressively more challenging to manage. To date, systemic therapies have proven mostly ineffective. We assessed if a rationale exists for the treatment of meningiomas with therapies targeting molecular subgroups. METHODS: Eight adult patients (3 women) with pathological diagnosis of atypical meningioma treated at the Tom Baker Cancer Center, Calgary, Canada, were included. They had signed informed consent and tissue was available from the initial surgery and at least one additional resection (median, 3 surgeries; range, 2 to 6). We investigated EGFR tyrosine kinase mutations (L858R; exon 19 deletions), EGFRIII expression, MGMT promoter methylation status, expression of the telomerase catalytic subunit hTERT, and mutational status of the IDH1 gene. RESULTS: There was no change in bromodomain status from the initial surgical specimens through subsequent resections with respect to EGFR, hTERT, and IDH1. No patients harbored EGFR or IDH1 mutations. No patient expressed the telomerase subunit hTERT. The MGMT promoter was found to be unmethylated in all specimens except for one patient with multiple radiation-induced meningiomas. MGMT promoter status was heterogeneous (some of them in 2 resections without clinical evidence of response to temozolomide). CONCLUSIONS: These results suggest that EGFR tyrosine kinase mutations and MGMT promoter deletion mutation are not manifested in meningiomas. Previous reports have shown hTERT to be present in atypical meningiomas; however, we were unable to detect any hTERT expression in our cohort. Mutations in the IDH1 gene were not observed in our study. Lastly, MGMT promoter methylation was found to be predominantly unmethylated. Our results support that primary and recurrent meningiomas do not express the target for molecular therapies that illicit beneficial response in other tumor types.

CB-30. MOLECULAR MECHANISMS OF ACQUIRED RESISTANCE TO EGFR TYROSINE KINASE INHIBITORS IN GBM
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EGFR-targeted therapies such as the tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib have had limited success clinically in GBM. However, some of the most common mechanisms of resistance to these agents in other solid tumors are rarely present in GBM. In an effort to identify molecular mechanisms of acquired resistance to EGFR TKIs in GBM, we utilized in vitro and cell line–based approaches to explore resistance mechanisms. Using cell lines resistant to gefitinib (CB-27, MOLECULAR MECHANISMS OF ACQUIRED RESISTANCE TO EGFR TYROSINE KINASE INHIBITORS IN GBM) and erlotinib, we have identified a novel mechanism of resistance. In CB-27, we observed EGFR tyrosine kinase expression in unresistant parental cells and reduced expression in TKI-resistant cells. This reduction was not due to changes in EGFR copy number, but instead was due to reduced expression of the EGFR subunit hTERT. The hTERT promoter is methylated in approximately 50% of glioblastomas and serves as a mechanism for downregulation of hTERT expression. In this study, we observed significant downregulation of hTERT expression in a subset of CB-27-resistant cells. Furthermore, we observed reduced phosphorylation of the downstream targets of EGFR, Akt, and Erk, consistent with reduced expression of hTERT. In conclusion, these results suggest that reduced expression of hTERT may contribute to acquired resistance to EGFR TKIs in GBM.

CB-31. MGMT PROMOTER METHYLATION AND DNA MISMATCH REPAIR IN PRIMARY AND RELAPSED GliOBLASTOMA
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Conventional treatments for newly diagnosed glioblastoma (GBM) include maximal surgical resection and radiotherapy with concomitant and adjuvant temozolomide (TMZ). However, nearly all patients inevitably experience tumor recurrence during or after the chemotherapy phase, and the relapsed lesion is typically refractory to further treatment. Both O6-methylguanine-alkyltransferase (MGMT) and mismatch repair proteins (MMR) are involved in the repair of methylating agents, such as TMZ. Patients with high levels of MGMT promoter methylation tend to have better clinical outcomes; however, MGMT promoter methylation is currently not used as a prognostic marker in clinical practice. The therapeutic potential for MMR inhibitors such as p53 or mismatch repair deficient glioblastoma is currently under investigation. In glioblastoma, MMR proteins are often mutated, and DNA mismatch repair (MMR) is frequently reduced, indicating that DNA mismatch repair (MMR) may be of potential therapeutic interest in glioblastoma.
(MMR) are involved in DNA repair. We have examined the incidence of MGMT methylation, assessed by pyrosequencing, in a retrospective cohort of 140 GBM specimens. An estimated 29% of patients were positive for MGMT methylated. While MGMT methylation did not affect overall survival in our cohort, progression-free survival was significantly prolonged. Interestingly, we also identified a proportion (36%) of unmethylated patients who showed a strong survival benefit of over 18 months. Among these patients, we performed cross-species comparison studies, using human GBM and murine glioma models, demonstrating that these models provide a valuable approach for refining lists of candidate genes that play a role in the evolution of GBM.

CB-34. ISOCENIC GLOBLASTOMA STEM CELL LINES WITH DIFFERENT EGFR AMPLIFICATION LEVELS ESTABLISHED FROM INDIVIDUAL TUMORS

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INTRODUCTION: EGFR amplification is present in almost 50% of all glioblastomas (GBM) and is frequently associated with expression of a truncated, constitutively active variant, EGFRvIII. Experimental approaches to study these alterations exist as both are rapidly lost when GBMs are taken into culture. We developed conditions facilitating the growth of cell lines that either maintain high-level EGFR amplification/EGFRvIII expression or not, allowing direct comparison between cells with a heterogeneous EGFR status derived from the same original tumor. METHODS: Fresh tumor material was cultured using glioma stem cell conditions with modifications. The resulting matched pairs of cell lines and original tumors were analyzed by immunohistochemistry, western blot, FACS, and FISH for different levels of EGFR gene amplification, EGFRvIII expression, EGFR/EGFRvIII protein, and in vivo tumorigenicity. RESULTS: From 5 freshly resected GBM, 2-3 primary cultures were generated that differed in EGFR gene amplification, EGFRvIII expression, and EGFR/EGFRvIII protein, depending on culture conditions. Cultures from 2 GBM developed into pairs of permanent cell lines. The first pair consisted of one cell line with high-level EGFR amplification and high EGFR protein expression, whereas the second cell line showed EGFRvIII expression. The EGFRvIII status was maintained in vivo. Analysis of xenograft tumors showed that they recapitulated the phenotype of the cell lines and resembled their parental primary tumors on protein and genomic levels. CONCLUSION: Our cell lines provide a model to study the function of EGFR amplification/EGFRvIII expression in glioma cells and assess the impact of intratumoral EGFR status heterogeneity on the response to EGFR-targeting and other agents.

