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

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January 2011

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Neuro-Oncology 13:iii10–iii25, 2011.
doi:10.1093/neuonc/nor148

NEURO-ONCOLOGY

Abstracts

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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Glioblastoma multiforme (GBM) is the most aggressive of the astrocytic malignancies and the most common intracranial tumor in adults. Although the epidermal growth factor receptor (EGFR) is overexpressed and/or mutated in at least 50% of GBM cases and is required for tumor maintenance in animal models, EGFR inhibitors have thus far failed to deliver significant responses in GBM patients. One inherent resistance mechanism in GBM is the co-activation of multiple receptor tyrosine kinases (RTKs), which generates redundancy in activation of phosphoinositide-3'-kinase (PI3K) signaling. Herein we present a novel mechanism by which fibroblast growth factor receptors (FGFRs) and src family kinases (SFKs) impact PI3K signaling in GBM by phosphorylating the PTEN tumor suppressor at a conserved tyrosine residue, Y240. Phosphorylation of Y240 is associated with shortened overall survival and resistance to EGFR inhibitor therapy in GBM patients and plays an active role in mediating resistance to EGFR inhibition in vitro. Experimentally, mutation of PTEN tyrosine 240 to phenylalanine generates an allele of PTEN that potently sensitizes cells expressing mutant EGFR to erlotinib. In contrast, activation of FGFR signaling promotes resistance to erlotinib in glioblastoma cells expressing wild type PTEN, concordant with phosphorylation of PTEN and activation of downstream signaling. Together, our findings identify a novel signaling connection between FGFRs and PTEN and provide a mechanistic link between PTEN regulation and drug resistance, suggesting that blocking PTEN phosphorylation by suppression of src and/or FGFR activity represents a potential strategy to re-sensitize tumors to EGFR inhibitors.

CB-02. GLIOBLASTOMA TUMOR MICROENVIRONMENT DRIVES SELECTION OF CELL SUBPOPULATIONS WITH DISTINCT RTK AMPLIFICATION GENOTYPES AND DRUG SENSITIVITIES

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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 GBM, its significance in maintaining cell populations is unknown. We investigated whether amplification of two 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, PDGFRA, and MET. Multicolor FISH was performed for EGFR, PDGFRA, and cen7 for 8 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 PDGFRA 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/PDGFRA coamplified tumors maintained distinct RTK-driven subpopulations that were subject to selection under EGF or PDGF ligand stimulation in vitro. Simultaneous inhibition of both EGFR and PDGFR 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 PDGFR.

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. This receptor/channel complex is not constitutively expressed but is upregulated in CNS astrocytes during ischemic or inflammatory stress via transcriptional activation of HIF-1alpha. When activated by natural ATP depletion or pharmacologically with diazoxide, this complex initiates a cascade leading to oncotic cell swelling and oncotic (necrotic) cell death that may be responsible for central necrosis observed in GBM. The naturally hypoxic state of brain tumors is expected to result in constitutive expression of this complex, which may serve as a tumor-specific target for therapy. METHODS: SUR1, TRPM4, and HIF-1alpha protein expression were examined by immunohistochemistry in 21 paraffin specimens of high-grade glioma. Normal brain specimens were used as controls. RESULTS: Immunohistochemistry showed that SUR1 and TRPM4 were expressed in all tumor specimens. Expression occurred broadly in normal support cells, including endothelial cells and reactive astrocytes as well as in tumor cells. *In situ* hybridization showed upregulation of ABCC8, the gene that encodes the SUR1 protein. SUR1 expression colocalized with areas of HIF-1alpha expression. In vitro luciferase assays confirmed that the human ABCC8 promoter is effectively 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 therapeutic 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.

CB-04. MISMATCH REPAIR-INDEPENDENT DNA DAMAGE DRIVES CHK1 ACTIVATION IN TEMOZOLOMIDE-TREATED GBM CELLS

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Temozolomide (TMZ) is a DNA methylating agent used in the treatment of brain cancer. TMZ-induced O6-methylguanine (O6MG) adducts, in the absence of repair by MGMT, mispair and trigger-futile mismatch repair (MMR), delayed DNA double-strand breaks (DSB), Chk1 phosphorylation (ser345)/activation, cell-cycle arrest, and ultimately cell death. Although Chk1 activation is considered to be a downstream biomarker of MMR activation and DNA DSB, we noted elevated levels of pChk1 within 3 hours of TMZ exposure in MGMT-proficient cells (G55 and U87 engineered to overexpress MGMT) as well as in their MGMT-deficient counterparts (U87 and benzylguanine-treated G55 cells). Furthermore, even in the MGMT-deficient cells, pChk1 levels peaked at 24 hours following TMZ exposure, long before the onset (48 hours) of H2AX foci, DNA double-strand breaks, and cell-cycle arrest. TMZ-induced pChk1 activation was also noted in MGMT-depleted, MMR-deficient cells that exhibited neither H2AX foci nor DNA DSB, dissociating pChk1 activation from MMR-driven processes. TMZ-induced pChk1 activation was not a result of O6MG-induced reactive-oxygen species (ROS) as ROS was not noted in MGMT-proficient cells. TMZ-induced pChk1 activation was, however, temporally associated with TMZ-induced alkali-labile DNA damage (ALD) detected by Comet

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 later 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 SENESCENCE 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 1000 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 glioma and glioblastoma cell lines and in interleukin-6 (IL-6)-stressed normal human 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 these induced effects. A strong positive correlation between the astroglial cell markers glial fibrillary acidic protein (GFAP) and vimentin and miRNA-125b abundance was noted in biopsied human glioma and glioblastoma samples. These results suggest that miRNA-125b contributes to the proliferation and premature aging of HAG cells, and that anti-miRNA (AM; antagomir) strategies may be clinically useful in treating astroglial cell proliferative disease. (Support: Translational Research Initiative [LSUHSC-NO] and an Alzheimer Association IIRG Award [WJL])

CB-06. KNOCKDOWN OF CARBONIC ANHYDRASE IX EXPRESSION IN MALIGNANT GLIOMA CELLS AUGMENTS EFFICACY OF RADIATION AND CHEMOTHERAPY

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OBJECTIVE: Concomitant radiochemotherapy is currently the standard postsurgical treatment for glioblastoma. However, despite improvement of radiation techniques and the advent of intensified chemotherapy dosage regimens, virtually all patients display tumor recurrence. The hypoxia-inducible carbonic anhydrase IX (CAIX) is highly expressed in glioblastoma, leading to effective buffering of intracellular pH. Since transient acidification of intracellular pH is required for the induction of apoptotic cell death, we hypothesized that inhibition of CAIX expression may contribute to an enhanced efficacy of radiation or chemotherapy. METHODS: U251 glioblastoma cells were transfected with a CAIX siRNA construct. A nonspecific sequence siRNA served as control. The cells were incubated in a humidified 5% CO_2 modular with either 21% oxygen and 25 mM glucose in the culture medium (ctrl.) or 0% oxygen plus 125 mM glucose (glycolysis). Cells were irradiated with 10 Gy; temozolomide treatment was performed at a concentration of 50 μM. Suppression of CAIX expression by siRNA was investigated by RTPCR and western blot. Cell toxicity was measured by a colorimetric assay (AQ assay, Promega); apoptotic cell death was investigated by annexin V labeling. ATP levels were measured using the Cell Titer Glo kit (Promega). RESULTS: Transfection with sequence-specific siRNA led to a 90% reduction of both CAIX mRNA and protein expression. Knockdown of CAIX expression caused significantly lower ATP levels combined with increased efficacy of radiation and chemotherapy, particularly under glycolytic conditions. Annexin V labeling demonstrated a significantly higher rate of apoptotic cell death upon CAIX knockdown in both treatment arms compared to the nonspecific control. CONCLUSION: Our data demonstrate that CAIX overexpression, which has been found in more than 90% of all glioblastomas, reduces efficacy of adjuvant treatment such as chemotherapy and radiation treatment. Blocking CAIX activity may therefore be an additional target in glioblastoma treatment.

