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

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(29%) tumors responding to TMZ, compared with 100%, 94%, and 90% response rates for paclitaxel, cis-platinum and vincristine, respectively. Length of survival in TMZ-treated patients who screened positive for a TMZ response averaged 301 days, compared with just 98 days in their TMZ-negative counterparts. CONCLUSIONS: In this study, we report a novel assay system that generates individual patient drug profiles and is able to predict patient survival in TMZ-treated patients. Further research, including a formal prospective clinical trial, may be warranted.

AI-28. ISOCITRATE DEHYDROGENASE-1 (IDH-1) EXPRESSION DOES NOT CO-LOCALIZE WITH HYPOXIA INDUCIBLE FACTOR-1ALPHA (HIF-1ALPHA) EXPRESSION IN GLIOMAS

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

AI-29. FOCAL ADHESIONS DYNAMICS IN MALIGNANT GLIAL CELLS WITH VARIABLE DRR EXPRESSION

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

AI-30. ONCOGENIC EGFRvIII SENSITIZES GBM CELLS TO PROANGIOGENIC EFFECTS OF THE COAGULATION SYSTEM

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INTRODUCTION: Tissue factor (TF) is a procoagulant receptor frequently overexpressed in human glioblastoma multiforme (GBM), in which thrombotic events are particularly frequent. Analysis of GBM cell lines suggests that TF is a regulatory target of several major genetic alterations associated with this disease, including activation of the epidermal growth factor receptor (EGFR) and expression of its mutant (EGFRvIII) (Milsom et al., 2008). Upon binding the coagulation factor VIIa, TF drives extracellular (procoagulant) and intracellular (signaling) events. The latter are mediated by protease activated receptors 1 and 2 (PAR-1/2) and are implicated in cancer progression and angiogenesis. This raises the question whether and how TF signals intersect with oncogenic effects of EGFRvIII. METHODS: U373 and U87 glioma cells lines and their EGFRvIII expressing or TF transfected counterparts were compared for growth in vivo and FVIIa-dependent procoagulant activities (Xa generation assay), expression of TF and PAR1/2 (RT-PCR, ELISA), and production of angiogenic factors (VEGF and IL-8). RESULTS: We found that EGFRvIII upregulates TF, PAR-1, PAR-2, and FVII in GBM cell lines, which also become highly procoagulant and hypersensitive to stimulation with FVIIa, PAR-1, and PAR-2 activating peptides. This stimulation evokes production of VEGF and IL-8 in EGFR/EGFRvIII-expressing GBM cells, but not in their indolent counterparts transfected with TF alone (TF-U373). However, TF transfection renders indolent GBM cells capable of forming tumors, but only after a long latency. CONCLUSIONS: TF signaling intersects with and amplifies the proangiogenic effects of EGFRvIII-driven oncogenic pathways, and TF also may have an independent but modest role in GBM progression.

CELL BIOLOGY AND SIGNALING

CB-01. REGULATION OF AMINOACYLASE EXPRESSION IN NEUROBLASTOMA

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Neuroblastoma, a cancer of the sympathetic nervous system, is the most common extracranial solid tumor in children. MYCN amplification and increased BDNF/TrkB signaling are features of high-grade tumors, yet only ~25% of malignant tumors display those features. Thus, the identification of additional biomarkers and therapeutic targets is essential. Since aminoacylase 1 (ACY1), an amino acid deacetylase, is a putative tumor suppressor in small cell lung and renal cell carcinomas, we investigated whether ACY1 or family members aspartoacylase (ASPA, aminoacylase 2) or aminoacylase 3 (ACY3) could serve a similar function in neuroblastoma. Aminoacylase expression was examined in TrkB-positive, MYCN-amplified SMS-KCNR and TrkB-negative, non-MYCN amplified SK-N-AS and SK-N-SH neuroblastoma cell lines. ACY1 and ASPA exhibited distinct spatial localization in SMS-KCNR and SK-N-SH cells, while ACY3 displayed nuclear expression in all three lines examined. ACY1 was the only aminoacylase whose expression was up-regulated upon neuronal differentiation of SK-N-SH cells in media containing 10% serum. ASPA expression was greater in the least aggressive SK-N-SH line and significantly reduced in the most aggressive SMS-KCNR line. Conversely, ACY3 expression was highly expressed in the most aggressive SMS-KCNR cells. *In vivo*, aminoacylases are expressed in common sites of neuroblastoma origin. Bioinformatics data mining of Kaplan-Meier survival data revealed that high ACY3 expression is correlated with poor prognosis and that low expression of ACY1 or ASPA is also correlated with poor prognosis, suggesting that the loss of these aminoacylases may contribute to neuroblastoma tumorigenesis.

CB-02. NPAS3 IS A LATE-STAGE-ACTING PROGRESSION FACTOR IN GLIOMAS WITH TUMOR SUPPRESSIVE FUNCTIONS

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BACKGROUND: Malignant astrocytomas, the most common primary brain tumors, are predominantly fatal with current therapies. In our effort to better understand the biology of astrocytomas, we explored new therapeutic targets. We previously cloned NPAS3, a transcription factor that maps to human chromosome 14. Our principal aim is to comprehend the disease associations of NPAS3, since we recently identified its expression in human astrocytes. We initially identified NPAS3 as an astrocytoma candidate based on the Cancer Genome Project reporting chromosome 14 deletions (with NPAS3) among ~20%-80% of astrocytomas and with >70% of our human astrocytoma panel (n = 433) having aberrant NPAS3 protein expression. Based on the findings from our precursory screen, we next undertook functional analyses of NPAS3 in human astrocytomas. METHODS-RESULTS: After undertaking extensive functional analyses, we now have evidence supporting NPAS3 as an astrocytoma tumor

suppressor involved in late-stage tumor progression, based on: 1) Aberrant NPAS3 expression is predominant in surgically resected high-grade astrocytomas compared with low-grade astrocytomas; 2) loss-of-function mutations in NPAS3, which are associated with loss of heterozygosity of the NPAS3 locus, are identified in surgically resected human glioblastomas; 3) absent NPAS3 expression is predominant in malignant human glioma cell lines; 4) over-expressed NPAS3 in malignant glioma cell lines suppresses transformation potential, while converse reduced expression promotes an increase in transformation potential; and 5) a reduced NPAS3 expression (efficiency >90%) in concert with other gliomagenesis genes can transform a well-characterized TERT immortalized human astrocyte cell line and promote the growth of malignant astrocytomas. CONCLUSIONS: Our data provide compelling evidence that the NPAS3 gene is involved in the cause of astrocytomas, with tumor suppressive and late-stage acting progression factor roles. Current research is focused on better understanding NPAS3 in gliomas using other pre-clinical models.