CB-35. KRTS IS THE TARGET GENE OF 41 OF 53 MICRORNA CLUSTERS IN CHROMOSOME 14q32.31

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BACKGROUND: We previously demonstrated that underexpression of a cluster of 53 microRNAs (miRNAs) on chromosome 14q32.31 is consistently present in gliomas. Our presumption was that this region operates as a tumor suppressor and that dysregulation of the related miRNAs may lead to subsequent abnormal expression of their targets, resulting in tumorigenesis. AIM: To identify the target genes of the miRNAs from the 14q32.31 region and their related oncogenic function. METHODS: Four bioinformatic algorithms (PITA, TargetScan, PicTar, and miRanda) were used to identify the potential target genes of miRNAs from this cluster. A reporter plasmid harboring the 3′-UTR region of putative target genes downstream of the luciferase coding region were constructed. To study the role of the target genes on tumorigenesis, mir-323-3p, mir-369-3p, and mir-433 from this region were introduced into mouse and human glioma cell lines, and their effects on proliferation and migration were evaluated. RESULTS: Among the putative identified candidates, KRT4 was selected for further analysis because 77.4% of the miRNAs from this cluster were predicted to target this oncogene. A 70%-80% reduction in luciferase activity was noted following cotransfection of KRT4 3′-UTR reporter plasmid with each of the individual pre-miRNAs 323-3p, 369-3p, and 433, and 90% for all of them together. Minor overexpression (>10 fold) of all miRNAs 323-3p and 369-3p significantly reduced cell proliferation and migration rate of both glioma cell lines.
CONCLUSIONS: Our study indicates that miRNA-cluster in chromosome 14q32.31 may function as a tumor suppressor gene through various mechanisms including inhibition of tumor cell growth and migration. It also suggests that the miRNA-cluster exerts its tumor suppression effect through post-transcriptional regulation of KRAS based on the account that all miRNA from this region are underexpressed in gliomas, while 77.4% of them putatively target the KRAS oncogene, and the findings that the 3 tested miRNA reduced luciferase activity following cotransfection with KRAS 3'UTR.

CB-36. DUAL INHIBITION OF HDACs AND KDM1A LEADS TO THE EXPRESSION OF GENES INVOLVED IN APOPTOSIS
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Glioblastoma multiforme (GBM) is a particularly aggressive brain tumor and, despite innovative therapies, remains a clinically devastating disease. Enzymes that control epigenetic alterations are popular targets for cancer therapy owing to the dynamic nature of these modifications and their ability to control cellular processes that lead to oncogenesis. Histone deacetylases (HDACs) are a family of enzymes that have been targeted for cancer therapy; however, HDAC inhibitors (HDACi) show only moderate success in GBM. Our previous studies evaluated the cellular consequences of simultaneously inhibiting HDACs and the lysine specific demethylase 1 (KDM1A), another enzyme that regulates epigenetic marks. We found that simultaneously inhibiting these enzymes increased cell death in GBM cells but not their normal counterpart counterparts. Since HDAC1/2 and KDM1A are found in similar complexes that function to regulate transcription, we hypothesized that these 2 enzymes cooperate to enhance cell death by controlling the expression of genes that play a role in apoptosis. Using a focused qRT-PCR array, we evaluated the expression profiles of 88 genes involved in apoptosis in GBM cells transfected with control or KDM1A-specific shRNA and left untreated or treated with the HDACi vorinostat. Our results reveal several genes that are altered upon treatment with vorinostat, some of which are further changed when both HDACs and KDM1A are inhibited. Surprisingly, mRNA expression of both p53 and p73 is reduced by 50% in KDM1A knockdown cells and further reduced (>90%) upon treatment with vorinostat for 24 hours. Similar to mRNA expression, this reduction is also decreased in KDM1A knockdown cells and almost undetectable with the addition of vorinostat. Further studies evaluating the molecular mechanism by which HDACs and KDM1A regulate p53 will provide molecular insight into the mechanism by which these epigenetic modifiers interact to control cell survival and may suggest novel options for GBM therapy.

CB-37. ENDOGENOUS DOWNREGULATION EFFECT OF DRUG RESISTANCE GENE MGMT BY MICRONA
Daisuke Oyama, Hiroshi Nakashima, Jakub Godlewski, and Antonio F. Choucair; The Ohio State University, Columbus, OH

INTRODUCTION: The drug resistance gene ß-O-methylguanine-DNA methyltransferase (MGMT) is thought to represent a mechanism of glioma resistance, countering TMZ treatment and possibly worsening patient prognosis. MicroRNAs are 22-nucleotide small RNAs that regulate translation and decay of their target mRNAs. OBJECTIVE: In this study, we searched for microRNAs that suppress translation of MGMT. METHODS AND FINDINGS: Based on our in silico analysis, we picked up 6 microRNAs that putatively target MGMT. Two of 6 selected microRNAs downregulated MGMT mRNA by more than 40% in the T98G glioma cell line that is usually resistant to TMZ. This led to downregulation of MGMT protein, assayed by western blot. MicroRNA-transfected T98G cells showed increased sensitivity to TMZ, similar to the sensitivity of U87MG cells that are very sensitive to TMZ. CONCLUSIONS: Suppression of the drug-resistance gene MGMT to increase chemosensitivity in a variety of tumors including GBMs.

CB-38. IDENTIFICATION OF EGFVR-II-INDUCED GENE SIGNATURES USING RAT GLIOMA MODEL AND HUMAN GBMS
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EGFRVIII, a constitutively active truncated mutant of epidermal growth factor receptor (EGFR) has been shown to increase neoplastic transformation and tumorigenesis in a variety of tumors including GBMs. However, transcriptional mediators of EGFVRVIII have not been fully elucidated. In the present study, we analyzed 32 primary human GBMs and a 9L EGFVRVIII rat brain tumor model to identify EGFVRVIII-specific gene signatures. Using 9L gliosarcoma cells and cells exhibiting increased transformation and invasion when compared to empty vector (EV) controls. MRI imaging of Fischer rats either bearing 9L.EV or 9L.EGFRVIII intracranial tumors revealed significantly enhanced tumor volume in EGFVRVIII tumors. Immunohistochemical analyses showed increased expression of Akt, Erk1/2, PLC-γ, Gab1, and SHP-2 in EGFVRVIII-bearing tumors. Gene expression analyses of 9L.EGFRVIII tumors demonstrated increased expression of ~1498 gene probes when compared to control tumors (p<0.05). David enrichment analyses revealed 9 clusters of genes that mediate increased transformation, invasion, glycolysis, and hypoxia in 9L.EGFRVIII tumors. Comparative evaluation of gene expression profiles from rat tumors and primary human GBMs revealed 9 novel genes (ckap4, lrp1, fat3, s3c7a1, cdk6, socs2, aqpl2, spry2, and aebp1) that were significantly upregulated in EGFVRVIII tumors. In the present study, we validated physiological significance of spry2 and aebp1 using RNAi-mediated gene silencing and immunoprecipitation assays and demonstrated that Spry2 and AEBP1 physically bind to EGFRVIII to inhibit its transforming efficiency. Collectively, our data presents a comprehensive EGFVRVIII-specific gene signature profile using a rat glioma model and human GBMs and characterizes potential targets to inhibit EGFVRVIII-mediated transforming phenotypes in malignant gliomas.

CB-39. NUCLEAR FIP200 AND RB EXPRESSION IN BRAIN METASTASIS FROM BREAST CANCER: POTENTIAL PREDICTORS OF SURVIVAL
Nooshin Hashemi Sadrzai, Monica Burgert, Mamnteelho Wolawa, Russell Lippins, Deepa Kheria, Robert Weil, Amy Nowacki, Richard Prayson, Ting Shi, and Candece Gladson; Cleveland Clinic Foundation, Cleveland, OH

Patients with brain metastasis from breast cancer have a poor outcome with significant variation in overall survival (OS). No marker to predict survival exists. FIP200 is a signaling node; in the nucleus, it inhibits cell proliferation by promoting Rbl-1 and p21 transcription, and in the cytoplasm it promotes cell survival by inhibiting Pyk2 activation and positively regulating autophagy. FIP200 cellular localization and genetic alterations have not been examined in brain metastasis from any cancer. In a retrospective analysis, brain tissues of 21 patients with brain metastasis from invasive ductal breast cancer obtained between 8/2000 and 3/2010 and randomly chosen based on availability of tissue were evaluated for FIP200 and Rb expression and localization by immunohistochemistry, along with 15 primary breast cancer samples. Genetic alterations were evaluated by DNA array analysis. Low levels of expression of nuclear Rb1 (<30%) and nuclear FIP200 (<20%) were seen in 11 and 13 of 21 patients with brain metastasis, respectively, and there was a trend towards shorter median OS in these patients as compared to patients with ≥ 30% nuclear Rb and ≥ 20% nuclear FIP200. The pattern of FIP200 in 15 primary breast cancers was very different; no nuclear FIP200 was detected. Previously other investigators reported FIP200 deletion or mutation in 20% of primary breast cancers. On DNA analysis for copy number variation and LOH in the brain metastases, we found loss of p53 in 4 of 11 patients, Erbb2 (Her-2) amplification in 4 of 11, no deletion/mutation in Rbl-1, and no deletion in FIP200. The pattern of nuclear expression of Rbl-1 and FIP200 in breast cancer metastasis to the brain is different in patients with a longer OS. An expanded study is underway to determine whether FIP200 and/or Rbl nuclear expression is predictive of OS in these patients and whether their expression is linked.