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

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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. MIB-1 (proliferation index) and microvascular density (MVD) were also analyzed on these samples. Copy number analysis (CNA) using TaqMan copy number assay for the 5 molecules was performed. Results of the IHC analysis showed protein expression (2+ to 4+) for VEGF, HIF-1, and CA9 correlating with higher grades of tumor in greater than 80% of pediatric and adult ependymomas. GLUT-1 and HIF-2 were expressed more strongly in pediatric ependymomas. Normal brain tissues showed no protein expression for any of the molecules. The grade II and III pediatric ependymoma samples showed higher MIB-1 and MVD indices than did their adult counterparts. There was no statistically significant difference in the MIB-1 values of recurrent and nonrecurrent pediatric ependymomas. CNA showed deletion to be the major aberration in the pediatric samples for all the target molecules except for HIF-1 in which the role of amplification seemed to predominate. The preliminary results of this study show for the first time the likelihood of hypoxia-driven pathways inducing tumorigenesis in ependymomas. Gene deletion of key molecules like VEGF and CA9 seems to be important in the etiopathogenesis of this tumor formation. Further evaluation of the hypoxia pathway and its signature molecules as potential therapeutic targets is warranted in larger prospective studies.

CB-08. EXTRACELLULAR HSP90 IS A CENTRAL MEDIATOR OF ONCOGENIC SIGNALING NETWORKS IN GLIOBLASTOMA

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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 terms of key signaling mediators, AKT is frequently activated in human GBM, either due to loss of PTEN or to RTK activation. We recently demonstrated that extracellular Hsp90 (eHsp90), in tandem with its receptor LRP1, comprises a signaling axis that is crucial for the stimulation and maintenance of AKT activation, which in turn contributes to the promotility and aggressive behavior of GBM. Given that the discovery of eHsp90 in GBM is completely new, knowledge is lacking concerning its broader signaling effects and downstream targets. Therefore, in order to investigate the potential role of eHsp90 in mediating signaling pathways in GBM, we have undertaken a comprehensive analysis of the effects of eHsp90 inhibition upon nearly all known cellular tyrosine kinases. Strikingly, we found that blockade of eHsp90 signaling potently suppressed basal AKT activity and overrode the ability of multiple growth factors to stimulate this kinase. Furthermore, eHsp90 targeting also diminished the activation status of several RTKs upon exposure to their respective ligands. Taken together, our results support the notion that eHsp90 regulates an extensive RTK signaling network that converges upon AKT function. Our results implicate eHsp90 targeting as a potentially novel therapeutic approach that may overcome some of the present challenges associated with RTK switching upon the molecular targeting of specific signaling receptors.

CB-09. CORTICOSTEROIDS IMPAIR GLIAL PROGENITOR CELL FUNCTION 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 subcortical white mater tracts of mice systemically exposed to methylprednisolone. Corticosteroid-associated impairment of progenitor cell proliferation was in part reversed by the powerful antioxidant N-acetylcysteine, suggesting a redox-dependent steroid effect on neural progenitor cells. Given the critical importance of 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 pHSP27 upregulation and promotes survival via AKT activation, suggesting it is a therapeutic target. Because pHSP27 also upregulates pAKT, we proposed that suppressing HSP27 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 [$n \geq 3$]). Two-fold changes were considered significant. 2) The cells were also subjected to clonogenic assays to assess survival ($n \geq 2$). Results indicate: 1) SPARC promotes prosurvival (HSP27, AKT) and prodeath (caspase 8, cleaved caspase 3) signaling, and SPARC and control cells survive similarly. 2) TMZ activates autophagy in both control and SPARC-expressing cells, but SPARC sensitizes cells to apoptotic signaling (cleaved caspase 7 and PARP). Despite increased apoptotic signaling, SPARC-expressing cells survive better in TMZ ($p = 0.0001$). 3) SPARC siRNA suppresses TMZ-induced caspase 7 and PARP cleavage and LC3-II, does not affect survival in TMZ, and paradoxically enhances survival in the absence of TMZ ($p = 0.018$). 4) AKT1/2/3 siRNA treatments mimic SPARC siRNA effects ($p = 0.003$), except AKT3 siRNA does not affect survival in the absence of TMZ. 5) HSP27 siRNA suppresses SPARC and AKT and suppresses survival ($p = 0.0001$) but does not further sensitize cells to TMZ. In conclusion, SPARC or AKT inhibition is not effective therapy as inhibition promotes survival, and inhibition in TMZ has a minor effect on survival. Although HSP27 inhibition does not sensitize cells to TMZ, suppression effectively decreases survival. Therefore, suppressing HSP27 is therapeutically 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 overexpression increases sensitivity to cell death and reduces cell invasion capacity. 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 assay. Mice injected with control (U87-NS) or miR-218 overexpressing 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 combinatorial use of miR-218 and TMZ significantly increased survival, and 50% of animals failed to develop tumors even after 288 days of treatment. ECOP, PLCγ1, and NAC-1 were identified as direct miR-218 targets and experimentally validated. Repression of ECOP and PLCγ1 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 PLCγ1 over-expression alone; however, reduced cell invasion was completely rescued with PLCγ1. Since PLCγ1 modulates PKC and NF-kappaB pathways, we analyzed and identified that miR-218 overexpression inhibits these cell survival pathways, indicating the role of PLCγ1. Our analysis of human GBM patient samples identified a reciprocal correlation between the expression of miR-218 and target genes. Finally, bioinformatic analysis revealed that low miR-218 expression correlates with poor patient survival with a mesenchymal gene signature. Altogether, these studies clearly reveal a critical role for miR-218 in GBM tumorigenesis.

CB-12. A SURVEY OF HUMAN CYTOMEGALOVIRUS GENOMIC LOCI 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, mRNAs, 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 chemotherapy. However, it is not known if these specific HCMV genes 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 nontumor 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 for HCMV DNA. We report that altogether the incidence of HCMV DNA in GBM is higher compared to that in the controls. This analysis provides the first evidence of the extent to which the HCMV genome is found in GBM. Future work would characterize the expression pattern of HCMV genes in GBM.

CB-13. GENOMIC ANALYSIS OF CYTOMEGALOVIRUS IN MALIGNANT GLIOMAS

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Studies show that most malignant gliomas are infected with cytomegalovirus (CMV). However, the genetic status, including DNA loads and genetic and genomic sequences of CMV in tumors has not been examined. CMV DNA was found in 16/17 (94%) tumor specimens. Viral DNA loads ranged from >10e2-10e6 copies/500ng of total DNA with a median log 10 viral load of 4.0 (±1.6). DNA loads did not correlate with tumor

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 tumor samples, but expression levels were not linked to viral DNA loads. These observations suggest that atypical infections occur in tumors. The viral UL83 gene, encoding pp65, was sequenced to determine whether specific sequences associate with tumor grade and because most anti-CMV T cell responses are directed against the protein. UL83 sequence networks suggest that GBMs and lower grade gliomas segregated away from each other, with amino acid changes observed in each GBM. To better understand the genomic nature of CMV in GBM, >80 kbp of the CMV genome was sequenced and analyzed. Sequence analysis revealed that a unique CMV strain exists in this tumor. Deep sequencing uncovered a viral population that was less variable ($\pi = 0.1\%$ and $\pi AA = 0.08\%$) than that observed in productive infections. In addition, 56/60 viral genes analyzed had dN/dS scores of <1, indicative of high sequence conservation. In conclusion, CMV DNA levels, while variable, result in readily detectable gene expression, but the virus does not appear to replicate in tumors. Gene level analyses suggest sequence biases separate low- and high-grade gliomas. Finally, near genome-scale sequencing of CMV indicates that a unique viral sequence is present in a GBM and pressures exist to retain CMV sequence information.