CB-03. 2-DEOXY-D-GLUCOSE INHIBITS N-GLYCOSYLATION IN GLIOBLASTOMA-DERIVED CANCER STEM CELLS

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

CB-04. UNDERSTANDING AND TARGETING KINASE-INDEPENDENT ACTIVITY OF EGFR AND EGFRVIII TO OVERCOME GBM DRUG RESISTANCE

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Glioblastoma (GBM) is the most common and most intractable brain malignancy in adults. Patients with GBM have a dismal prognosis, with a median survival of 12–14 months. Epidermal growth factor receptor (EGFR) and its constitutively activated variant EGFRvIII are linked to GBM resistance to therapy; the mechanisms underlying this association, however, are still unclear. Also unclear are the mechanisms underlying the resistance of GBM to EGFR-targeted monotherapy and combination therapy. We report here that in GBM cell lines, xenografts, and primary specimens (N = 101), EGFR and EGFRvIII paradoxically co-express with p53-upregulated modulator of apoptosis (PUMA), a proapoptotic member of the Bcl-2 family of proteins primarily located on the mitochondria, unlike other BH3-only proteins. Mitochondrial PUMA is known to bind to and antagonize antiapoptotic Bcl-2/Bcl-xL/Mcl-1 and also to associate with and activate the apoptotic executor Bax, together leading to apoptotic response upon appropriate stress. Our results showed that both EGFR and EGFRvIII bind to PUMA constitutively and under apoptotic stress, subsequently sequestering PUMA in the cytoplasm. EGFR siRNA-mediated expression knockdown relocates PUMA from the cytoplasm onto the mitochondria. The EGFR-PUMA interaction is independent of epidermal growth factor (EGF)-induced EGFR activation and is sustained under treatment with an EGFR kinase inhibitor, Iressa. Although GBM cells express several proapoptotic members of the Bcl-2 protein family, our results indicate that PUMA is essential for therapy-induced apoptosis and thus for drug sensitivity. Importantly, we found that Bcl-2/Bcl-xL/Mcl-1 inhibitors (BH3 mimetics) that mimic PUMA's antiapoptotic activity sensitize EGFR- and EGFRvIII-expressing GBM cells to Iressa. Collectively, we uncovered a novel kinase-independent function of EGFR and EGFRvIII that contributes to GBM resistance to EGFR kinase inhibition and apoptosis-inducing agents and also provides a rationale for targeting kinase-dependent and -independent activities of EGFR as a novel combination therapy for GBM.

CB-05. NEW THERAPEUTIC APPROACH FOR BRAIN TUMORS: INTRANASAL ADMINISTRATION OF RAS INHIBITOR PERILLYL ALCOHOL

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PURPOSE: The monoterpene perillyl alcohol (POH), a Ras inhibitor with the potential capacity to arrest gliomagenesis, is being used in a phase I/II clinical trial in adults with recurrent malignant glioma. The present study aimed to investigate the efficacy of the intranasal administration of POH and the survival rate in patients with recurrent glioblastoma (GBM) in comparison with a historical control group of GBM patients. PATIENTS AND METHODS: The study included 89 adults with recurrent GBM who received daily intranasal administration of 440 mg POH and 52 matched GBM patients as the historical control group. RESULTS: The 6-month progression-free survival (stable disease) rate was 48.3% for POH-treated patients, with a significant ($p = 0.0001$) survival advantage compared with the untreated historical control group. The median survival time for patients with secondary GBM was 11.2 months, longer ($p = 0.0002$) than for patients with primary GBM (5.9 months). Age-adjustment multivariate analysis showed a significant difference ($p = 0.0002$) in the survival rate between primary and secondary GBM patients. Patients with tumors localized in deep regions (e.g., thalamus, basal ganglia) survived longer ($p = 0.0083$) than those with tumors in lobar regions. Radiographic improvement and reduction of corticosteroid dosage (36%) was further associated with a delay in progression. CONCLUSION: Intranasal administration of POH increased the overall survival of patients with recurrent GBM compared with historical controls, especially of patients with secondary GBM and those with tumor localized in deep regions of the brain, without clinical evidence of side effects for more than a year.

CB-06. ROLE OF A NOVEL NF1-LRPPRC INTERACTION IN RNA GRANULE TRANSPORT

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

CB-07. KETOGENIC DIET AS AN ADJUVANT THERAPY TO RADIATION AND THE EFFECTS ON SAPK/JNK PROTEIN EXPRESSION

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Glioblastoma multiforme (GBM) is an aggressive tumor. Despite surgery, radiation, and chemotherapy, median survival is 12–18 months. More effective therapy is needed, and one promising treatment is the ketogenic diet (KD), which has been shown to increase survival in mouse models. To dissect KD's mechanism of action, we did gene expression profiling on tumor and contralateral normal brain from animals fed KD or standard diet (SD). Mitogen-activated protein kinase (MAPK) is one pathway affected by the diet. Within the MAPK pathway, stress-activated protein kinase/c-jun N-terminal kinase (JNK) decreased 1.8-fold in animals fed KD. Total and phosphorylated JNK were evaluated through Western blot (WB) analysis and immunofluorescence. Although there was no obvious difference shown by WB, immunofluorescence suggested an increase in total JNK in SD versus KD. Phospho-SAPK/JNK(p-46) (phospho-JNK) was over-expressed in tumor versus non-tumor, irrespective of diet. Additionally, immunofluorescence results showed that the JNK2 isoform translocated into the nucleus in tumors in SD animals but not in KD animals. Because therapy for GBMs typically includes radiation and/or chemotherapy, we used radiation with and without KD. KD plus radiation extended the survival of animals compared with those given either treatment alone. Expression profiling analyses demonstrated a global effect on gene expression following radiation. We have shown that the expression of some genes reverts closer to control (SD) levels when animals are fed KD. Expression analysis of diet plus radiation treatment indicates that some of the expression altered by radiation is minimized by KD. Total JNK is increased in tumors in animals that receive KD plus radiation. Phospho-JNK is increased in all groups treated with radiation, irrespective of diet. Protein expression alterations and activation of JNK can alter growth, apoptosis, and angiogenesis. Our data suggest a complex interaction between metabolic alterations such as those that occur with KD and treatment response.

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

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We hypothesized that multiple neurofibromin-regulated small G-proteins from the classic Ras (H, N, and K-Ras) and R-Ras (R-Ras, R-Ras2, and M-Ras) subfamilies promote the proliferation and migration of malignant peripheral nerve sheath tumor (MPNST) cells. We found that H-Ras, N-Ras, and R-Ras2 proteins were uniformly expressed in 8 MPNST lines; their expression of K-Ras2b and R-Ras was variable, while M-Ras protein was not detected in these lines. RT-PCR analyses demonstrated that the guanine nucleotide exchange factors necessary to activate these Ras proteins were also present. Using ^{3}H-thymidine incorporation and Transwell migration assays, we assessed the effects that dominant negative (DN) H-Ras and R-Ras mutants exerted on MPNST cells. We found that DN H-Ras and DN R-Ras both inhibited MPNST mitogenesis, while only DN R-Ras inhibited migration. Raf-1 RBD affinity assays performed in MPNST cells transiently transfected with Myc-tagged H-Ras, N-Ras, and K-Ras demonstrated that all 3 classic Ras proteins were constitutively activated. However, shRNA-mediated ablation of N-Ras or K-Ras had no effect on proliferation. We conclude that both classic Ras and R-Ras subfamily members contribute to MPNST pathogenesis. Inhibition of multiple Ras isoforms will therefore likely be required to achieve an optimal therapeutic effect. (This work was funded by R01 CA122804 and F30 NS063626.)