CB-40. PREDICTION OF THERAPY RESPONSE IN SHORT-TERM TREATED PRIMARY TUMOR-INITIATING CELLS IN VITRO
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The increasing knowledge of the pathogenesis of tumor formation and progression in high-grade gliomas has led to the development of a novel group of therapeutic agents that includes small molecule kinase inhibitors. These novel agents often directly interfere with growth factor signaling pathways that are upregulated in brain tumors and are supposed to interfere with oncogenesis. Despite promising preclinical studies, results of pilot trials have been generally disappointing. One reason may be the enormous molecular and genetic heterogeneity between individual tumors. Therefore, it is demanding to correlate individual response to the general susceptibility of a certain tumor entity to certain substances. In our preclinical study, we...
examined the effect of the receptor-tyrosine kinase (RTK) inhibitor Sunitinib in 20 tumor-initiating cell lines using the following approach: culture under serum-free conditions; short-term culture and treatment; and hypothesis-free evaluation with a signature by mutual validation of independent datasets. Dose responses were evaluated by western blot and response was evaluated by functional assays. Cells were treated with Sunitinib or DMSO (control) alone or together with VEGF-A/ PDGF-AB for 6 hours. The phosphorylation of signal transducers and activators of transcription (STATs) downstream of these RTKs was assessed by western blot. The results reveal that stimulation as well as inhibition of particular RTKs has different downstream effects in distinct cell lines. We are on our way to further correlate this data to the expression profiles obtained from microarrays and the outcome of functional assays to define a molecular signature that can predict treatment response in vitro. So far our data clearly demonstrate the heterogeneity of treatment response on a molecular level and underlines the importance to preselect patients for clinical trials. We mandate that the concept of a personalized treatment requires an in vitro drug testing tool that enables the prediction of therapy response within a single short-term assay.

CB-41. DIFFERENTIAL EXPRESSION OF G PROTEIN-COUPLED RECEPTOR KINASES IN GLOBLASTOMA MULTIFORME
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G protein-coupled receptor kinases (GRKs) comprise a family of 7 transmembrane serine/threonine protein kinases that regulate signal transduction by phosphorylating activated G protein-coupled receptors, leading to their desensitization, endocytosis, intracellular trafficking, and resensitization. Recent studies have highlighted differential expressions of GRKs in breast, ovarian, and thyroid cancers, suggesting their potential role in tumorigenesis and tumor growth. Furthermore, GPCRs, including EGFR, PDGFR, and VEGFR, are well known for roles in regulating GBM proliferation and intratumoral angiogenesis. Therefore, it is crucial to investigate the role of GRKs in GBM. To analyze the expression of GRKs in GBM, we established 20 glioma stem cell lines in serum-free condition along with more differentiated GBM cell lines grown in the presence of serum from 20 freshly collected GBM specimens. We compared GRKs protein and mRNA expression levels in 2 types of cell lines. We found that GBM-derived glioma cells have higher expression of GRK3. On the other hand, more differentiated tumor-derived cells have higher levels of GRK2 and GRK6. However, GRK5 was expressed equally in both types of tumor cell lines. In contrast, normal human astrocytes expressed relatively equal but lower levels of GRK2, 3, 5, and 6. To further evaluate the functional role of GRKs in GBM clinically, we utilized the TCGA database, and the resulting Kaplan-Meier survival analysis demonstrated that GRK3 downregulation, compared to its upregulation in GBM, correlated with significantly increased the tumorogenicity, or stemness, of GSCs within the GBM microenvironment enhance the tumorigenic and proliferative properties of GSCs through the IL6/STAT3 pathway.

CB-43. AN ESSENTIAL ROLE FOR EGFR WILD TYPE IN EGFRVIII-MEDIATED GLIOGENESIS
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EGFR gene amplification and mutation is a signature lesion in glioblastoma (GBM). EGFRvIII is the most common EGFR mutation in GBM and is highly oncogenic. EGFRvIII is usually coexpressed with EGFR wild type (EGFRwt) in GBMs. Previous studies have suggested a role for EGFRwt in EGFRVIII-mediated oncogenicity. In order to study the role of EGFRwt in EGFRVIII-mediated oncogenicity, we used a tetracycline-inducible model of EGFRvIII expression in an isogenic GBM cell line that also expresses endogenous EGFRwt. To perturb EGFRvIII signaling, we either silenced or overexpressed wild-type EGFR in U251MG cells expressing tetracycline-inducible EGFRVIII. Silencing the wild-type EGFR resulted in a striking inhibition of EGFRVIII-induced tumorigenecity in an orthotopic mouse model while increasing EGFRwt resulted in accelerated EGFRVIII-mediated tumor formation. EGFRwt in the absence of EGFRVIII had only a weak tumorigenic effect. To examine the effect of EGFRwt in EGFRVIII-mediated signal transduction, we conducted gene expression analysis in tetracycline-induced EGFRIII-expressing cells with silenced EGFRwt, low endogenous EGFRwt, and overexpressed EGFRwt. Consistent with the biological phenotype, we found that the level of EGFR wild-type expression has a profound effect on EGFRVIII signaling with major shifts in gene expression profiles. In general, the signal transduction profile of EGFRVIII signaling in the presence of EGFRwt includes a higher number of genes involved in cell proliferation compared to cells with silenced EGFRwt. In addition, in the presence of EGFRwt the top canonical pathway activated is “the role of tissue factor in cancer”. This highly oncogenic pathway is not activated by EGFRvIII in cells with silenced EGFRwt. Thus, our studies strongly suggest an important role for EGFRwt in EGFRVIII signaling and may help to identify key EGFRVIII effector signals.