CB-14. THERAPEUTIC POTENTIAL OF AZD1480 FOR THE TREATMENT OF HUMAN GLIOBLASTOMA

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Aberrant activation of the janus kinase (JAK)/signal activator of transcription (STAT) pathway has been implicated in glioblastoma (GBM) progression. STAT-3 is persistently activated in human GBM samples, which correlates with elevated levels of many STAT-3 target genes that promote tumor progression. To develop a therapeutic strategy to inhibit STAT-3 signaling, we have evaluated the effects of AZD1480, a pharmacological inhibitor of JAK1 and JAK2. AZD1480 treatment inhibits the growth of multiple tumor types, but the effect on GBM tumors has not been reported. In this study, the in vitro efficacy of AZD1480 was tested in human and murine glioma cell lines. AZD1480 treatment effectively blocks constitutive and stimulus-induced JAK1, JAK2, and STAT-3 phosphorylation in both human and murine glioma cells and leads to a decrease in cell proliferation and induction of apoptosis. Furthermore, we utilized human xenograft GBM samples as models for the study of JAK/STAT-3 signaling in vivo. In these xenograft tumors, JAK2 and STAT-3 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 STAT-3 in these GBM xenograft tumors in vitro downstream gene expression and inhibits cell proliferation. Furthermore, AZD1480 suppresses STAT-3 activation in the stem cell population in GBM tumors. In vivo, AZD1480 inhibits the growth of subcutaneous tumors and increases survival of mice bearing intracranial GBM tumors by inhibiting STAT-3 activity. However, we found that xenograft tumors that display EGFR amplification are less sensitive to AZD1480 therapy, indicating EGFR status may be an important determinant of responsiveness to AZD1480 and will need to be considered in future studies. Overall, our data indicate that pharmacological inhibition of the JAK/STAT-3 pathway by AZD1480 should be considered for study in the treatment of patients with GBM tumors.

CB-15. beta$_3$-ADRENERGIC AGONISTS MIMIC EUSTRESS RESPONSE AND REDUCE LEPTIN-MEDIATED PROLIFERATION IN A GBM CELL LINE

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A great deal of mental stress, depression, and anxiety often overwhelm cancer patients; those diagnosed with glioblastoma multiforme (GBM) are no exception. Different types of stress invariably impact what has been termed "the brain-adipocyte BDNF/leptin axis" (Dr. Cao and colleagues of the Comprehensive Cancer Center at The Ohio State University). For example, eustress (good stress) and distress (bad stress) both lead to increased sympathetic activity and adrenal gland stimulation, yet eustress reduces leptin levels and attenuates tumor growth while distress increases leptin levels and augments tumor growth. Complicating matters in GBM is that 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 cell proliferation of the established GBM cell line T98G. We hypothesized that elevated sympathoadrenal activity would increase cell proliferation and be additive to leptin's effects. To the contrary, adding 300 pg/mL of epinephrine to leptin-conditioned media blocked leptin-mediated proliferation. Because beta$_3$-adrenergic receptor stimulation suppresses leptin gene expression and release in adipocytes, we hypothesized that a beta$_3$-adrenergic agonist would counteract leptin's effects on T98G cell proliferation. Use of the beta$_3$-adrenergic agonist BRL 37344 did not only counteract leptin's effects but also significantly reduced T98G cell proliferation in unconditioned media. This has immediate translational value in that treating GBM with a beta$_3$-adrenergic agonist may reduce tumor proliferation through receptor activation and by blocking the leptin-leptin receptor autocrine loop. Moreover, recent reports indicate that beta$_3$-adrenergic agonists capable of crossing the blood-brain barrier (like SR 58611A) 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

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GBM is characterized by rapid proliferation, angiogenesis, hypoxia, and necrosis at the primary site, yet a small portion of the tumor invades and infiltrates into normal brain parenchyma. Tumor cells in response to this challenging and dynamic microenvironment face hypoxia, acidity, and limited nutrient availability as the tumor grows. Glioma cells often utilize glycolysis, 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 periods of metabolic stress. We previously identified a single microRNA (miR-451) that controls glioma cell proliferation, migration, 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 the expression of miR-451 is regulated by stress, as we were able to demonstrate profound effect of glucose withdrawal, radiotherapy, 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 modulates glioma cell invasiveness *in vivo*, we performed series of experiments co-injecting invasive (+/− miR-451) and non-invasive cells into nude mice intracranially. We demonstrate that miR-451 expressing cells have severely limited invasive phenotype, which is consistent with *in vitro* findings. We believe that our in vivo model is highly relevant as it recapitulates clinical characteristics of glioblastoma: highly proliferative tumor core co-existing with highly invasive sub-population of cells; and as such is valuable tool to explore glioma biology. The dynamic regulation of microRNAs in response to fluctuating glucose levels in the glioblastoma microenvironment is essential for rapid tumor growth and dissemination and is revealing novel targets for therapeutic intervention.

CB-17. MITOSIS INTERFERENCE OF CANCER CELLS DURING ANAPHASE BY ELECTRIC FIELD FROM NOVOTTF-100A

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Chemotherapy and radiation initiate cancer cell death by triggering defined cell cycle checkpoints. Unlike these treatments, the NovoTTF-100A device, which is approved for the treatment of recurrent glioblastoma, kills tumor cells by a novel mitosis interference mechanism. Our research has revealed that the electric field (TTField) affects processes during mitosis proximal to the point of the metaphase to anaphase transition, leading to cell cycle arrest and ultimately cell death. Time-lapse movies of cells exposed to the TTField revealed membrane blebbing and oscillation that was initiated near the time of metaphase to anaphase transition. In synchronous cell cultures exposed to the TTField, phospho-histone H3 was elevated while securin levels were decreased, suggesting that cells exposed to the TTField pass through the metaphase to anaphase transition but may be delayed in mitotic exit. Immunofluorescence of cells treated with TTField

for increasing 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 absence of cytokinesis, and daughter cells with asymmetric chromosomal segregation, with derangement of cells increasing with the time of exposure. These data suggest that TTField exposure resulted in perturbation of processes following entry into anaphase and interfered with processes necessary for orderly exit from mitosis. Furthermore, cells exposed to TTField showed no detectable p53 induction, indicating that cell death is achieved by a p53-independent mechanism. NovoTTF-100A appears to effect 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 GLIOMAGENESIS DRIVEN BY PDGFRA 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. PDGFRA 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 PDGFRalpha confers tumorigenicity to Ink4a/Arf-deficient mouse astrocytes and human glioma cells in the brain. Restoration of p16INK4a but not p19ARF suppresses PDGFRalpha-promoted glioma formation. Mechanistically, abrogation of signaling modules in PDGFRalpha that lost capacity to bind to SHP-2 or PI3K significantly diminished PDGFRalpha-promoted tumorigenesis. Furthermore, inhibition of SHP-2 by shRNAs or pharmacological inhibitors disrupted the interaction of PI3K with PDGFRalpha, 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, PDGFRalpha 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, overexpressed PDGFRalpha 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 PDGFRA overexpression.

CB-19. FUNCTIONAL CHARACTERIZATION OF MICRORNAS IN PDGF-DRIVEN GLIOMAS

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MicroRNAs (miRNAs) are a class of small, noncoding RNAs that regulate gene expression on a pretranslational 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 appears to play a crucial role in the initiation and maintenance of both low- and high-grade diffuse gliomas. An improved understanding of how PDGF signaling mediates its oncogenic effects and the mechanisms for its regulation would be of obvious benefit to the development of effective targeted therapies. Based on an analysis of miRNA involvement in the phenotypic expression and regulation of oncogenic PDGF signaling, we have identified a group of miRNAs whose expression levels are responsive to PDGF pathway activation in vitro and have recapitulated these findings in human glioblastomas, particularly those driven by aberrant PDGF signaling. Similarly, using data from The Cancer Genome Atlas (TCGA), we found that these microRNAs are differentially expressed in gliomas with amplification of PDGFRA and/or in tumors of the proneural subtype compared to other tumors in TCGA. By transiently and constitutively changing miRNAs levels in gliomasphere cells, we have demonstrated that miRNAs from this group specifically affect growth of PDGF driven gliomas, whereas the same miRNAs have no effect on non-PDGF driven gliomas. We are now evaluating the functional properties of these miRNAs in a variety of in vivo systems and are investigating the mRNA targeting profiles of each using a combination of differential protein expression, mRNA expression arrays, and bioinformatics.