CB-09. IN SITU PROXIMITY LIGATION-BASED ANALYSIS REVEALS ABERRANT DIMERIZATION AND ACTIVATION OF EPIDERMAL GROWTH FACTOR RECEPTORS PREVALENT IN GLIOBLASTOMA MULTIFORME

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Aberrations in epidermal growth factor receptor (EGFR/ErbB1) signaling are the most common oncogenic stimuli in human glioblastoma multiforme (GBM). Interactions between mutant and wild-type ErbB family members in GBMs are of biological and potential therapeutic importance. Recently, we have described our work developing and optimizing a novel in situ proximity ligation assay (PLA) for dimerization and activation analysis of EGFR mutants prevalent in GBMs. Utilizing our novel in situ platform for EGFR dimerization analysis, we seek to directly ascertain the dimerization capacity and activation status among EGFR mutants and ErbB members in GBM operative specimens and whether this feature has important prognostic or diagnostic value in GBMs harboring them. Our initial in vitro analysis using this platform demonstrates the aberrant homo-/hetero-dimeric properties of EGFRvIII and EGFRc958 mutants, the two most common mutants associated with EGFR amplification in GBMs. In addition, dimer phospho-activation status can be detected with >16-fold sensitivity and >17-fold SNR than phospho-EGFR measurements currently undertaken with IHC or IF. Dimer activation analysis indicates the aberrant activation properties of mutant dimers that may be associated with oncogenic downstream signaling. Furthermore, we report for the first time the detection of wild-type and EGFRvIII dimerization in GBM specimens, in keeping with our prior cell line data. GBM tissue microarray analysis for the presence of this mutant heterodimer has commenced for statistical examination with patient survival. We propose the PLA analysis platform as an alternative diagnostic modality to evaluate the expression, dimerization, and activation of wild-type and mutant EGFRs prevalent in GBMs and whether these features have important prognostic or diagnostic value. Moreover, since PLA allows specimen assessment of not only expression and activation but also dimerization, which is not evaluated by current IHC techniques, it will likely serve as a way to evaluate promising anti-EGFR strategies directed at preventing EGFR dimerization and activation.

CB-10. RAS REGULATED OVER-EXPRESSION OF INHIBITORS OF APOPTOSIS PROTEIN (IAPS) IN GLIOMAGENESIS

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

CB-11. MIR-21 AND MIR-128 DEREGULATION IN GLIOBLASTOMA: EFFECT OF MIRNA MODULATION ON CELL VIABILITY

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The discovery of microRNAs (miRNAs), small non-coding RNAs that regulate post-transcriptionally the expression of messenger RNAs, has revealed an additional level of finetuning of the genome. miRNA alterations

have been associated with development and progression of cancer, and several miRNAs were found to be implicated in the modulation of glioma oncogenesis. In this work, it is demonstrated that miR-21 and miR-128 expression is markedly altered in a retrovirally-induced murine model of glioblastoma. Furthermore, miR-21 and miR-128 expression is shown to be significantly altered in human glioblastoma specimens (n = 22) and cell line (U87) compared with control brain tissues. miR-21 is overexpressed in 80% of the tumor samples, with an average fold change value of 7.0, whereas miR-128 is highly downregulated in all samples, with an average fold change value of 15.6. Remarkably, analysis of the Cancer Genome Atlas Research Network data on 252 human glioblastomas reveals miR-21 upregulation and miR-128 downregulation in all the cases, corroborating our experimental data. Identifying the regulatory mechanisms of these aberrantly expressed miRNAs in glioblastoma may be critical to better understand their role in initiation and progression of human glioblastoma, thus helping in the development of effective RNA-based therapeutic approaches for this disease.

CB-12. DOES THE MICRORNA CLUSTER OF 53 MIRNAS ON CHROMOSOME 14Q32.31 PLAY A ROLE IN GLIOMAS?

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

CB-13. KIAA0495/PDAM IS FREQUENTLY DOWN-REGULATED IN OLIGODENDROGLIAL TUMORS, AND ITS DOWN-REGULATION BY SIRNA INDUCES CISPLATIN RESISTANCE IN GLIOMA CELLS

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Co-deletion of chromosomes 1p and 19q is a common event in oligodendroglial tumors (OTs), suggesting the presence of OT-related genes. The aim of this study was to identify target genes residing in the minimally deleted regions on chromosome 1p36.31-p36.32 that might be involved in OTs. A novel gene, KIAA0495/PDAM (for p53-dependent apoptosis modulator), was found to be frequently deregulated, with 37 of 58 (63.8%) OTs examined showing reduced PDAM transcript by at least two-fold and 19 of those exhibiting >10-fold reduced expression relative to the mean expression level of eight normal brain samples. PDAM down-regulation was associated with chromosome 1p loss status ($P = 0.001$). Promoter hypermethylation of PDAM was detected in 30 of 37 (81.1%) OTs with reduced PDAM expression, and the two parameters were significantly associated ($P = 0.0004$). Glioma cells (A172 and TC620) treated with 5-azacytidine exhibited significant enhancement of PDAM expression. Notably, combined treatment with 5-azacytidine and trichostatin A showed a synergistic effect on PDAM level. These findings suggest that chromosome 1p loss and epigenetic modification are the major mechanisms contributing to PDAM down-regulation. The role of PDAM in chemosensitivity was also evaluated. PDAM knockdown had no effect on sensitivity to vincristine, lomustine, temozolomide, and paclitaxel but could induce cisplatin resistance in glioma cells harboring wild-type p53. BCL2L1 exhibited significant up-regulation, while BCL2 showed partial derepression in PDAM-silenced cells after cisplatin treatment, suggesting that alteration of antiapoptotic genes contributed in part to cisplatin resistance. Knockdown of BCL2L1 abrogated the induced cisplatin-resistant phenotype. Collectively, these findings suggest that PDAM deregulation may play a role in OT development and that PDAM may possess the capacity to modulate apoptosis via regulation of p53-dependent antiapoptotic genes.

CB-14. THE HUMAN CYTOMEGALOVIRUS IE1 PROTEIN CONFERS RESISTANCE TO TEMOZOLOMIDE IN THE U87MG CELL LINE

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

CB-15. TARGETING MICRORNAS IN GLIOMA CELLS WITH ANTINEOPLASTONS

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MicroRNAs (miRNAs) are short, endogenous, non-protein-coding, single-stranded sequences of RNA that have been found to play an important regulatory role in gene expression. The genes encoding miRNA are often located in genomic regions associated with cancer; hence, it has been suggested that miRNAs may be tumor suppressors or oncogenes. Recently, several miRNAs that are deregulated in glioblastomas have been studied, and their target genes and pathways have been identified. In this study, we report the changes in expression of several miRNAs in U87 glioblastoma cells in response to exposure to antineoplaston AS2-1. This study was done using the Dharmacon miRNA profiling array (Thermo Fisher Scientific). The miRNAs 125a-5p and 125a-3p are some of the miRNAs up-regulated in our study; 125a-5p has recently been shown to be regulated by the epidermal growth factor receptor and to function as a tumor suppressor in lung cancer. It has also been shown that the over-expression of miRNA 125a or miRNA 125b caused reduced migration and invasion of SKBR3 breast cancer cells. Using the total human microarray screen (Affymetrix) we have noted the reduced expression of AKT2 and the enhanced expression of genes involved in apoptosis in U87 cells exposed to antineoplaston AS2-1. Antineoplastons will be used in phase III U.S. Food and Drug Administration-regulated clinical trials this year. Once approved, these amino acid derivatives may offer promising treatment in many types of brain tumors.