CB-44. A NOVEL MECHANISM OF GLIOMA RESISTANCE TO ONCOVIRAL VIROTHERAPY: IFN-INDUCED ISGLYATION BY HDAC6
Tran Nguyen, Hiroshi Nakashima, and E. Antonio Chiocca; The Ohio State University, Columbus, OH

Novel strategies, including oncolytic viral (OV) therapy using conditionally replicating virus, have shown promise in various neurological tumors, but there is resistance to the lytic effect of the OV. We have been attempting to identify interferon-response pathways and genes that may be important in this response. In this study, we hypothesized that one interferon-stimulated gene (ISG15) was important in this cellular antiviral response through its interactions/ binding with a particular histone deacetylase (HDAC6), which also participates in antiviral response. METHODS: To investigate the relationship between HDAC6 and ISG15, enzyme overexpression was induced in U251 glioma cells under IFNβ treatment. Analyses were performed using communoprecipitation assay. RESULTS: We confirmed the binding of HDAC6 to ISG15, and also found that HDAC6 was capable of binding to ISG15 enzymes (UbE1L, UbCH8 and HERC5), suggesting that HDAC6 is involved in ISGLylation mediation. We then proceeded to identify the binding region of ISG15 E1-E3 enzymes on HDAC6. CONCLUSION: The novel discovery of HDAC6-ISG15 interaction and antiviral inhibition via inhibition of the STAT3 pathway. In conclusion, TA-MSCs within the GBM microenvironment enhance the tumorigenic and proliferative properties of GSCs through the IL6/STAT3 pathway.
CB-45. USE OF DOXORUBICIN AND DOXORUBICIN/IMIPRAMINE BLUE COLORED NANOPARTICLES YIELDS SURVIVAL IN AGGRESSIVE HUMAN GLIOBLASTOMA IN MICE
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Glioblastoma invasion is a major reason for recurrence after treatment. Recently, we saw in a rat glioblastoma model (RT2) that combination of doxorubicin and imipramine blue, a novel anti-invasive agent that acts via inhibition of Nox4, enhances survival as compared to doxorubicin alone. In the current study we use conventional enhanced delivery (CED) of doxorubicin (DXR) and imipramine blue (IB) and human glioblastoma line transformed to express the EGFR-VIII receptor, which is more clinically relevant than the rat glioblastoma previously studied. In this study, we used liposomal nanoparticles containing DXR and IB for CED to an aggressive human glioblastoma U87MG-EGFRVIII. Mice were intracranially injected with tumors into the brains of immunocompromised mice and treated 7 days later via CED delivery with 5 μL of nanoparticles containing: saline (n = 5), IB (16 μg, n = 5), DXR (20 μg, n = 8), or IB-DXR (16 μg IB, 20 μg DXR, n = 8). Mice were monitored by MRI before and after treatment. Postmortem, brains were sectioned and stained for hematoxylin and eosin. Further, the combinatorial treatment was tested in vitro on the cell line to determine any synergistic effects on cell viability. Both the DXR and IB groups showed enhanced efficacy by increasing debris above control groups (p < 0.001 for each as compared to control using Mantel-Wilcoxon test). There was no benefit for codeelivery of the IB with DXR. However, there was complete survival and remission in 3 animals prior to DXR-loaded nanoparticles. The results indicate benefit to this therapeutic in CED against EGFRVIII positive glioblastomas. However, this data is striking in the ability of the nanoparticles delivered via CED to yield complete survival in this highly aggressive model of glioblastoma.

CB-46. THERAPEUTIC IMPLICATIONS OF PROTEIN KINASE CK2 IN GLIOBLASTOMA
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Protein kinase CK2 is a serine/threonine kinase composed of 2 catalytic subunits (alpha or alpha') and 2 beta regulatory subunits. CK2 phosphorylates over 300 substrates involved in DNA replication, gene transcription, signal transduction, cell growth, and apoptosis. CK2 expression and activity is upregulated in tumors including kidney, breast, prostate, and head and neck cancers, and it has been suggested that a common denominator of diverse cancers may be an addiction to CK2. The NF-kappaB, Wnt, Notch, PI3K/AKT, and Hedgehog pathways are all positively regulated by CK2 in a manner that promotes cell survival. We have recently demonstrated another important function of CK2 as a novel interaction partner of JAK1 and JAK2 that promotes JAK and STAT-3 activation. Aberrant activation of the JAK/STAT-3 pathway is implicated in glioblastoma (GBM) progression, as well as propagation of the stem cell population in GBMs. We have evaluated the expression/function of CK2 in the context of GBMs. CK2alpha is overexpressed in brain tissue from GBM patients compared to control tissues. TCGA data reveals gene dosage gains in CK2alpha in 33% of 219 GBMs. Functionally, we inhibited CK2 expression by use of siRNA against CK2alpha and beta, and the activity of CK2 by pharmacological inhibitors such as TBB. These strategies resulted in inhibition of both constitutive and stimulus-induced JAK2 and STAT-3 activation and inhibition of STAT-3 target genes such as SOCS3, IL-6, Pim-1, and Mcl-1. CK2 inhibition also suppressed survival of GBM cell lines as well as primary GBM xenograft tumors, suppressed colony formation, and induced apoptosis. CK2 inhibitors are in phase I clinical trials in multiple hematological malignancies and pancreatic cancers as well as multiple myeloma. Pharmacological inhibition of the JAK/STAT-3 pathway by CK2 inhibitors, which will likely also negatively impact other signaling pathways, should be considered for treatment of patients with GBM tumors.

CB-47. GALECTIN-3 SELECTIVELY KILLS TUMOR CELLS THROUGH THE INTERACTION WITH B1 INTEGRIN VIA ENHANCED N-GLYCAN-DEPENDENT MECHANISM
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Galectins are a family of animal lectins, which bind beta-galactose moieties. Galectin-3 (Gal3) is the only chimeric family member of galectins and possesses a carbohydrate recognition domain (CRD), which has binding affinity for galactose and N-acetylactosamine. We found that extracellular Gal3 shows a tumor-specific apoptotic effect that in a variety of tumor cell types (malignant glioma, breast, colon, pancreas, GI) are sensitive to Gal3-mediated killing, while normal cells (fibroblast, endothelial, astrocyte) are not affected. Furthermore, extracellular Gal3 significantly reduces in vivo tumor volume. We hypothesize that Gal3 selectively kills tumor cells through a tumor-specific modified caspase receptor system. First, we found that Gal3 kills tumor cells through caspase-9 dependent apoptotic induction and that their killing is neutralized with lactose, a Gal3-CRD binding ligand. Second, we identified that Gal3-induced tumor-specific apoptosis is mediated through Gal3-beta1 integrin interaction through a couple of affinity assays and beta1 integrin siRNA neutralization. Third, we discovered that enhanced Gal3-beta1 integrin interaction is mediated through the aberrantly N-linked glycosylated beta1 integrin on tumor cells in that the expressions and the activities of N-acetylgalactosaminyltransferase V (MGATS5), a tetraantennarybranching glycosyltransferase, and upstream glycan-branich enzymes such as beta1, 4 galactosyltransferases (beta4GalT1), and beta1, 3 acetylgalactosami nyltransferase 2 (beta3GalT2) are significantly enhanced in tumor cells. The manipulation of MGATS5 affects the sensitivity of Gal3-mediated apoptosis. Our data showed Gal3 selectively kills tumor cells through the interaction with aberrantly glycosylated beta1 integrin and consequent induction of caspase-9 dependent apoptosis. This study gives new insight into the development of selective and safe tumor therapeutics.