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

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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 endocrine side effects. The development of new and 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 dysregulation in either WNT, sonic hedgehog (SHH), Myc, 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. Using in vitro as well as 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. Finally, 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.

CB-21. TARGETED DISRUPTION OF STAT3 SIGNALING INHIBITS TUMOR GROWTH AND BRAIN METASTASES BY CAVEOLIN-1 UPREGULATION

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Studies have demonstrated that Stat3 activation has a critical role in tumorigenesis. However, the role and mechanisms of Stat3 activation in brain metastases of breast cancer are unknown. In this study, we found that activation of Stat3 and downregulation of caveolin-1, a novel downstream target of Stat3, are important for brain metastases. In human tissues, there was increased expression of activated Stat3 but decreased expression of caveolin-1 in brain metastases compared to that in primary breast cancer, while increased expression of activated Stat3 correlated with decreased caveolin-1 expression in brain metastases. Genetically engineered overexpression of activated Stat3 suppressed caveolin-1 promoter activity and gene expression, whereas both knockdown of Stat3 expression and inhibition of Stat3 activation increased caveolin-1 promoter activity and gene expression. Moreover, we have identified putative Stat3-binding elements on caveolin-1 promoter and functionally verified a high affinity

Stat3-binding site by EMSA, ChIP analyses, and promoter mutagenesis assays. Furthermore, increased caveolin-1 expression by either caveolin-1 gene transfection or Stat3 knockdown attenuated breast cancer invasive ability, while decreased caveolin-1 expression by either caveolin-1 knockdown or Stat3 activation promoted breast cancer invasive ability. Finally, by using stable SOCS-1-transfected 231-BR cells, we demonstrated that suppression of Stat3 activation but upregulation of caveolin-1 inhibited tumor growth and total abrogation of brain metastases of breast cancer in animal models. Collectively, our findings provide both clinical and mechanistic evidence that activated Stat3 directly binds to caveolin-1 promoter and inhibited its transcription, hence contributing to breast cancer invasion and brain metastases.

CB-22. BREVICAN, A PRO-INVASIVE MATRIX PROTEIN, ACTIVATES PDGFR SIGNALING AND STIMULATES OLIGODENDROCYTE PRECURSOR RECRUITMENT IN THE GLIOMA MICROENVIRONMENT

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Malignant gliomas are virtually impossible to treat with conventional therapies due to their chemoresistance and invasive ability. Glioma invasion may be regulated in part by interactions with normal neural cells that would facilitate tumor dispersion. Brevican is a brain-specific matrix protein secreted by glioma cells and cleaved by ADAMTS metalloproteases in the tumor matrix. This cleavage releases a 50-kDa fragment ("B50") that promotes glioma invasion through autocrine/paracrine EGFR activation. To better understand the pro-invasive molecular mechanisms of B50, we generated U87MG glioma cells stably transfected with this bioactive fragment and implanted them intracranially. B50-overexpressing tumors were larger than controls and had invasive borders, as expected from the pro-invasive role of B50. Interestingly, these tumors were surrounded by a large number of NG2-positive and PDGFR-alpha-positive cells resembling oligodendrocyte precursors (OPCs). Analysis of brevican and PGFDR expression in culture showed that brevican mRNA matched the expression of PDGFR-alpha, but not PDGFR-beta, in glioma cell lines and glioma stem cells. In addition, U87MG and U251MG cells transfected with brevican or B50 showed elevated levels of total and phosphorylated PDGFR-alpha. To investigate how brevican could activate PDGFR, we tested its binding to PDGF. We observed that B50 could bind to PDGFaa and protect the dimer from enzymatic degradation. Finally, we analyzed the effect of purified brevican on Oli-Neu cells, an Erb2-immortalized OPC line. We confirmed that brevican increased PDGFR signaling in these cells as well as expression of the proliferative gene Olig2. Together, these results suggest that brevican secreted by glioma cells may activate PDGFR signaling both in the tumor and its microenvironment, partly through PDGF stabilization. This signaling could recruit nontumor cells, producing a favorable environment for glioma cell invasion into the surrounding brain tissue. This finding highlights the importance of interactions between glioma and tumor microenvironment for tumor progression.

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 Nuovo, 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 blockade of Notch signaling using pharmacological inhibitors 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-24. POLYVALENT GOLD NANOPARTICLES FUNCTIONALIZED WITH RNAI AS ANTIGLIOMA THERAPEUTICS

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Glioblastoma (GBM) is a lethal brain tumor characterized by intense resistance to extant chemotherapy, radiation, and targeted therapies. Bcl2L12 (for Bcl2-Like 12) is a cytoplasmic and nuclear glioma oncoprotein that is overexpressed in greater than 90% of primary GBM and confers resistance toward therapy-induced apoptosis. On the molecular level, Bcl2L12 binds and inhibits caspase-7 and blocks caspase-3 maturation 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 gliomagenic activities, we employed a gene silencing approach using small interfering RNA (siRNA)-conjugated polyvalent gold nanoparticles (RNA-Au-NPs) to knockdown and inactivate Bcl2L12 signaling in glial cells. Our studies document that RNA-Au-NPs exhibit superior biological stability, highly significant knockdown efficacies, 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 could show that RNA-Au-NPs are single entity agents, which are 500 times more effective in knocking down Bcl2L12 than conventional RNAi-based methods and do not require auxiliary transfection agents. MRI studies using Gd(III)-conjugated RNA-Au-NPs confirmed that these nanomaterials penetrate intracranial 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-26. S100A8/A9 PLAY AN IMPORTANT ROLE IN ENDOGENOUS ONCOGENIC KRAS-MEDIATED ASTROGLIOSIS

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Previous studies suggest that upregulation of Ras signaling in neurons promotes astrocytoma formation in a cell nonautonomous manner. However, the underlying mechanisms remain unknown. We hypothesized that these genetically altered neurons secret growth factors that promote astrocyte proliferation and/or survival. To test our hypothesis, we generated compound mice (LSL Kras G12D/+; CamKII-Cre) that express endogenous oncogenic Kras in postmitotic neurons since P1. These mice developed progressive astrogliosis, which is associated with hyperactivation of Ras signaling pathways. Microarray analysis using control and mutant cortexes identified that both S100A8 and S100A9 are significantly overexpressed in the mutant cortex. We further validated this microarray result using semiquantitative RT-PCR and western blot analysis. S100A8 and A9 are chemokines predominantly expressed in myeloid cells (e.g., neutrophils and macrophages) and have been implicated in promoting tumor cell growth and metastasis through their expression in the stromal myeloid cells. However, our preliminary results indicate that overexpression of S100A8 and A9 in the mutant cortex is not due to increased microglias or contaminated circulating myeloid cells in our cortex protein extractions. Rather, the mutant neurons and their surrounded astrocytes overexpress these proteins. Consistent with this observation, we found that astrocytes express both TLR4 and RAGE, receptors for S100A8/A9. Moreover, purified S100A8/A9 heterodimer but not S100A8 or S100A9 alone promotes growth of primary astrocytes in vitro. In summary, our results reveal a novel role of S100A8/A9 in endogenous oncogenic Kras-associated astrogliosis.

CB-27. MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF MIR-137 IN OLIGODENDROGLIAL 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 astrocytomas and glioblastomas. The aims of this study were to investigate whether miR-137 was involved in oligodendroglial 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%) oligodendrogliomas 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 a mutated miR-137 binding site. These results strongly suggest that CSE1L is a target of miR-137. Immunohistochemical analysis further demonstrated that CSE1L was overexpressed in oligodendroglial tumors. 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 oligodendroglial tumors and suggest that the miR-137/CSE1L axis may serve as a potential therapeutic target for treatment of oligodendroglial tumors.