CB-16. SHP-2/PTPN11 IS A CRITICAL MEDIATOR OF GLIOMAGENESIS DRIVEN BY PDGFR-ALPHA AND INK4A/ARF ABERRATIONS

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Glioblastoma is the most common and aggressive cancer of the central nervous system. Recent collaborative efforts have classified glioblastomas into four clinically relevant subtypes based on signature genetic lesions in the tumor specimens. In a large number of proneural glioblastomas, PDGFR-alpha over-expression is concomitant with a loss of CDKN2A locus (encoding p16INK4a and p14ARF). In this study, we demonstrate that activation of PDGFR-alpha and/or PDGF-A confers tumorigenicity to Ink4a/Arf-deficient mouse astrocytes or human glioma cells in the brain. Restoration of p16INK4a but not p19ARF suppresses PDGFR-alpha-promoted glioma formation. Conversely, cellular depletion of endogenous p16/INK4a by short hairpin RNA (shRNA) in glioma cells with wild-type INK4a/ARF markedly enhanced PDGFR-alpha-induced transformation. Mechanistically, the abrogation of intrinsic tyrosine kinase activity of PDGFR-alpha or the inhibition of signaling modules in PDGFR-alpha that lost the capacity to bind to PI3K or SHP-2 (encoded by PTPN11) significantly diminished PDGFR-alpha-promoted tumorigenesis in vitro and in vivo. Furthermore, the inhibition of SHP-2 by shRNAs or inhibitors disrupted the interaction of PI3K with PDGFR-alpha, suppressed Akt activation, and impaired tumorigenesis of Ink4a/Arf-null cells. Additionally, SHP-2 inhibition attenuated mammalian target of rapamycin (mTOR) activation of S6 kinase, and the inhibition of mTOR abrogated PDGFR-alpha-promoted tumorigenesis. Taken together, our data suggest that in proneural glioblastomas with Ink4a/Arf deficiency, PDGFR-alpha promotes tumorigenesis through the PI3K/Akt/mTOR-mediated pathway regulated by SHP-2 activity. These findings not only functionally validate the genomic analysis of proneural glioblastomas with PDGFR-alpha over-expression but also identify SHP-2 as a potential target for treatments against malignant glioblastomas.

CB-17. TARGETING POLO-LIKE KINASE 1 (PLK1) FOR TREATMENT OF PEDIATRIC BRAIN CANCERS

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Brain tumors are the leading cause of cancer death in children. Pediatric brain cancer is extremely aggressive, is difficult to treat, and has high mortality/morbidity rates. Drug resistance and disease relapse are two major challenges to long-term survival. Thus, there is an immediate need to investigate novel therapeutic strategies that improve patient outcome. In a genome-wide siRNA library screen of 691 kinases, we recently identified polo-like kinase 1 (PLK1) a lead target; silencing PLK1 resulted in 80%-90% growth suppression in pediatric rhabdomyosarcoma cells. Further target validations revealed that PLK1 inhibition by siRNA and small molecular inhibitor BI2636 significantly inhibited cell growth, caused G2/M arrest, and induced apoptosis in pediatric glioblastoma multiforme (GBM) and medulloblastoma cell lines ($IC_{50} = \sim 5$ nM). Of note, PLK1 inhibition is cytotoxic to the GBM cell line SF188, which is notably TMZ-resistant, suggesting that this kinase may be a potential molecular target for the treatment of TMZ-refractory brain tumors. Further, we are examining the possibility that PLK1 inhibition has the potential to eliminate brain tumor-initiating cells (BTICs). We have preliminary results indicating that the level of activated PLK1 is much higher in neurospheres than in cells in monolayer. Importantly, BI2536 inhibited neurosphere growth by as much as 90% in 7-day cultures, where the number and size of the spheres were affected. Given that clinical trials have already begun to address the promise of BI2536 in adult cancers, we propose that it may also be considered for the treatment of pediatric brain tumors, particularly for those that are TMZ-resistant.

CB-18. EFFECTS OF BONE ENVIRONMENT ON MENINGIOMA BIOLOGY

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PURPOSE: Meningiomas are considered benign tumors, and in the majority of cases, complete surgical resection will be curative. However, invasion of the adjacent bone is a known risk factor for recurrence. We hypothesized that the bone environment contributes to a more aggressive behavior in meningiomas. We therefore exposed meningioma cells cultured from resected tumor tissue to bone-specific factors and observed the cells' growth rate, bone invasiveness, and susceptibility to hydroxyurea (HU) treatment. METHODS: Meningioma cell culture was established from 21 surgical meningioma specimens. The purity of the cultures was defined by immunofluorescent staining for epithelial membrane antigen (EMA), vimentin, and GFAP. The cells were treated with IGF I IGFII at a concentration of 100 ng/mL, TGF beta 1 and TGF beta 2 at 10 ng/mL, and extracellular calcium concentration of 2, 4, and 8 mmol/L. Cell growth rate was analyzed by a colorimetric cell proliferation assay. Bone invasion was analyzed by co-cultivation of meningioma cells with neonatal mouse calvariae. The invading cells were visualized with immunofluorescent staining for human HLA and quantified by cell count. HU treatment was performed at 100 μmol/L, Apoptotic cell death was analyzed via TUNEL assay. RESULTS: Immunofluorescent staining showed a 98% purity of the cultures. Treatment with increasing extracellular calcium concentrations and IGF I and IGFII induced a significant increase in cell proliferation, whereas TGF beta 1 and TGF beta 2 had no proliferative effects. Bone invasion was significantly enhanced in all treatment groups compared with controls. All bone-specific growth factors significantly diminished the induction of apoptotic cell death by HU. CONCLUSION: Our data show that that the bone environment can induce cell proliferation, enhanced bone invasion, and resistance to HU treatment in meningioma cells. This suggests that the bone matrix provides a permissive environment for a more aggressive biological phenotype in meningiomas.

CB-19. EGFRVIII PROMOTES GLIOMA INVASION THROUGH SRC-DEPENDENT PHOSPHORYLATION OF DOCK180Y722 AND STIMULATES RAC1 ACTIVITIES

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Acquisition of insidiously invasive behaviors by malignant glioblastoma cells involves the aberrant activations of signal pathways via multiple genetic alterations, including mutation of the epidermal growth factor receptor (EGFR). EGFRvIII, a constitutively activated EGFR mutant, is the most commonly occurring and amplified mutant form of EGFR in glioblastomas. We recently reported that Dock180 and ELMO1, a bipartite Rac1 guanidine nucleotide exchange factor (GEF), promotes glioma cell invasion. We identify a regulatory mechanism by which EGFRvIII promotes glioma cell invasion through phosphorylation of Dock180-stimulating Rac1 activities. Exogenously expressed EGFRvIII significantly promotes SNB19 and LN444 glioma cell migration in vitro and tumor growth and invasion in the brains of animals. EGFRvIII induces an Src-mediated phosphorylation of Dock180 at tyrosine residue Y722 and stimulates Rac1 activities. Inhibition of Src by pharmaceutical inhibitors or a dominant negative Src mutant abrogates EGFRvIII-induced p-Dock180Y722, whereas a constitutively activated Src induces p-Dock180Y722 without EGFRvIII stimulation. In vitro, cellular depletion of Dock180 or expression of a Dock180Y722F mutant inhibits EGFRvIII-promoted glioma cell growth and invasion activating Rac1, whereas wild-type Dock180 markedly enhances EGFRvIII-promoted tumor growth and invasion in the brain. A homologous search revealed that Y722 is highly conserved in Dock180 proteins among various species. Significantly, when primary human glioblastoma specimens were analyzed by immunohistochemical staining using specific antibodies against EGFRvIII and p-Dock180Y722, p-Dock180Y722 was found to be co-over-expressed with EGFRvIII in the invasive areas but not the central regions of glioblastoma specimens. Taken together, these data indicate that EGFRvIII induces glioma cell invasion through Src-dependent p-Dock180Y722 and stimulates Rac1 activity, suggesting that the aberrant activation of p-Dock180Y722-Rac1 could be an alternative therapeutic target in the treatment of glioblastomas.