CB-48. MATRICELLULAR PROTEIN CYR61 ACTIVATES A CELLULAR ANTIVIRAL RESPONSE BY BINDING TO INTEGRIN A6 IN LIMITING ONCOLYTIC VIRAL THERAPY FOR GLIOMA
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Oncolytic viruses (OV) have been increasingly recognized as an effective therapy against glioma. The efficacy of this therapy, however, is limited by the host cellular innate immune response characterized by activation of type 1 interferons (IFN). We have previously identified a significant induction of the secreted, extracellular matrix protein Cyr61 following OV therapy. Here, we tested the impact of Cyr61 in the tumor microenvironment on OV efficacy. Using glioma cells transiently transfected with Cyr61 plasmid and tetracycline-inducible glioma cell lines that express Cyr61 in the presence of doxycycline, we show that Cyr61 expression leads to a significant reduction in OV transgene expression. Additionally, reduction in OV transgene expression is also observed in glioma cells plated on purified Cyr61, and this inhibition is reversed when cells are incubated with neutralizing antibodies specific to Cyr61. Attesting to Cyr61’s effect on viral oncolysis, we show that Cyr61 expression reduces viral toxicity and inhibits viral replication both in vitro and in vivo. Microarray and real-time qPCR analyses revealed a significant induction of the type 1 IFNs and IFN responsive genes when cells were induced to express Cyr61; activation of the Jak/Stat signaling pathway was functionally verified by phosphorylation of Stat1 and Stat2. Employing function-blocking antibodies to various integrin receptors known to bind to Cyr61, we show that neutralization of the alpha6beta1 integrin receptor rescues the Cyr61 mediated OV inhibition. Indeed, we also show that activation of the alpha6beta1 integrin receptor on glioma cells by laminin also results in a reduction in OV transgene expression. Collectively, the results from this study indicate that the interaction of the extracellular matrix protein Cyr61 with the alpha6beta1 integrin receptor on glioma cells results in the induction of type 1 interferons, activating an innate antiviral response to OV and limiting its efficacy.

CB-49. INFLUENCE OF ACID SPHINGOMYELINASE ON THE BIOLOGY OF GLIOBLASTOMA AND ITS RESPONSE TO STANDARD THERAPEUTICS
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Ceramide is widely known for its influence in cell signaling, specifically in relation to programmed cell death. Interestingly, ceramide exists in decreased levels in glioma compared to normal brain, and levels have even been correlated inversely with tumor grade. Ceramide can be generated de novo from serine and palmitoyl-CoA or by the hydrolysis of sphingomyelin by the phosphodiesterase sphingomyelinase. Acid sphingomyelinase (ASM) has been shown to be activated by numerous stimuli including chemotherapy and γ-irradiation but is significantly downregulated in malignant astrocytomas compared to normal glial tissue by analysis p < 0.001. Furthermore, our
data show that primary glioma neurosphere cultures express significantly lower levels of ASM protein compared to traditional serum cultured glioma cell lines. In this study, we describe the effects of ASM expression on glioma therapeutic resistance. We created 2 stable glioma cell lines, which constitutively overexpress ASM. Although ASM overexpression had no significant effects on proliferation in vitro, intracranial tumor formation was delayed significantly in vivo (p < .01). We also observed significant changes in the migratory and invasive properties of these cells. Cells overexpressing ASM migrated significantly less in a standard Boyden chamber assay compared to transfection controls (p < .005). Invasion was also abrogated as these glioma cells showed significant inhibition in their migration through Matrigel-coated Boyden chambers (p < .005). Next tested the influence of ASM expression on standard therapeutics. Glioma cells overexpressing ASM were shown to have significant sensitivity to temozolomide (p < .001) and radiation in a standard clonogenic assay (p < .005). This increase in sensitivity was not dependent on traditional caspase signaling but did result in a reduction of prosurvival signaling as displayed by diminished levels of phosphorylated AKT post-treatment. Here, we show for the first time that expression of ASM has substantial effects on the biology of glioblastoma and its response to therapy.

CB-50. NOVEL PHOSPHORYLATION SITE IN GLIOMA EXPRESSED GALECTIN-1 MODULATES INVASIVE PHENOTYPE
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High-grade gliomas express high levels of galectins (predominately Galectin-1, -3, and -7). Galectins comprise a family of carbohydrate-binding proteins, which bind beta-galactosides. Galectin-1 (Gal-1) is differentially expressed in normal tissues and tumors. It appears to be functionally polyvalent and displays a wide range of biological activities. We and others have previously shown that Gal-1 is highly expressed in high-grade glioma cell lines and in glioma stem-like cell lines, almost without exception. It has also been shown that increased Gal-1 expression is highly correlated to going.

CB-52. MOLECULAR DETERMINANTS OF DICHOTOMY BETWEEN PROLIFERATION AND INVASION OF GLIOMA CELL LINES
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Classic histological features of glioblastoma include dense proliferative areas rich in angiogenesis as well as centripetal dissemination of neoplastic cells and a distinct invasive zone adjacent to the tumor. Distinct transcription factors are discernible between GBM cells at the tumor core and invasive rim, and many of the differentially expressed genes are co-associated with migration and proliferation. Our studies of glioma cells from paired core and rim human biopsy specimens reveal a higher proliferative index (Ki67 Mib-1 IHC score) at the core as compared to the rim (19 of 35 specimens; p < 0.002). Analysis of activation states of transcription factors revealed that c-Myc activity is up in the core while NF-kappab activity is down in the rim. Immunohistochemical validation using a glioma tissue microarray containing paired core and rim biopsy specimens showed that phospho-c-Myc staining in the nucleus was higher in the core than in the rim for 24 of 39 biopsy specimens scored, whereas phospho-NF-kappab staining was lower in the core than in the rim in 25 of 39 biopsy specimens scored. Depletion of c-Myc expression resulted in a decrease in migration rate and decrease in the proliferation rate of glioma cells in vitro. Conversely, inhibition of NF-kappab by pharmacological inhibitors resulted in a decrease in the migration and invasion rates of GBM cells in vitro and ex vivo. However, inhibition of NF-kappab actually did not increase the proliferation rate of glioma cells, suggesting that some basal expression level of NF-kappab is necessary to negate pro-apoptotic characteristics of c-Myc and to drive the malignant phenotype of the glioma cells. The “Go versus Grow” hypothesis suggests cell proliferation and migration are temporally exclusive behaviors and tumor cells postpone cell division for migration. Our findings argue that differential suppression/activation of c-Myc and NF-kappab underlies the shift of glioma cells from growing to going.

CB-53. FOXO CROSS-GREDUCTION OF TSC1-MTORC1 IN GLIOMA
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The Forkhead Box subclass O (FOXO) transcription factors govern the cellular programs of quiescence, redox stress responses, and apoptosis. We show that FOXOs regulate glycolysis through transcriptional control of the TSC1 tumor-suppressor protein. FOXO inactivation reduced TSC1 expression in multiple cell types, triggering mTOR complex 1 (mTORC1) activation and increased cellular glycolysis (the Warburg effect). FOXO inactivation was counteracted by the allosteric inhibitor of mTORC1, rapamycin, reducing glycolysis. Oncomine analysis suggested that decreased TSC1 mRNA expression frequently occurs in glioma. In mRNA harvested from gliomEPI1, a paired reduction in TSC1 mRNA expression, which correlated with decreased FOXO3 mRNA (R² = 0.779, p < 0.001). Immunoblot analysis of a small panel of tumors suggested increased activation of mTORC1 in samples under inhibitory phosphorylation of FOXO3, together with the data indicates that FOXO3 is required for mTORC1 activation and altered metabolism in glioma. This suggests that FOXO inactivation contributes to mTOR activation and therefore re-activating FOXO may be a target for treatment of gliomas.
Injecting PDGF-expressing retrovirus into the subcortical white matter of adult rats induces the formation of brain tumors with the histological features of GBM, including diffuse infiltration of tumor cells, glomeruloid vascular proliferation, and pseudopalisading necrosis. In contrast, when the same retrovirus is injected into the spinal cord of adult rats, the resulting tumors do not resemble GBM but rather showed a unique histology characterized by nests of tumor cells separated by a dense vascular network without areas of necrosis. To examine if these differences were intrinsic to the tumor cells or owing to the effects of the microenvironment, we performed a series of tumor cell transplantation studies. Cells were isolated from forebrain or spinal cord tumors and then injected into the forebrain or spinal cord of naïve adult rats. Injections into the forebrain by cells isolated from either forebrain or spinal cord tumors formed secondary tumors with a morphology closely resembling GBM. In contrast, cell injections into the spinal cord by either spinal cord or forebrain tumor cells resulted in tumors that resembled the retrovirus driven spinal tumors. These results suggest that microenvironment is affecting the tumor histology. To explore the effect of the microenvironment on radiation resistance, we examined the difference in response of glioma cells to radiation between groups that contained or did not contain recruited glial progenitors expressing PDGFRalpha and pericytes expressing PDGFRbeta, both of which were known to promote GBM-PDGF-BB stimulation from the retrovirus infected cells. Tumors generated in the forebrain were more sensitive to radiation whereas cells from the spinal cord tumors were more resistant to radiation. These results merit further investigation into NNMT's role in GBM treatment resistance.