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 RhoG 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 RhoG 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, in contrast to Rac1, the functions of RhoG 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 initially treated by surgical resection. However, atypical and anaplastic meningiomas have a higher risk 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 biomarkers. 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), *EGFRvIII* 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 biomarker 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 was methylated in one of them in 2 resections without clinical evidence of response to temozolomide). CONCLUSIONS: These results suggest that *EGFR* tyrosine kinase mutations and the *EGFRvIII* deletion mutant 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 suggest 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 ink4a/arf $-/-$ astrocytes overexpressing delta-EGFR and having wild-type or deleted PTEN. To model TKI-resistance in vitro, cells were seeded in soft agar with 1 μM gefitinib or erlotinib for 2 weeks; during this time the growth of colonies was inhibited. The dose was escalated to 2 μM, and after 3 weeks TKI-resistant colonies began to form and were isolated to form clonal cell lines. In 3 PTEN wild-type TKI-resistant lines, delta-EGFR expression was not decreased, and gefitinib inhibited receptor phosphorylation in a manner identical to that observed in parental cells. Interestingly, 2 of these 3 cell lines demonstrated decreased PTEN expression and increased AKT phosphorylation. Removal from the maintenance dose of TKI resulted in restoration of delta-EGFR and ERK phosphorylation to a level several-fold greater than that in parental cells. Notably, the TKI-resistant phenotype was reversible, and these cells became more sensitive to gefitinib and erlotinib than pretreatment parental cells. Overall, we have generated and characterized a model system for the identification of mechanisms of resistance to EGFR TKIs in GBM. We have identified at least 2 different resistance scenarios in cells with wild-type PTEN: decrease in PTEN expression, and another as-of-yet unknown mechanism that does not appear to involve PI3K. The reversibility of resistance and subsequent increased sensitivity to TKIs have potential clinical implications. This work forms the basis for our effort to uncover novel targets or approaches to therapy that may increase the utility of these drugs for treatment of 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 O^6 methylguanine methyltransferase (MGMT) and mismatch repair pathway

(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* methylation. While *MGMT* methylation did not associate with an overall survival advantage 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 on average. In our preliminary in vitro studies, using a panel of 8 commercially available GBM cell lines, reduced protein levels of MSH6 and MLH1 specific to the recurrent GBM cell line DBTRG-05MG were observed, but not in any of the primary cell lines. We sequenced 5 regions of *MSH6* genome, covering 6 mutation hot spots, as previously reported [1], in all 8 cell lines and DNA extracted from 15 recurrent GBM clinical specimens. All 5 regions appeared very well conserved, and no mutations were identified. To determine relative sensitivity to treatment, we treated the cell lines with TMZ and radiation therapy (6 Gy). DBTRG-05MG showed a slightly increased resistance to TMZ but not to combined TMZ/radiation or radiation alone. These results indicate that other mechanisms are involved in mediating resistance to treatment. Analyses are underway to decipher the protein expression levels of MMR in relapsed clinical specimens and to look for novel mechanisms associated with this acquired resistance. [1]Yip S *et al.*, Clin Cancer Res 15(14), 4622-4629, 2009

CB-32. NEUROTROPHIN RECEPTOR SIGNALING IN GLIOMA

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Glioblastoma multiforme (GBM) is a highly invasive disease that is refractory to current treatments. Previous work by this lab has identified and characterized the role of the neurotrophin receptor p75NTR in promoting neurotrophin-dependent glioma cell invasion. Neurotrophins are a group of growth factors that signal through the p75NTR receptor (TNFR family) and Trk receptors (receptor tyrosine kinases) to regulate diverse functions such as neuronal survival and cell motility. Here, we have further investigated the role of p75NTR and Trk receptors in mediating the response of patient-derived gliomasphere cultures (commonly referred to as brain tumor initiating cells [BTICs]) to neurotrophins. BTIC cultures express a diverse repertoire of neurotrophin receptors, and the expression of specific Trk receptors (TrkA, TrkB, and TrkC) correlates with the response of each cell line to specific neurotrophin ligands (NGF, BDNF, and NT3). In particular, BDNF and NT3 promote the growth of BTICs and stimulate MAPK pathway activity. Perturbation of Trk receptor function by pharmacological inhibition or by lentiviral-mediated shRNA knockdown renders BTICs unresponsive to neurotrophins in terms of growth promotion and MAPK stimulation. The contribution of p75NTR to these phenotypes is being investigated by utilizing shRNA and by FACs fractioning of BTIC cultures into populations positive and negative for p75NTR expression. The in vivo relevance of these observations is being examined through orthotopic injection of neurotrophin receptor knockdown BTICs and by treatment of gliomas with Trk receptor inhibitors, which are currently in clinical trials for a variety of hematological and solid tumors.

CB-33. DECRYPTING CHROMOSOME 10Q DELETION IN GLIOBLASTOMA: AN INTERSPECIES COMPARISON OF GENE COPY NUMBER ALTERATIONS

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Human glioblastomas (GBM) acquire genetic deletions that are considered critical for tumor development, presumably because these deletions result in the loss of critical tumor suppressor genes. The distinction between these functionally relevant genes and bystanders within the same loci is difficult since oftentimes a single deletion includes many genes. Chromosome 10q deletion, the most common found in GBM, includes the tumor-suppressor gene PTEN, which is well known to play an important role in development of GBM. However, several groups have proposed that the loss of other tumor-suppressor genes, in addition to PTEN, may also contribute to the functional significance of chromosome 10q deletion in GBM. To explore this possibility, we performed comparative genomic hybridization of a retrovirus-induced mouse model of GBM in which PTEN was experimentally deleted by Cre-recombinase, in combination with PDGF overexpression, as tumor initiating alterations. The resulting mouse tumors consistently developed a series of deletions that included several genes that map to human chromosome 10q and are found among the most common genetic deletions seen in human GBM. Interestingly, these genes are found in multiple independent deletions localized in 3 different mouse chromosomes, suggesting that there is independent selective pressure for deletion of these different genetic loci. Furthermore, the mouse tumors did not suffer frequent copy number alterations within the chromosomal region that contains PTEN, consistent with the fact that this gene was experimentally inactivated by Cre-mediated deletion during tumor induction. These results identify multiple distinct genetic deletions, in addition to PTEN, that provide independent selective advantage during the development of GBM. Cross-species comparisons between human GBM and murine glioma models constitute a valuable approach for refining lists of candidate genes that play a role in the evolution of GBM.

CB-34. ISOGENIC GLIOBLASTOMA 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 limitations 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 RT-PCR, 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 levels, expression of EGFRvIII, and levels of 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 its isogenic sister-line lacked EGFR amplification. The second pair comprised one line showing EGFRvIII expression, EGFR amplification and high EGFRvIII/EGFR protein levels, whereas these alterations were absent in the sister-line. Nude mice injected with EGFR-amplified cell lines died significantly earlier than those xenografted with nonamplified lines, and the EGFR/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. KRAS 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, KRAS 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 KRAS 3-UTR reporter plasmid with each of the individual pre-miRNAs 323-3p, 369-3p, 433, and 90% for all of them together. Minor overexpression (>10 fold) of miRNAs 323-3p and 369-3p significantly reduced the 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 this 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 one 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 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 84 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, p53 protein 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 MICRORNA

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INTRODUCTION: The drug resistance gene O^6-methylguanine-DNA methyltransferase (MGMT) is thought to represent a mechanism of glioma resistance, counteracting 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 to TMZ can be achieved with microRNAs.

CB-38. IDENTIFICATION OF EGFRVIII-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 tumorigenicity in a variety of tumors including GBMs. However, transcriptional mediators of EGFRvIII have not been fully elucidated. In the present study, we analyzed 52 primary human GBMs and a 9L.EGFRvIII rat brain tumor model to identify EGFRvIII-specific gene signatures. EGFRvIII-expressing 9L gliosarcoma cells exhibited 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 EGFRvIII tumors. Immunohistochemical analyses showed increased phosphorylation of Akt, Erk1/2, PLC-γ, Gab1, and SHP-2 in EGFRvIII-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, lrp5, fat3, slc7a1, cdk6, socs2, aqp1, spry2, and aebp1) that were significantly upregulated in EGFRvIII-expressing 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 EGFRvIII-specific gene signature profile using a rat glioma model and human GBMs and characterizes potential targets to inhibit EGFRvIII-mediated transforming phenotypes in malignant gliomas.