CB-20. NEDD9 IS REQUIRED FOR MESENCHYMAL INVASION OF GLIOBLASTOMA CELLS

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RATIONALE: Glioblastoma is the most common primary brain tumor, with an average patient survival of 12–15 months. Despite advances in technology and therapies over the last two decades, there has been little improvement in survival, and the final mortality rate remains at almost 100%. Therefore, there is an urgent need to develop new drugs to target specific molecules in signaling pathways that have been implicated in glioblastoma.

Individual glioblastoma cells are able to diffusely infiltrate into surrounding healthy brain tissue, leading to recurrence of the tumor and ultimately death. Thus, targeting migration signaling molecules may prove promising. An exciting candidate molecule is NEDD9 (neural precursor cell expressed, developmentally down-regulated 9). The docking molecule NEDD9/HEF1/Cas-L is an in vivo regulator of cell migration, has been linked to metastasis of melanoma and lung cancer, and has recently been demonstrated to promote migration in glioblastoma cells. OBJECTIVE: The aim of this study was to investigate the role of NEDD9 in glioblastoma cell migration using three-dimensional (3D) culture systems to better recapitulate the in vivo microenvironment. METHODS AND RESULTS: We screened seven human glioblastoma cell lines and observed high-level NEDD9 expression in half the cell lines, correlating with mRNA levels as determined by quantitative real-time polymerase chain reaction (PCR). We also established a 3D-collagen gel model that revealed distinct morphological differences when compared with cells on 2D substrates. Subsequently, siRNA targeting of NEDD9 was shown to cause an ~80% decrease in NEDD9 expression in 3 high-expressing cell lines, and siRNA-mediated down-regulation of NEDD9 significantly inhibited glioblastoma cell migration and invasion in 3D collagen gels. CONCLUSIONS: Our data are the first demonstration that NEDD9 plays a role in the 3D invasion of glioblastoma cells.

CB-21. CIRCULATING TUMOR CELLS (CTCS) IN PATIENTS WITH GLIOBLASTOMA MULTIFORME (GBM)

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INTRODUCTION: Expanding evidence from circulating tumor cells (CTCs) in numerous solid tumors, including glioblastoma multiforme (GBM), has led to techniques that isolate and characterize these cells from peripheral blood. We hypothesized that GBM CTCs would be detectable and distinguishable from normal blood cells using a cluster of differentiation (CD) antibody panel and fluorescence activated cell sorting (FACS). Once isolated, these cells would be characterized for cell signaling and genetic abnormalities. METHODS: Under IRB approval, GBM cell lines were screened with 100 CD antibodies. The antibodies that had positive expression (>90% cells) or low expression (<10% of cells) were then used to screen normal peripheral white blood cells (WBC) from control subjects. The resulting CD antibody panel was then used to detect CTCs in the peripheral blood of GBM patients using FACS. RESULTS: Following the screening process, the CD antibody panel consisted of CD45 (negative in GBM, positive in WBC), CD8 (negative in GBM, positive in WBC), CD81 (positive in GBM, negative in WBC), and CD63 or CD56 (positive in GBM, negative in WBC). Testing the ability of this panel to isolate GBM cells mixed with WBC, a subpopulation (CD8−/CD45−/CD81+/CD56+/CD63+) was found only in the mix of cells and not in the WBC alone. The CD antibody panel was then used to test for CTCs in the peripheral blood of GBM patients. A distinct population of putative GBM cells was identified, representing <1% of the total number of cells. We have used this strategy to isolate CTCs in three GBM patients. These cells have not been seen in six control samples. Currently, additional GBM CTCs are being characterized with cell culture and PCR and correlated clinically. CONCLUSION: GBM CTCs exist and can be isolated and characterized from the peripheral blood of GBM patients.

CB-22. BREAKING HYPOXIA ADAPTATION AND BLOCKING GLIOMA CELL GROWTH BY INHIBITING AMP KINASE ACTIVITY

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

CB-23. EXPRESSION OF SRC FAMILY KINASES IN GLIOMA STEM CELLS AND THEIR ROLE IN CELL PROLIFERATION AND MIGRATION

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Src family kinases (SFKs) are highly expressed and active in clinical glioblastoma multiforme specimens. SFKs inhibitors have been demonstrated to inhibit proliferation, migration, and invasion of primary glioma cells and are currently in clinical trials for the treatment of glioblastoma. However, the expression of SFKs and their functional role in glioma stem cells (GSC) are unclear. We examined the expression pattern of individual members of SFKs in several CD133+ glioma stem cells as well as their corresponding (CD133-) primary glioma cells isolated from the same human glioblastoma xenografts. We found that the SFKs were highly expressed and activated in glioma stem cell lines, and the expression pattern of individual kinase members may or may not have been similar to that of their corresponding primary glioma cells. Members of SFKs expressed in GSC include Fyn, c-Src, c-Yes, Lck, and Lyn. The SFK inhibitor dasatinib had little effect on the growth of glioma stem cells, although it effectively inhibited the proliferation of primary glioma cell lines at a concentration comparable to that of clinical therapeutic serum levels. However, SFK inhibition by dasatinib significantly suppressed the migration of glioma stem cells in a laminin-coated Transwell migration assay. These results suggest that an SFK inhibitor alone is unlikely to completely control tumor growth, and combination with other medications will be needed for the effective inhibition of cancer stem cells. (This work was supported in part by NCI Grants 3P30CA013148-38S5, P50CA097247-05S4.)