CB-57. THE REGULATORY ROLE OF MICRORNA IN MALIGNANT GLIOMAS AND THEIR POTENTIAL ROLE AS NOVEL TUMOR BIOMARKERS
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INTRODUCTION: Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. MicroRNAs (miR) are important regulators of gene expression through posttranscriptional silencing of target mRNA. MiR roles in cell proliferation, invasion, angiogenesis, and glioma stem cell activity are unknown. METHODS: Using RNA extracted from GBM patient tumor specimens, miR 338-3p expression, relative to normal brain and control serum, was examined using RT PCR. Regional miR expression within a single tumor was identified. A DNA sequence containing the hsa-miR-338-3p locus was amplified and cloned into a lentiviral vector, then transduced into GBM established neurospheres, primary patient samples, and human neuronal stem cells. MiR 338-3p and its downstream target expression was assessed by RT PCR and western blot. GBM neurosphere formation and cellular proliferation were examined in vitro and vivo. Using serum obtained from GBM patients, RNA was extracted identifying miR levels relative to normal controls. RESULTS: Overexpression in MiR 338-3p is associated with decreased glioblastoma proliferation, cellular invasion, and neurosphere formation both in vitro and in vivo. MiR 338-3p displays decreased expression levels in the tumors core with increased expression at the tumor’s rim and migratory edge. MiR 338-3p overexpression induces cell apoptosis as determined by cleaved caspase 3 expression levels in GBM. MiR 338-3p overexpression shows no effect on proliferation in human neural stem cells; however, it does result in induced neuronal differentiation as determined by TUJ1 expression levels. MiR 338-3p and its downstream target expression was assessed by RT PCR and western blot. MiR 338-3p is detectable in the serum of glioma patients as well as normal surgical controls. Serum miR 342-5p levels seem to correlate with response to therapy. CONCLUSION: MiR 338-3p inhibits GBM neurosphere growth and invasion both in vitro and in vivo through reducing proliferation and inducing apoptosis possibly via targeting of HDAC4.
CB-59. LYN PROMOTES MALIGNANT GLIOMA CELL SURVIVAL BY PROMOTING AUTOPHagy

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We reported previously that LYN activity and protein are significantly elevated in glioblastoma (GBM) biopsies. Here, we investigated the function of LYN in regulating the survival of GBM cells by expressing a dominant-negative LYN (DN-LYN) or a constitutively active-LYN (CA-LYN) construct, followed by analyses of cell survival and proliferation. Using both monolayer viability assays in serum-free media and soft-agar growth assays, we found that expression of DN-LYN resulted in decreased survival and decreased colony number, whereas expression of CA-LYN resulted in increased survival and increased colony number as compared to control LV cells. To determine whether LYN regulates cell-cycle progression, we labeled cells with BrdU followed by cell-cycle analysis; CA-LYN increased the percentage of cells in G0/G1 and decreased the percentage of cells in S phase relative to the control cells consistent with quiescence. We also investigated apoptosis and found an increase in annexin V labeling with expression of DN-LYN and a decrease in annexin V labeling with expression of CA-LYN. Finally, using western blot analysis, we found LYN promotes autophagy, which was blocked by LC3B protein in lysates and found an increase in the normalized LC3BII band in the CA-LYN lysates and a decrease in the DN-LYN lysates, suggesting LYN promotes survival through autophagy in the GBM cells. Furthermore, when LYN was propagated in the nude mouse brain, a dramatic increase in tumor volume was seen, accompanied by a decrease in tumor cell TUNEL-labeling, and a decrease in tumor volume was seen in the DN-LYN GBM tumors with an increase in TUNEL-labeling. Akt has recently been shown to regulate autophagy; we found an increase in Akt activity in the CA-LYN cells suggesting CA-LYN may promote autophagy and cell survival through an increase in Akt activity. In summary, LYN promotes autophagy of GBM cells thereby enhancing survival both in vitro and in vivo.

CB-60. DEFINING AND TARGETING TUMOR SIGNALING NETWORKS IN NF1 DEFICIENT GBLOASTOMA

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Glioblastoma (GBM) is a genetically heterogeneous group of tumors that remains largely refractory to treatment. The Cancer Genome Atlas (TCGA) has described 4 transcriptional signatures of primary GBM, 3 of which—clonal, proneural, and mesenchymal—are associated with alterations in EGFR, PDGFR, and NF1, respectively. NF1 loss—either by mutation, deletion, and/or underexpression—is found in up to 25% of primary GBM and is associated with sarcomatous histology, suggesting that these tumors represent a distinct subclass of GBM. NF1 deficiency in other cancer types has been associated with diverse pathway dysregulation and, in some cases, sensitivity to MAP kinase pathway inhibition. We sought to investigate this in GBM. Using reverse phase protein array technology (RPPA), we have profiled signaling network proteins in a panel of 80 GBM and lower grade astrocytomas and compared patterns of activation with NF1 status determined by resequencing, array-CGH, mRNA expression and western blot. Consistent with NF1-mediated regulation of the MAPK pathway, we observed upregulation of pErk and pMEK and modulation of other signaling nodes such as STAT3, Src, p38, and mTOR target S6K in NF1-deficient tumors. We defined the phenotype associated with key signaling nodes in NF1-expressing versus deficient tumor sphere lines and assessed the comparative effects of MEK inhibitor PD98059 biochemically and in terms of their growth and viability. These findings will help inform the selection of candidate “synthetic lethal targets” for synergistic inhibition in MEK-resistant NF1-deficient GBM.

CB-61. IDENTIFICATION OF BCL2L13 AS A NOVEL GBM ONCOPROTEIN

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Using in silico analysis of 272 GBM samples in the TCGA data set to identify important cell death mechanisms in gliomapathogenesis, we identified differential expression of the novel BCL2L13 gene. BCL2L13 expression was associated with increased chemotherapeutic and alternative therapy resistance in GBM and is associated with sarcomatous histology, suggesting that these tumors may be more sensitive to MAP kinase pathway inhibition. We sought to investigate this and determined by flow cytometric analysis of cell proliferation and apoptosis and tumor xenograft formation in NSG mice. Using Western Blot, we demonstrated that expression of BCL2L13 significantly increased overall glioma-free survival, which was associated with enhanced intratumoral apoptosis and decreased proliferative indices. To molecularly elucidate the mechanism by which BCL2L13 exerts its anti-apoptotic and anti- proliferative effects, we performed a yeast 2 hybrid screen and identified a select number of tumor suppressors and oncoproteins including the tumor suppressor c-Myc (C-myc, 2 (Cer52), a regulator of MOMP, and O-6-methylguanine-DNA methyltransferase (MGMT), an important prognostic indicator of temozolomide effectiveness, as BCL2L13 interaction partners. Taken together, these results reveal that BCL2L13 represents a novel anti-apoptotic, anti-proliferative oncoprotein that inhibits apoptosis progression and promotes tumor growth by impacting mitochondrial membrane physiology.