CB-39. NUCLEAR FIP200 AND RB EXPRESSION IN BRAIN METASTASIS FROM BREAST CANCER: POTENTIAL PREDICTORS OF SURVIVAL

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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 Rb1 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 Rb1, and no deletion in FIP200. The pattern of nuclear expression of Rb1 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 Rb1 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 of a response signature by microarray. In parallel signaling pathways 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 signaling molecules downstream of these RTKs was assessed by western blots. 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 GLIOBLASTOMA 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, PDFGR, and VEGF-R, 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 GRK5 downregulation, compared to its upregulation in GBM, correlated with significantly reduced overall survival ($p < 0.0001$). For the remaining GRKs (GRK2, GRK3 and GRK6), there were no data available on TCGA to assess their significance in GBM. These findings suggest that altered GRK protein expression in GBM could be involved in the pathogenesis and proliferation of this malignant tumor.

CB-42. TUMOR-ASSOCIATED MESENCHYMAL STROMAL CELLS INCREASE PROLIFERATION AND MAINTAIN STEMNESS OF GLIOMA STEM CELLS THROUGH THE IL6/STAT3 PATHWAY

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Though there has been considerable progress in the understanding of the genetics and biology of glioblastoma multiforme (GBM), little is known about the tumor microenvironment. Mesenchymal stromal cells (MSC) are components of the tumor microenvironment in many cancers, including GBM. To explore the role of MSCs, we isolated tumor-associated mesenchymal stromal cells (TA-MSC) from GBM specimens using established markers (CD73 + /90 + /105+). We also isolated glioma stem cells (GSC) based on marker expression and sphere formation. To identify the effect of TA-MSCs on GSCs, we cocultured them in transwell plates. GSC coculture with bone marrow-derived MSCs served as a positive control and coculture with human brain-derived microvascular endothelial cells (HBMEC) served as a negative control. GSCs cocultured with TA-MSCs had increased proliferation and self-renewal compared with GSCs cocultured with HBMECs or in NSC media alone. GSCs that had been cocultured with TA-MSCs were implanted into mouse brains (104 and 105 cells/mouse). Mice implanted with TA-MSC cocultured GSCs died earlier than control. A significantly higher percentage of mice (80%) implanted with low numbers of TA-MSC cocultured GSCs (102 and 103 cells/mouse) developed tumors compared with controls (20-40%) indicating that TA-MSCs increased the tumorigenicity, or stemness, of GSCs. GSCs mixed with TA-MSCs formed larger tumors than GSCs mixed with HBMECs or GSCs alone in an orthotropic xenograft model. Phosphorylation of STAT3 was increased in GSCs cocultured with TA-MSCs than control and pharmacological inhibition of STAT3 blocked the TA-MSCs action on GSCs. TA-MSCs secrete high levels of IL6 and neutralizing antibody against IL6 or knockdown of the IL6 receptor (IL6R) or its coreceptor GP130 in GSCs abolished the ability of TA-MSCs to increase GSC proliferation and stemness 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-43. AN ESSENTIAL ROLE FOR EGFR WILD TYPE IN EGFRVIII-MEDIATED GLIOMAGENESIS

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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 GBM. 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 EGFRwt 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 tumorigenicity 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 EGFRvIII-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 ONCOLYTIC VIROTHERAPY: IFN-INDUCED ISGYLATION BY HDAC6

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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 IFNb treatment. Analyses were performed using coimmunoprecipitation 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 ISGylation 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 ISGylation in viral infection may be important in enhancing glioma cell responses against the OV by providing a previously unknown effector mechanism of interferon signaling.

CB-45. USE OF DOXORUBICIN AND DOXORUBICIN/IMIPRAMIME BLUE COLOADED 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 this study we use convention enhanced delivery (CED) of doxorubicin (DXR) and imipramine blue (IB) and human glioblastoma line transformed to express the EGFRvIII 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 implanted 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-DXR groups showed enhanced efficacy by increasing survival times above control groups ($p < 0.001$ for each as compared to control using Mantel-Wilcox test). There was no benefit for codelivery of the IB with DXR. However, there was complete survival and remission in 3 animals per group that received the DXR-loaded nanoparticles, indicating a 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, lung, prostate, and head and neck cancers, and it has been suggested that a common denominator of diverse cancer cells 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, which potentiates 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 of breast, prostate, 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 consists of a collagen-like N-terminal region and a C-terminal carbohydrate recognition domain (CRD), which has binding affinity for galactose and N-acetyllactosamine. We found that extracellular Gal3 shows a tumor-specific apoptotic effect in that a variety of tumor cell types (malignant glioma, breast, colon, prostate, and lung) are sensitive to Gal3-mediated killing, while normal cells (fibroblast, endothelial, and astrocyte) are not affected. Furthermore, extracellular Gal3 significantly reduces in vivo tumor volume. We hypothesize that Gal3 selectively kills tumor cells by interaction with tumor-specific modified cell surface receptor(s). 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. beta1 integrin has multiple glycans on its extracellular domain. Gal3 is supposed to interact with beta1 integrin through glycans. Third, we discovered that enhanced Gal3-beta1 integrin interaction is mediated through the aberrant N-linked glycans of tumor cells in that the expressions and the activities of N-acetylglucosaminyltransferase V (MGAT5), a tetraantennary branching glycosyltransferase, and upstream glycan-branching enzymes such as beta1, 4 galactosyltransferases (beta4GalT), and beta1, 3 acetylglucosaminyltransferase 2 (beta3GnT2) are significantly enhanced in tumor cells. The manipulation of MGAT5 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 A6B1 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 virus 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 $alpha_6beta_1$ integrin receptor rescues the Cyr61 mediated OV inhibition. Indeed, we also show that activation of the $alpha_6beta_1$ 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 $alpha_6beta_1$ 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 y-irradiation but is significantly downregulated in malignant astrocytomas compared to normal brain (oncomine analysis $p < .001$). 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 the biology and therapeutic response of human glioma cells. 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 ($p < .005$). We 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 cancer stem-like cell lines, almost without exception. It has also been shown that increased Gal-1 expression is highly correlated with poor patient survival, thus indicating that Gal-1 could represent a good therapeutic target. Glioma cell motility involves Gal-1-induced increases in rhoA expression and alterations of the polymerization of actin cytoskeleton. In a lactose-independent fashion, Gal-1 is recruited from the cytosol to the cell membrane by H-Ras-GTP with the subsequent stabilization of the H-Ras-GTP complex. To date, there have been no reports on specific phosphorylation sites in Gal-1 to explain the multiple subcellular and extracellular locations to which the protein can be found. We report for the first time that a threonine residue appears to be a unique location of Gal-1 phosphorylation. Furthermore, this phosphorylation event appears to be associated with a phenotypic shift in glioma cell lines to promote invasion. In addition to designing siRNA probes to both the 5'- and 3'- untranslated regions of endogenous Gal-1 mRNA, we also constructed both dominant negative and positive mutants at this phosphorylation site to interrogate the phenotypic shifts that this phosphorylation site confers. Discovery of the specific kinase that is responsible for this phosphorylation event could lead to therapeutic approaches to abate or modulate high-grade glioma invasiveness.