CB-24. INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 2 (IGFBP2) PROMOTES GROWTH OF MEDULLOBLASTOMA CELL LINES

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The insulin-like growth factor (IGF) signaling pathway has an important role in proliferation in many tissues and a variety of tumors. The effects of IGF on tumorigenesis are partly modulated by the six secreted IGF-binding proteins (IGFBPs). Circulating IGFBPs can be inhibitory by reducing available free IGFs or may enhance the action of an IGF by increasing ligand presentation to its receptor. Additionally, in some cases, IGFBP2 is also thought to utilize IGF1R-independent mechanisms, although these mechanisms are mostly unknown. Of clinical relevance, IGFBP2 overexpression is observed in many neoplasms and is associated with a more malignant state in prostate cancer, breast cancer, colon cancer, leukemia, and gliomas. In this project, we are working to understand the function of IGFBP2 in medulloblastoma, the most common malignant brain tumor in children. Microarray analysis (U133 plus 2) revealed an 85.8-fold overexpression of IGFBP2 in 31 patient medulloblastoma tissue samples when compared to 13 normal cerebella samples and a mean overexpression of 16.5-fold in four medulloblastoma cell lines. Immunohistochemistry of 7/7 of these samples demonstrated high IGFBP2 expression (nuclear, intra-, and extra-cellular), while in normal cerebellum, IGFBP2 expression was limited to a small population of pericytes. Addition of human recombinant IGFBP2 (1–10 ng/mL, 72 hours) in the presence of 0.5% fetal bovine serum to two medulloblastoma cell lines increased cell number as measured by MTT by 75%–100% for CHLA-259 ($n = 10$ per experiment in two separate experiments with $p < 0.001$ in each) and by 35%–50% for D283MED ($n = 10$ per experiment in three separate experiments with $p < 0.001$). However, IGFBP2 did not affect phosphorylation of two of the IGF-receptor downstream targets, AKT (ser473) and ERK, suggesting possible IGF-independent effect(s) of

IGFBP2 in these medulloblastoma cell lines. Through ongoing work, we are now seeking to understand the mechanism of the growth-promoting effect of IGFBP2 in medulloblastoma cell lines.

CB-25. INVESTIGATING THE ROLE OF NEUROTROPHIN SIGNALING IN BRAIN TUMOR STEM CELLS

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Glioblastoma multiforme (GBM) is a highly invasive disease that is refractory to current treatments. The cancer stem cell hypothesis states that tumor growth is driven and maintained by a rare subset of self-renewing cells that are highly tumorigenic and capable of differentiation. Understanding the biology of these cells could aid the development of therapies designed to eliminate them, either through conventional inhibition of key growth and survival factors or through more novel approaches, such as the promotion of BTSC differentiation. Previous work by this lab has identified and characterized the role of the neurotrophin receptor $p75^{NTR}$ in promoting glioma cell invasion and shown that this function of $p75^{NTR}$ is sensitive to treatment with gamma-secretase inhibitors (GSIs). It was also demonstrated that $p75^{NTR}$ is expressed in brain tumor stem cells (BTSCs). The role of $p75^{NTR}$ and other neurotrophin receptors in BTSCs is being further investigated by means of in vitro and in vivo functional studies (e.g. tumorigenicity, differentiation potential, and invasion) following lentiviral shRNA knockdown of receptor expression, the pharmacological inhibition of receptor function, and the application of relevant receptor ligands. It is hoped that these specific methods of $p75^{NTR}$ manipulation will help discriminate the relative contribution of GSI-mediated targeting of $p75^{NTR}$ in the context of inhibiting other gamma-secretase targets, such as Notch.

CB-26. ANALYSIS OF PHOSPHOTYROSINE SIGNALING IN GLIOBLASTOMA IMPLICATES NUCLEAR EGFRVIII-STAT5B COMPLEX IN THE INDUCTION OF BCL-XL EXPRESSION

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Aberrant EGFR signaling is a major contributing force to glioma progression and treatment resistance. The most prevalent mutation, ΔEGFR/EGFRvIII, is an inframe deletion of the extracellular domain, occurring in the about 40% of glioblastomas and promoting growth and survival of tumor cells. The signaling of ΔEGFR is ligand-independent, does not involve receptor dimerization, and is of low intensity. We have analyzed ΔEGFR signaling using shotgun phosphoproteomics based on recovery of phosphotyrosine-containing peptides and mass spectrometry. Two glioma cell lines expressing ΔEGFR and wild-type EGFR and with different PTEN backgrounds were compared by this approach, leading to the identification of 249 tyrosine phosphorylated proteins. Of these, 30 showed statistically significant differences in intensity when ΔEGFR was present, including the previously described Gab1 and c-Met, and a newly identified phosphorylation of STAT5b on Y699 in cells expressing ΔEGFR. In human glioblastoma samples, pSTAT5 levels correlated positively with EGFR expression and were associated with reduced survival. Phosphorylated STAT5b and ΔEGFR associated in the nucleus, bound DNA, and were found on promoters known to be regulated by STAT5, including that of the Aurora A gene, which they positively regulated. Interestingly, the activation of STAT5b downstream of ΔEGFR was dependent on Src, in contrast to the signal from EGF stimulated EGFR to STAT5b, which showed the involvement of Jak2. ΔEGFR cooperated with STAT5b to positively regulate the Bcl-XL promoter, and knockdown of STAT5b suppressed anchorage- independent growth, reduced the levels of Bcl-XL, and sensitized glioblastoma cells to cisplatin. Taken together, these observations support the conclusion that the nuclear association of ΔEGFR with STAT5b promotes oncogenesis and treatment resistance in glioblastoma via up-regulation of Bcl-XL.

CB-27. FORCED DIMERIZATION AMPLIFIES EGFRVIII SIGNALING AND INCREASES ITS ONCOGENICITY

YeoHyeon Hwang, Vaibhav Chumbalkar, Khatri Latha, and Oliver Bogler; The University of Texas MD Anderson Cancer Center

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

CB-28. ACCESS TO THE NUCLEUS IS REQUIRED FOR THE FULL ONCOGENIC POTENTIAL OF EGFRVIII

Anupama Gururaj and Oliver Bogler; The University of Texas MD Anderson Cancer Center

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

CB-29. PHOSPHOPROTEOMIC ANALYSIS OF NOVEL JAK/STAT INHIBITORS IN GLIOMA CELLS

Vaibhav Chumbalkar, Jaykumar Arumugam, Lixia Dao, Keith Baggerly, Waldemar Priebe, and Oliver Bogler; The University of Texas MD Anderson Cancer Center

INTRODUCTION: Signal transducer and activator of transcription (STAT) molecules are constitutively activated in many cancers, including gliomas. Targeting STATs is a promising therapeutic approach, and WP1066 and WP1193 are small molecule inhibitors of the JAK/STAT pathway that have shown potential in preclinical studies. Here we used phosphotyrosine-directed shotgun phosphoproteomics to profile the impact of WP1066 and WP1193 on glioma cell signaling. APPROACH: LNZ308 glioma cells were treated with WP1066 and WP1193, followed by peptide extraction and enrichment of phosphotyrosine peptides by immunoaffinity using P-TYR-100 antibody. These peptides were further enriched on titanium dioxide resin on a special nano-fluidics chip (Phosphochip, Agilent Technologies) and analyzed on an ETD-enabled ion trap mass spectrometer. After identifying the phosphopeptides with Spectrum Mill software from resultant spectra, these phosphopetides were quantified based on their intensities. Pathway analysis was performed to integrate the findings. RESULTS: We identified and quantified 256 phosphotyrosine peptides from 99 proteins. We observed a significant change in intensity for 20 (for WP1066) and 16 (for WP1193) proteins. As expected, levels of STAT3 phosphopeptide

Y705 were reduced in drug-treated samples. We also observed an increase in ERK2 phosphorylation, which was confirmed by Western blot. Proteins whose phosphorylation changed significantly included kinases such as ERK, CDC2, GSK3alpha, HCK-1, FAK, and transporter molecules including Connexin 43 and Solute carrier family 38 proteins. SIGNIFICANCE: This analysis confirmed that WP1066 and WP1193 suppress the activity of the known targets, Jaks, in glioma cells. In addition, we found changes in pathways not directly associated with Jak signaling, such as the ERKs, which may help us understand potential failures or limitations of drug therapy. Further investigations of such phosphorylation changes will help us design smart combinations and anticipate and monitor treatment failures.