CB-62. FREQUENT EPIGENETIC INACTIVATION OF XAF1 AND ITS IMPLICATION FOR TUMOR CELL RESISTANCE TO APOPTOTIC STRESSES IN HUMAN Glioblastoma

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OBJECTIVE: XIAP-associated factor 1 (XAF1) is a tumor suppressor that has been known to exert proapoptotic effects by interfering with the caspase-inhibiting activity of XIAP. In this study, we investigated the XAF1 status and its role in human glioblastoma. METHODS: Expression and promoter methylation status of XAF1 was examined using 16 human glioblastoma tissues and 7 cell lines. The effect of XAF1 on tumor growth was determined by flow cytometric analysis of cell proliferation and apoptosis and xenograft formation assay using T98G cells. Furthermore, the impact of XAF1 on the progression of gliomagenesis in vivo, we orthotopically injected glioma cell lines with enforced expression of BCL2L13 targeting shRNAs into immunocompromised SCID mice. Interestingly, neutralization of BCL2L13 significantly reduced overall glioma-free survival, which was associated with increased intratumoral apoptosis and decreased proliferative indices. To molecularly elucidate the mechanism by which BCL2L13 exerts its anti-apoptotic effects, we performed a yeast 2 hybrid screen and identified a select number of tumor suppressors and oncoproteins including the tumor suppressor c-Myc (Cer52), a regulator of MOMP, and O-6-methylguanine-DNA methyltransferase (MGMT), an important prognostic indicator of temozolomide effectiveness, as BCL2L13 interaction partners. Taken together, these results reveal that BCL2L13 represents a novel anti-apoptotic, anti-proliferative oncoprotein that inhibits apoptosis progression and promotes tumor growth by impacting mitochondrial membrane physiology.

CB-63. CLASSIFICATION OF ADULT MALIGNANT GLIOMA SUBTYPES WITH AN ACTIVATED AND OPERATIONAL HEDGEHOG PATHWAY

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Abstracts

The Hedgehog (Hh) signaling pathway regulates the growth of a subset of adult malignant gliomas. Thus, correlating the operational status of the Hh pathway with the clinical status of malignant glioma subtypes and incidence of the clinical utility of monitoring and targetting this pathway in patients. Our prior studies indicating that Hh pathway activation is confined to WHO grade II and III astrocytomas and oligodendrogliomas are at odds with reports of pathway activation in IV glioblastomas. We further the subtypes of malignant glioma in which the Hh pathway is operational, we conducted a more extensive survey of adult glioma specimens and primary glioma cell cultures and included mutational analysis of the IDH1 and IDH2 genes. Here, we report that the Hh pathway is commonly activated and operational in astrocytomas and oligodendrogliomas and rarely in glioblastoma. Screening for mutations in IDH1 and IDH2 within WHO Grade II and III gliomas does not enhance the predictive impact for identifying Hh-responsive gliomas beyond the use of histological classification. With respect to glioblastoma, however, we found that an operational Hh pathway is confined to clinically defined secondary GBM and primary GBM bearing IDH mutation.

CB-64. MICRORNA-182 ACTS AS A CHEMOSENSITIZER IN GBM BY REPRESSING BCL2L12
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Glioblastoma multiforme (GBM) is the most prevalent and aggressive brain tumor, exhibiting high mortality rates and increased resistance to current forms of treatment. GBM development has been linked to deregulation of several signaling cascades involved in cell proliferation and apoptosis. Bcl2-like 12 (Bcl2L12) is a multifunctional glioma oncoprotein that is overexpressed in the majority of GBM and plays pivotal roles in driving tumor progression and resistance to therapy-induced apoptosis. Mechanistically, we identified Bcl2L12 as a potent inhibitor of postmitochondrial effector caspases and the p53 tumor suppressor. To begin to molecularly dissect cellular mechanisms that regulate Bcl2L12 expression and function, we interrogated whether Bcl2L12 is under the control of microRNAs (miRs). In silico analysis of 272 primary GBM samples of the multidimensional TCGA data set aimed to identify miRs displaying expression levels that anticorrelate with Bcl2L12 mRNA abundance, as such candidates represent potential Bcl2L12-targeting miRs. This oncogenic approach revealed a significant anticorrelation between Bcl2L12 mRNA and miR-182 expression, preferentially in the proneural subtype, a GBM class with established low Bcl2L12 genomic amplification and mRNA expression and a trend toward longer survival compared to patients with mesenchymal, neuronal, or classical tumors. Enforced expression of miR-182 in various glioma cell lines confirmed robust downregulation of Bcl2L12 mRNA and protein levels, identified the miR-182 binding site within the 3’UTR of Bcl2L12, and documented that miR-mediated downregulation of Bcl2L12 is highly specific for miR-182, but not for other related miRs, such as miR-96 and miR-183. Finally, transfection of pre-miR-182 significantly sensitized cells towards various apoptosis-inducing agents enhanced effector caspase activities. Taken together, our studies confirmed a Bcl2L12- miR-182 axis on genetic and biological levels and identified miR-182 as a potential therapeutic agent to halt GBM progression.

CB-65. SIGNIFICANTLY ALTERED PLASMA LEVELS OF SOLUBLE TNFR1 AND TNFR2 IN GЛИОБЛАСТОМА PATIENTS
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Plasma biomarkers are needed to identify patients with recurrent glioblastoma (GBM) and to detect lack of a response to therapy. Soluble TNFR1 and TNFR2 (sTNFR1 and sTNFR2) in blood are thought to bind and inhibit TNF alpha; however, in several other types of cancer plasma/serum levels of TNF alpha do not correlate with sTNFR1/2, suggesting the levels of sTNFR1/2 are not reflective of the levels of TNF alpha. We evaluated the levels of sTNFR1 and sTNFR2 in the plasma of 29 GBM patients as compared to 19 normal controls by specific ELISA analyses. We found a significant increase in the level of sTNFR1 in the GBM patients (mean, 1208 pg/ml ± 823; mean ± SD) compared with normal controls (mean, 878 pg/ml ± 288; Wilcoxon rank-sum test, p < 0.0004). In contrast, we found a significant decrease in the level of sTNFR2 in the GBM patients (mean, 2658 pg/ml ± 1357) compared with normal controls (mean, 3572 pg/ml ± 927; p = 0.0004, Wilcoxon rank-sum test). In several other cancers, sTNFR2 has been reported to be significantly elevated in plasma/serum and to be associated with tumor depth and metastasis. Our finding of reduced sTNFR2 in GBM may in part reflect the absence of metastatic tumor. No correlation of survival with the level of sTNFR1 or of sTNFR2 was found; we are expanding our sample numbers (power) to further examine this. In summary, measurement of plasma/serum levels of sTNFR1 and sTNFR2 may be useful biomarkers to follow GBM patients.