CB-51. INTEGRATIVE ANALYSIS OF GENOME EXPRESSION AND PROTEIN PROFILING REVEALS MULTIPLE CORE PATHWAYS AFFECTED BY EFEMP1 IN GLIOMA

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Gliomas are infiltrating primary brain tumors with higher grades characterized by increasing neoangiogenesis and changes of extracellular matrix components. We carried out a study of EGF containing fibulin-like extracellular matrix protein 1 (EFEMP1) and demonstrated a strong tumor-suppression function of EFEMP1 partially via suppression of proangiogenic factor VEGFA expression. Other signaling pathways should be involved that we are investigating using whole genome oligo expression arrays on RNA samples of glioma cells with or without EFEMP1 overexpression and phosphorylated AKT antibody array on whole cell lysate of the corresponding cells after a 48-hour serum starvation. The candidate EFEMP1 targets were further verified by real-time qRT-PCR and western blot. Expression array showed that EFEMP1 suppressed the expressions of multiple genes encoding extracellular matrix proteins (*FN1*, *COL4A1*, *COL6A1*, *COL21A1*, *LAMA4*, and *LAMB3*), extracellular ligands (*VEGFA*, *EGF*, *FGF*, and *EGR2*), and membrane receptors (*PDGFRA*, *FGFR2*, *FGFR3*, *EGFR*, *ITGAV*, *ITGB1*, and *IL11RA*). Antibody array revealed that EFEMP1 blocked multiple target protein phosphorylations downstream of AKT, which was consistent with finding of exogenous EFEMP1 protein in blocking AKT phosphorylations in multiple glioma cell lines. It includes FOXO transcription factors, NFKBIA, CHUK, BCL2 protein family that phosphorylation may ensure glioma cell survival under stressful environments. EFEMP1 also reduced phosphorylations of PTK2, PTK2B, and PTK2 downstream target PXN, which together form the FAK signaling pathway. Overall, data revealed multiple ways of EFEMP1 in suppressing high grade glioma malignant behaviors, including blockage of AKT- and FAK-signaling pathways and normalized tumor microenvironment.

CB-52. MOLECULAR DETERMINANTS OF DICHOTOMY BETWEEN PROLIFERATION AND INVASION OF GLIOMA CELLS

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Classic histological features of glioblastoma include dense proliferative areas rich in angiogenesis as well as centripetal dissemination of neoplastic cells into adjacent brain tissue. Distinct transcriptomes are discernable 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 up at the invasive rim of the tumor. 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 was higher in the rim than the core for 30 of 43 biopsy specimens scored. Depletion of c-Myc expression resulted in an increase in the 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-REGULATION OF TSC1-MTORC1 IN GLIOMA

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The Forkhead Box subclass O (FOXO) transcription factors govern the cellular programs for 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 gliomas, we observed a frequent reduction in TSC1 mRNA expression, which correlated with decreased FOXO3 mRNA ($R^{2=0.779,}$ $p < 0.0001$). Immunoblot analysis of a small panel of tumors suggested increased activation of mTORC1 in samples upon inhibitory phosphorylation of FOXOs. Together the data indicate that FOXO inactivation contributes to 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.

CB-54. DIFFERENCES IN FOREBRAIN AND SPINAL CORD TUMORS REFLECT UNIQUE MICROENVIRONMENTAL RESPONSES TO PARACRINE STIMULATION IN PDGF-DRIVEN MODELS OF GLIOMA

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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 naive 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 nature of these microenvironmental influences, we examined the ratio between recruited glial progenitors expressing PDGFRalpha and pericytes expressing PDGFRbeta, both of which respond to paracrine PDGF-BB stimulation from the retrovirus infected cells. Tumors generated in the forebrain contained PDGFRalpha+ recruited glial progenitor populations that far outnumbered the PDGFRbeta+ pericytes, whereas in the spinal cord tumors the pericytes outnumbered the recruited glial progenitors. Thus, the forebrain and spinal cord tumors differ in the relative abundance of PDGF responsive cells (glial progenitors and pericytes) that are being recruited from the microenvironment, and these differences in cellular composition give rise to differences in tumor histology.

CB-55. NEUTROPHILS PROMOTE THE MALIGNANT GLIOMA PHENOTYPE IN VITRO

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Solid tumors, including glioblastoma, contain large populations of cancer-related inflammatory cells. Among these inflammatory cells, infiltration of tumor-associated neutrophils (TANs) has been found to be significantly correlated with glioblastoma grade. However, the potential roles of TANs and the molecular mechanisms by which these inflammatory cells promote glioma growth are not well established. In this study, we found increased neutrophil infiltration into tumor cells during glioma xenograft progression in vivo. To determine the effect of neutrophils on glioma cells, we next took advantage of an in vitro coculture model to mimic communication between these 2 types of cells. Our data demonstrate that neutrophils increased the proliferation rate of stem cell-like cells (GSCs). Condition media (CM) collected from cultured neutrophils had a similar effect, suggesting that growth factor secretion by neutrophils exerts this paracrine effect. After GSCs were cultured in CM for 48 hours, there was a statistically significant increase in the proportion of cells in the S + G2/M compartment and a concomitant decrease in apoptosis as measured by PI staining. We next evaluated the impact of neutrophils on glioma invasion. CM from neutrophils significantly increased GSC transwell migration compared to controls. cDNA microarray and immunoblotting experiments confirmed that the expression levels of Cyclin D2 and c-Myc were upregulated in GSCs cocultured with neutrophils. This upregulation could be abrogated by MEK/ERK inhibitor (U1026), suggesting a possible role of the MAPK pathway in the proliferative-promoting effects of neutrophils on GSCs. Collectively, these data suggest that recruitment of neutrophils could promote glioma proliferation and invasion. Targeting granulocytes may be an effective approach to inhibit the glioma malignant phenotype.

CB-56. NICOTINAMIDE-N-METHYLTRANSFERASE IN GLIOBLASTOMA CONFERS RESISTANCE TO RADIATION TREATMENT AND EXPRESSION LEVEL INVERSELY CORRELATES WITH SURVIVAL

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PURPOSE: Glioblastoma multiforme (GBM) is the highest grade and most aggressive form of glioma. Average patient survival is about 14 months after diagnosis. The relative resistance to conventional therapy displayed by GBM leaves opportunity for vast improvement in survival. Elucidating mechanisms of radiation resistance or uncovering novel targets to sensitize tumors to radiation could significantly benefit patient outcomes. Nicotinamide-*N*-methyltransferase (NNMT) is an enzyme involved in the nicotinamide pathway as well as in the metabolism of drugs and xenobiotic compounds. While the exact role NNMT plays in the malignant phenotype is poorly understood, it has been investigated for its potential use as a prognostic marker or therapeutic target in other cancers. NNMT is highly expressed in glioblastoma compared to either lower grade astrocytomas or normal brain tissue. Furthermore, Kaplan-Meier plots from publically available clinical data sets have shown that relatively higher NNMT expression is correlated with adverse patient outcome. Gaining a better understanding of NNMT's role in GBM could uncover exciting advances in neuro-oncology. EXPERIMENTAL DESIGN: We explored the expression profile of NNMT in established (ATCC) and in our panel of patient-derived primary GBM cell lines. We generated an isogenic, in vitro model by knocking down as well as overexpressing NNMT in U87 cells. We conducted various functional experiments such as MTS assay, clonogenic survival assay, annexin V, ATP assay, comet assay, and western blotting analysis. In vivo experiments were also used to determine differences in relative tumorigenicity. RESULTS AND CONCLUSION: Intracranial implantation of NNMT knockdown cells in mice showed a delayed tumor growth compared to NNMT overexpression cells. Silencing NNMT leads to an enhanced sensitivity to radiation in vitro, whereas cells overexpressing NNMT are 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 in 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 migrating edge. MiR 338-3p overexpression induces cellular apoptosis as determined by cleaved caspase 3 expression levels in GBM. MiR 338-3p overexpression shows no effect on proliferation in human neuronal stem cells; however, it does result in induced neuronal differentiation as determined by TUJ1 expression levels. MiR 338-3p targets histone deacetylase 4 (HDAC4). MiR 542-5p is detectable in the serum of glioma patients as well as normal surgical controls. Serum miR 542-5p 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-58. ENHANCED ONCOLYTIC HSV-1 THERAPY BY HDAC6 INHIBITION

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INTRODUCTION: Oncolytic viruses (OVs) have been used as a treatment modality for malignant gliomas, yet efficient replication of OVs to tumor regions may be a challenge owing to innate immune responses to gliomas. Previously, we have shown that pan-HDAC inhibitors can enhance HSV-1 replication in human glioma cells by escaping IFN-mediated antiviral effects (Otsuki et al., Mol. Therapy 2008). In this study, we hypothesized that a novel glioma defensive mechanism against

oncolytic HSV1 is provided by histone deacetylase 6 (HDAC6). RESULTS: Catalytic inactivation of HDAC6 by pharmacological inhibitor or shRNA knockdown increased oHSV1 replication. Pharmacologic inhibition of HDAC6 induced hyperacetylation of alpha-tubulin and enhanced viral capsid trafficking toward the nucleus while impairing lysosomal encapsulation of the HSV-1 capsids in human glioma cells. Furthermore, HDAC6 inhibition suppressed interferon-stimulated genes (ISGs) expression upon HSV-1 infection, suggestive of HDAC6 function involving antiviral immune response. CONCLUSION: Our data show for the first time that HDAC6 is a novel antiviral mechanism in gliomas and uncovers pharmacological maneuvers to help viral replication.