CB-30. CLEAVAGE OF THE BRAIN-SPECIFIC PROTEIN BREVICAN RELEASES A FRAGMENT THAT PROMOTES EGFR-DEPENDENT GLIOMA CELL MOTILITY

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

CB-31. IDENTIFYING MODIFIER GENES OF MPNSTS IN THE *NF1;P53CIS* MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 1

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The current study aimed to identify modifier genes of malignant peripheral nerve sheath tumors (MPNSTs) in the *Nf1;p53cis* (*NPcis*) mouse model of NF1. Previous studies have shown that the incidence of MPNST development in the *NPcis* mouse model is affected by the parental transmission of the mutant chromosome 11. In this study, microarray analysis was used to examine gene expression differences between MPNST primary tumors derived from *NPcis* mice varying in inheritance of the *NPcis* chromosome from the mother (*NPcis* maternal) or father (*NPcis* paternal). *Grb10* was found to be more highly expressed in *NPcis* maternal MPNSTs. *Zrsr1* was found to be more highly expressed in *NPcis* paternal MPNSTs. qPCR was used to validate both gene expression differences. We chose to focus first on *Grb10* due to its role as a cytoplasmic signaling adapter protein. Fluorescence *in situ* hybridization was used to examine the presence of *Grb10* on chromosome 11. *Grb10* was found to be lost more frequently in *NPcis* paternal MPNST cell lines, potentially contributing to the decrease in *Grb10* gene expression seen in these tumors. *Grb10* is paternally imprinted in the periphery of the mouse; therefore, we examined *Grb10* isoform expression and found paternal and maternal isoforms expressed in the MPNSTs. Due to these results, we are examining whether loss of imprinting is contributing to tumorigenesis. In order to study the function of *Grb10* in vitro, more than 200 clones of *Grb10* isoforms were sequenced from the *NPcis* MPNSTs, and several new isoforms were identified. Finally, we have generated *NPcis;Grb10cis* mutant mice. Preliminary studies indicate that *NPcis;Grb10cis* mice have an increase in tumor volume and a decrease in survival. Additionally *NPcis;Grb10cis* maternal mice have an increase in MPNST incidence. Our studies indicate that *Grb10* is a candidate modifier gene of MPNSTs, acting in a growth-suppressive manner.

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

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

The forkhead box M1 (FoxM1) is a key transcription factor regulating multiple cell cycle-related genes that control G1-S and G2-M phase progression. Our previous studies have shown that FoxM1 is over-expressed in human brain tumors and other solid tumors and is correlated with cancer progression and invasion. Knocking down FoxM1 inhibited cancer cell growth in vitro and brain tumor formation in vivo. However, how FoxM1 is regulated in either normal or malignant cells still is not clear. In this study, our results showed that FoxM1 was up-regulated by heat shock factor 1 (HSF1) during heat shock stress conditions. Knocking down HSF1 with HSF1 siRNA or inhibiting HSF1 with an HSF1 inhibitor abrogated heat shock-induced expression of FoxM1. Similarly, heat shock stress did not induce FoxM1 expression in mouse embryo fibroblast cells with *Hsf1* knockout (MEF *hsf1* $-/-$). Chromatin immuno-precipitation assay and promoter reporter assay confirmed that HSF1 directly binds to the FoxM1 promoter. Furthermore, our results demonstrated that FoxM1 is required for the G2-M phase progression through regulating cyclinB1, CDC25B, and CDC2 in a mild heat shock stress condition while enhancing cell survival during lethal heat shock stress. Finally, immunohistochemical analysis of 34 human glioblastoma specimens also showed a significant correlation between FoxM1 overexpression and elevated HSF1 expression. Our results indicated that FoxM1 is critical for HSF1-mediated heat shock response, which increases cell survival and protects cells from stress-induced cell death.

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

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

BACKGROUND: Malignant gliomas are characterized by extensive hypoxic areas and innate resistance to treatment. Hypoxia-inducible factor HIF 1-α is an indicator of malignant angiogenesis and abnormal proliferation, but less studied is HIF1-α involvement in the metabolic shift to glycolysis required for survival in low-oxygen environments. One of the HIF 1-α regulated genes is the mitochondrial Lon, which plays an important role in mitochondrial bioenergetics and mitochondrial DNA maintenance. Here, we demonstrate that Lon controls metabolic adaptation to hypoxia in glioma cells in direct response to HIF1-α activation. Also, Lon induction is used by glioma cells to increase resistance to radiation and chemotherapy by direct repair of mitochondrial DNA. RESULTS: Two malignant glioma cell lines (D-54-MG and U-251-MG) were exposed to hypoxia, which increased HIF1-α protein levels. The increase of HIF1-α levels was paralleled by a doubling in both the mRNA and the protein Lon levels, while HIF 1-α down-regulation was associated with a brisk decrease of Lon expression. Lon protease up-regulation in D54-MG cells caused increased invasion and resistance to starvation. Treatment with temozolomide (TMZ) led to a four-fold induction of Lon, while Lon down-regulation led to increased sensitivity to TMZ. TMZ-resistant lines D54-TR and U251-TR had twice the level of Lon of their parent TMZ-sensitive lines. Lon protease in the D54-MG cells induced resistance to TMZ at levels similar to that of the resistant line D54-TR. D54 cells with Lon over-expression also had increased resistance to radiation. Both the radiation and TMZ resistance in the D54 MG/Lon over-expression cells was caused by an increase in the repair of the treatment-induced mitochondrial DNA damage. CONCLUSIONS: The data presented show that Lon is one of the principal mediators connecting hypoxia with invasion, resistance to starvation, chemo-resistance, and radio-resistance, supporting our current research of Lon inhibition as a possible therapeutic target in malignant gliomas.

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

Alexander H. Stegh; Northwestern University

Glioblastoma multiforme (GBM) is a highly lethal and neurologically debilitating brain tumor characterized by intense apoptosis resistance and

extensive necrosis. Bcl2L12 (for Bcl2-Like 12) is a cytoplasmic and nuclear protein that is consistently over-expressed in primary GBM and functions to inhibit post-mitochondrial apoptosis signaling at the level of effector caspases by directly binding and blocking caspase-7 maturation (Stegh et al., Genes Dev. 21:98–111) and by up-regulating alpha-B-crystallin expression to inhibit caspase-3 activation (Stegh et al., PNAS 105:10703–10708). In the course of studying Bcl2L12's nuclear functions, we determined that nucleoplasmic Bcl2L12 co-localizes and physically interacts with p53 tumor suppressor protein as evidenced by the capacity of Bcl2L12 to (i) enable bypass of replicative senescence without concomitant loss of p53 or p19Arf, (ii) inhibit p53-dependent DNA damage-induced apoptosis, (iii) abrogate p53 binding to its target gene promoters, and (iv) block endogenous p53-directed transcriptomic changes following genotoxic stress. Correspondingly, TCGA profile and tissue protein analyses of human GBM specimens show significantly lower Bcl2L12 expression in the setting of genetic p53 pathway inactivation. Thus, Bcl2L12 is a multi-functional protein that contributes to intense therapeutic resistance of GBM through its ability to operate on two key nodes of cytoplasmic and nuclear signaling cascades.