CB-66. BETACATENIN/TCF4 REGULATES BIOLOGICAL BEHAVIOR BY REGULATING AKT1/AKT2 AND MMIR-21 IN HUMAN BRAIN GLIOMA
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It is very clear that the Wnt/beta-catenin pathway takes part in some pathologic process such as tumorigenesis, cell cycle, development, and differentiation. beta-catenin and Tcf-4 are important transcription factors in the Wnt/beta-catenin pathway; they form a beta-catenin/Tcf-4 complex to regulate the expression of a series of proto-oncogenes at the transcriptional level. A number of studies have found that the wnt pathway closely links with many pathways, such as the EGFR-Pi3K-AKT pathway. Preliminary work in our laboratory found that aspirin can inhibit the activity of beta-catenin and Tcf-4 in glioma, but it does not affect expression of the beta-catenin and Tcf-4 protein. After treating with beta-catenin/Tcf-4 inhibitors in glioma cells and detecting the downstream target genes, it was found that AKT1, AKT2, miR-21, EGFR, c-mycare all decreased. TOP/FOP luciferase reporter experiments prove the transcriptional activity of beta-catenin/Tcf-4 complexes after treatment with aspirin or beta-catenin/Tcf-4 inhibitors. Fluorescence levels were found to decrease after treatment. To further determine the transcription start sites of promoter region between beta-catenin/Tcf-4 complex with AKT1, AKT2, and miR-21, we constructed plasmids of the wild type and mutant type, which contained expression luciferase in the promoter region of gene that bind to AKT1, AKT2, and miR-21. To determine the specific binding site that bind to AKT1, AKT2, and miR-21 directly. (Supported by NSFC 30971136, NCET-07-0615, TJSRC. 09JZCJD17600). "Chunsheng Kang, PhD, Laboratory of Neuro-Oncology, Tianjin Neurological Institute, 152, Anshan Road, Heping, Tianjin 300052, PR China. Fax: 022-27813550. Tel:022-60362662. E-mail: kang97061@yahoo.com

CB-67. DIFFERENTIAL EXPRESSION OF AQUAPORIN 1 AND 4 IN HUMAN GLIOMAS
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Cerebral edema is a common feature of primary brain gliomas and contributes to significant morbidity and mortality. Additionally, increased cerebral edema is often associated with more aggressive tumors. The mechanism of cerebral edema may involve several pathways, such as increased vascular permeability, increase in intracellular volume, and increased interstitial volume. The role of aquaporins (AQP) in cerebral edema is not well understood by aquaporins (AQP). AQP channels play a role in water homeostasis. AQP-1 and AQP-4 expression has been demonstrated in glioma astrocytes. Furthermore, increased expression of AQP-1 and AQP-4 has been reported in human glioblastoma. These findings suggest a regulatory role for AQP in the tumor-associated edema. Nevertheless, previous studies have failed to demonstrate a causal relationship between AQP upregulation and glioma histologic grade. We hypothesize that increased expression of AQP-1 and AQP-4 is directly correlated with the degree of tumor-associated edema and the tumor histologic grade. METHODS: Formalin-fixed, paraffin-embedded brain tumor specimen, including low-grade astrocytomas, low-grade oligodendrogliomas, anaplastic oligodendrogliomas, anaplastic astrocytomas, and glioblastomas, were analyzed (n = 20). Immunohistochemistry was used to assess tumor expression of AQP-1 and -4. These specimens were double stained with GFAP, NSE, vWF, and VEGF to colocalize to cells of astrocytic, neuronal, and endothelial origin. Secondary analysis correlated the expression of AQP-1 and AQP-4 directly correlated with the degree of volume of extracellular brain edema based on preoperative MR imaging. RESULTS: Immunohistochemical analysis demonstrated a direct correlation between increased AQP expression and higher tumor histologic grade. Additionally, upregulation of AQP-1 and AQP-4 was highly correlated with the volume of extracellular brain edema based on preoperative MR imaging. CONCLUSION: This project establishes a framework for future research involving cerebral aquaporin channels in human gliomas. We demonstrate an association between AQP-1 and AQP-4 expression and aggressive glioma tumor phenotype and tumor-associated cerebral edema volume. Further undersanding of AQP's role in tumor-associated edema may lead to new therapeutic targets for the reduction of brain edema and its associated comorbidities.
CB-68. NHERF-1 FUNCTIONS AS A MOLECULAR SWITCH BETWEEN CELL MIGRATION AND PROLIFERATION IN Glioblastoma

We previously showed that NHERF-1, a gene was significantly overexpressed in the invading rim of the tumor specimens when compared to matched, more proliferative, core regions. In this study, we demonstrated that NHERF-1 functions as a critical ‘switch’ for GBM cells in the differential adoption of a migratory versus proliferative phenotype, potentially regulating the EGFR signaling pathway. Specifically, depletion of NHERF-1 expression by siRNA oligonucleotides suppresses GBM migration but surprisingly increases cell proliferation. In addition, depletion of NHERF-1 expression increases Grb2 binding to the EGFR receptor and enhances MAPK activation. In contrast, inhibition of Grb2 expression by siRNA oligonucleotides decreases EGFR-stimulated MAPK signaling and cell proliferation but enhances cell migration. Moreover, since NHERF1 has been shown to stabilize EGFR at the cell surface and retard receptor downregulation, our study also suggests that NHERF-1 may facilitate exclusion of Grb2 from EGFR proximity to drive GBM migration. These results suggest that NHERF-1 functions as a novel molecular switch that regulates the dichotomy between the migratory and proliferative phenotypes in GBM. Elucidating the mechanisms by which NHERF-1 controls and enables GBM tumor migration and proliferation is essential for understanding tumor cell progression and raises the possibility of targeting NHERF-1 in tumor cells for the development of novel anti-invasive therapies.

CB-69. THE DIFFERENTIAL EFFECT OF CONDITIONAL DELETION OF BETA-1 INTEGRIN AND FAK ON NEURAL STEM CELL MIGRATION FROM THE SUBVENTRICULAR ZONE THROUGH THE ROSTRAL MIGRATORY STREAM TO THE OLFACTORY BULB

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The objective of this study was to investigate the role of beta-1 integrin-dependent mechanisms and Fak (a known downstream mediator of integrin signaling) dependent mechanisms effecting adult stem cell migration in the subventricular zone (SVZ), the rostral migratory stream (RMS), and the olfactory bulb (OB). In the adult mammalian brain, neurogenesis is restricted to the SGZ and SVZ. Neural stem cells self-renew and produce transient amplifying progenitors that generate neuroblasts. SVZ neuroblasts migrate a considerable distance along the wall of the lateral ventricle and through the RMS to the OB. A critical component of stem cells is the use of integrin receptors to communicate with extracellular matrix molecules, but their precise role in migration is unclear. This study employed a genetic strategy using a tamoxifen-inducible Nestin-CreERT2/Rosa26-YFP reporter mouse line crossed to either a conditional floxed beta-1 integrin or a conditional floxed Fak mouse. In beta-1 integrin deficient cells, fewer YFP-labeled cells from the Neuro-epithelial boundary were observed to migrate through the RMS and to the OB compared to wild type. Some YFP-labeled beta-1 integrin deficient cells were also observed to aberrantly migrate to the adjacent cortex. However, in the Fak deficient cells, a similar number of YFP labeled cells migrated from the SVZ to the OB. Taken together, our data show that Beta-1 integrin is necessary for astro progenitor cell migration from the SVZ to the OB; downstream signaling appears not to involve Fak. Beta-1 integrin restricts neuroblast migration to the confines of the RMS. Understanding molecular mechanisms of progenitor cell migration in the RMS may provide crucial insight into strategies adopted by malignant glioma cells.