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 significant decrease in annexin V labeling with CA-Lyn. To evaluate whether Lyn promotes autophagy, we blotted for 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 CA-Lyn cells were 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 GLIOBLASTOMA

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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 transcriptomal signatures of primary GBM, 3 of which—classical, 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 basal activation state of 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-i 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 TGCA data set to find important cell death mechanisms in gliomapathogenesis, we identified differential expression of the novel Bcl2-like13 protein (Bcl2L13). Bcl2L13 shares significant structural homology with Bcl-2 and Bcl-xL, but contains a unique 250 amino acid sequence that may point to unique, nonclassical functions. Several studies have shown that Bcl2L13 expression correlates with increased chemotherapeutic resistance and unfavorable treatment outcome in leukemia and ZIC1-driven liposarcomas. Those clinical data suggest that Bcl2L13 likely acts as an oncoprotein in multiple cancers and may act in a similar manner in GBM. Using qRT-PCR and immunohistochemical analyses, we found that Bcl2L13 was highly overexpressed in >90% of GBM samples but was absent in adjacent normal brain. Using RNAi-loss and cDNA complementation studies in glioma cell lines, we determined that Bcl2L13 potently inhibits therapy-induced apoptosis by inhibiting mitochondrial outer membrane permeabalization (MOMP) and postmitochondrial caspase-3 and -7 cleavage, likely acting in a similar manner as canonical Bcl-2 family proteins. To study the impact of Bcl2L13 on the progression of gliomagenesis in vivo, we orthotopically injected glioma cell lines with enforced expression of Bcl2L13-targeting shRNAs into immunocompromised SCID mice. Strikingly, neutralization of Bcl2L13 signaling 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 effects, we performed a yeast 2 hybrid screen and identified a select number of tumor suppressors and oncoproteins including ceramide synthase 2 (CerS2), 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, Bcl-2-like GBM 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 colony formation assay. RESULTS: While *XAF1* transcript was easily detectable in all normal brain tissues we tested, its expression was undetectable or very low in 86% (6 of 7) of cell lines and 75% (12 of 16) of primary tumors. Moreover, *XAF1* reduction was significantly more common in high-grade tumors versus low-grade tumors. Following treatment with the demethylating agent 5-aza-dC, hypermethylation at 7 CpG sites in the 5' proximal region of the *XAF1* promoter was highly prevalent in glioblastomas versus normal tissues and tightly associated with reduced gene expression. XAF1 expression suppressed tumor cell growth and enhanced cellular response to apoptotic stresses in a XIAP-independent manner, while knockdown of its expression protected cells from the stresses. Intriguingly, it was found that XAF1 expression is upregulated by chemotherapeutic drugs, such as temozolomide and etoposide, and XAF1 induction leads to Erk suppression. Furthermore, under apoptotic stress conditions, Erk elevation by XAF1 depletion was associated with enhanced tumor cell resistance to apoptotic stresses. In addition, the apoptosis-sensitizing effect of XAF1 was attenuated by ectopic overexpression of Erk, indicating that XAF1's proapoptotic effect is associated with its ability to inhibit Erk elevation under apoptotic stress condition. CONCLUSION: Collectively, our study demonstrates that epigenetic alteration of *XAF1* is frequent in human glioblastoma and may contribute to the malignant tumor progression.

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

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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 classifications of malignant glioma subtypes might enhance the clinical utility of monitoring and targeting 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 other reports of pathway activation in grade IV glioblastoma. To evaluate 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 oncogenomic 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, neural, 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 antiglioma therapeutics via enhancement of effector caspase activation. 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 GLIOBLASTOMA 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 $\pm$ 823; mean $\pm$ SD) compared with normal controls (mean, 878 pg/ml $\pm$ 288; $p = 0.0335$, Wilcoxon rank-sum test). Surprisingly, we also found a significant decrease in the level of sTNFR2 in the GBM patients (mean, 2658 pg/ml $\pm$ 1357) compared with normal controls (mean, 3572 pg/ml $\pm$ 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. beta-CATENIN/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 AKT1, AKT2, and miR-21, to determine the specific binding site that bind to AKT1, AKT2, and miR-directly.(Supported by NSFC 30971136,NCET-07-0615, TJSTC 09JCZDJC17600). *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 mechanisms of cerebral edema are complex and are mediated in part by aquaporin proteins (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 glioblastoma, 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 to the 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 is directly correlated with volume of extracellular brain edema. 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. This improved understanding 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

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Genetic alterations in malignant gliomas affect cell proliferation and cell-cycle control and are currently the targets of most chemotherapeutic agents. However, the efficacy of treating advanced glial tumors with adjuvant therapies remains largely unsuccessful owing to the inability to effectively target invading cells. Primary glioblastomas that arise de novo often show amplification and overexpression of EGFR (>50% of glioblastomas). As with many tumors, a temporal dichotomy between growth and invasion is evident; however, key intracellular factors responsible for regulating the 'switch' between proliferation and migration remain poorly discerned. We previously have shown that the Na + /H+ exchanger regulatory factor (NHERF-1) 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 mechanism 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-CreER^{T2}/Rosa26-YFP reporter mouse line crossed to a either a conditional floxed beta-1 integrin gene or a conditional floxed Fak mouse. In beta-1 integrin deficient cells, fewer YFP-labeled cells from the Nestin compartment were observed to migrate from the SVZ to the OB compared to wild type. Some YFP-labeled beta-1 integrin deficit 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 adult 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.

CB-70. ASYMMETRY-DEFECTIVE OLIGODENDROCYTE PROGENITORS ARE GLIOMA PRECURSORS

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Postnatal oligodendrocyte progenitors self-renew, generate mature oligodendrocytes, and are a cellular origin of oligodendroglioma. The mechanism for the neoplastic transformation of NG2+ oligodendrocyte is largely unknown. Using novel cell culture and in vivo assays, we show that normal murine and non-neoplastic human oligodendrocyte progenitors undergo asymmetric cell divisions. To achieve asymmetric cell division, they segregate the proteoglycan NG2 asymmetrically during oligodendrocyte progenitor mitosis to generate cells of distinct fate. By analyzing NG2-dificient oligodendrocyte progenitor cells, we found that the NG2+ progeny undergoes EGF-dependent self-renewal, which depends on endogenous NG2 expression. In contrast, the NG2- progeny differentiates. In a mouse model for oligodendroglial tumors, the S100beta-verbB p53 deleted mice, increased symmetric oligodendrocyte progenitor divisions coincide with abnormal self-renewal rather than differentiation at premalignant stages and leads to neoplastic transformation. Asymmetric cell divisions are prevalent in NG2+ cells from human non-neoplastic tissue but not from low-grade oligodendrogliomas, which misexpress regulators of asymmetric cell division. Taken together, we unraveled loss of asymmetric division in premalignant, aberrantly self-renewing, differentiation-defective murine oligodendrocyte progenitors and human NG2+ oligodendroglioma cells, thereby explaining initial steps by which oligodendrocyte progenitors transform into oligodendroglioma cells. Our data suggest that asymmetric cell divisions maintain homeostasis in the postnatal oligodendrocyte lineage. In contrast, loss or disruption of asymmetric cell division might be causal to the neoplastic transformation of oligodendrocyte progenitor cells and provide novel points of disruption to which specific glioma therapies can be targeted.