CB-35. TUMOR HETEROGENEITY IS AN ACTIVE PROCESS DRIVEN BY A MUTANT EGFR-INDUCED PARACRINE CIRCUIT IN GLIOBLASTOMA

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A powerful oncogenic event in GBM is the amplification and rearrangement of EGFR (EGFRvIII, de2-7EGFR, hereafter ΔEGFR), yet notably, only a minority of tumor cells possess this lesion, while the remainder maintain expression of wild-type EGFR (wtEGFR). This disconnect between tumorigenic potential and the frequencies and proportions of these receptors might arise simply from a stochastic process wherein independent genetic events arise in these rapidly fatal tumors, which never have the time to become homogeneous. Another possibility is that ΔEGFR occurs later in tumor progression, where the minority of cells that express it not only enhance their own intrinsic tumorigenic abilities but also potentiate through a paracrine mechanism the proliferation of neighboring majority cells expressing amplified wtEGFR. To test these hypotheses, we engrafted wtEGFR-expressing glioma cells into mice and examined the ability of various amounts of co-injected ΔEGFR-expressing cells to influence tumorigenic growth. We also examined conditioned media produced from ΔEGFR-expressing cells (ΔEGFR-CM) for its ability to activate signaling pathways in wtEGFR-expressing cells and for the presence of potential paracrine factors. From these experiments we determined that human glioma tissues, glioma cell lines, glioma stem cells, and primary mouse astrocytes that express ΔEGFR each secrete IL-6 and/or LIF cytokines. This then prompts a novel interaction between the receptor that is common to these cytokines, gp130, and wtEGFR in neighboring cells that express amplified levels of EGFR, resulting in co-receptor activation and tumor growth enhancement mediated through the activation of gliomagenic signature molecules Akt, MAPK, and STAT3. siRNA knockdown of IL-6, LIF, or gp130 uncouples this cellular crosstalk and potently attenuates tumor growth enhancement. These findings demonstrate that the heterogeneity that characterizes GBM does not occur stochastically but instead can be an actively maintained feature and illuminates a heterotypic cancer cell interaction of potential therapeutic significance.

CB-36. ALTERED APKC SIGNALING CONTRIBUTES TO GLIOBLASTOMA CELL MIGRATION

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Asymmetry along an apical-basal axis or apical-basal polarity is essential for a number of biological processes, including asymmetric segregation of cell fate and differentiation, regulation of cell-cell adhesion, and vectorial cell migration. These processes are fundamental for organogenesis and embryonic development as well as for tumorigenesis and metastasis. We have demonstrated that atypical protein kinase C (aPKC) provides an instructive signal for apical-basal polarity in NSC during embryonic development of the chick CNS. aPKC is compartmentalized at the apical membrane of neural progenitors, and disrupting the endogenous localization of this kinase results in a loss of apical cell adhesion junctions, increased proliferation of neural progenitors, and abnormal migration of these cells within differentiated cell layers. Can aPKC and apical-basal signaling pathway be also associated with abnormal cell proliferation and invasiveness of central nervous system tumors such as glioblastoma (GBM) We have demonstrated that aPKC staining is significantly enhanced in clinical samples of GBM. aPKC functions downstream of the oncogenic EGFR-Ras-PI3-K cascade in glioma cells. The knockdown of aPKC in GBM-derived cell lines decreases cell migration. Our studies suggest that aPKC, a protein kinase and thus a highly attractive target for rational drug design, may be a novel therapeutic target in GBM.

CB-37. EGFR AS A PREDICTOR OF RELAPSE AND A POTENTIAL THERAPEUTIC TARGET IN RECURRENT DISSEMINATED MYXOPAPILLARY EPENDYMOMA

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INTRODUCTION: Myxopapillary ependymomas (MEPN) are a rare subtype of ependymoma in children, occurring most commonly in the lumbosacral region. Although these tumors are considered Grade I per the World Health organization, having a tendency for slow growth and local recurrence, they are capable of leptomeningeal dissemination. The rarity of these tumors and the unpredictable behavior of relapse have made these tumors a therapeutic challenge. To date, no defined molecular markers have been established at the gene or protein level that could be predictive of disseminated recurrence in MEPN. METHODS: We reviewed medical records and identified seven patients with MEPN from 1995 to 2009. Of these, four had recurrent tumors, and three did not have a recurrence. We looked into protein expression of Mib-1, survivin, and EGFR using immunohistochemistry (IHC) to see whether they could be predictive of relapse. RESULTS: EGFR expression was found to be a likely predictor of relapse. IHC showed strong EGFR protein expression in four patients with recurrent tumors, both at diagnosis and at recurrence. However, the three patients without recurrence did not show any EGFR expression. Mib-1 and survivin expression did not differ between tumors with and without recurrence. CONCLUSION: This small case series demonstrates for the first time that EGFR protein expression by IHC could be a potential biological marker of recurrence and may play an important role in tumorigenesis in MEPN. A large prospective study is warranted to determine whether EGFR protein expression at diagnosis in MEPN is predictive of recurrence, which would help in profiling an optimal follow-up pattern. This study also shows that EGFR could be a potential therapeutic target in these tumors when they relapse, as no standard therapy exists beyond surgery.

CB-38. REVERSAL OF EFFECT OF U87 DERIVED MICRO-VESICLES ON BIOLOGICAL PROCESSES OF GLIOBLASTOMA MULTIFORME

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BACKGROUND: Micro-vesicles, also known as exosomes, are extracellular, membrane-bound vesicles derived from the intralumenal membranes of multivesicular bodies (MVBs) of the endocytic pathway. These MVBs fuse with the plasma membrane, which causes the release of vesicles into the extracellular environment. Various cell types, including tumor cells, have been shown to produce micro-vesicles, which are believed to play a role in signal transduction. Recently, glioblastoma-derived micro-vesicles have been shown to contain proteins and RNA. Since micro-vesicles from different tumor cells appear to be involved in different biological processes, we set out to investigate the influence of U87 micro-vesicles on multiple biological processes. METHODS: First, we isolated (for the first time) micro-vesicles from U87 cells by step-wise centrifugation. Next, we assessed the influence of these vesicles on proliferation, angiogenesis, and migration by WST-1 assay, endothelial cell tubule formation on matrigel, and invasion assays, respectively. After this, U87 cells were treated with interferon beta, and their micro-vesicles were isolated and used to assess differential effects on those biological processes and changes in protein and RNA content. RESULTS: Microvesicles derived from untreated but not from treated U87

cells stimulate growth, angiogenesis, and migration of glioblastoma cells. The contents of micro-vesicles derived from treated and untreated U87 cells show differences, which explain the observed effects. CONCLUSION: U87 micro-vesicles influence the behavior of glioblastoma cells. This can be reversed by treating the cells with interferon beta.

CB-39. STUDYING THE ROLE OF EXOSOMES IN GLIOBLASTOMA TUMOR BIOLOGY

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

CB-40. THE ROLE OF WILM'S TUMOR-1 (WT-1) IN GLIOMA BIOLOGY

Archana Chidambaram, Catherine I. Dumur, Martin Graf, Timothy E. Vanmeter, Helen L. Fillmore, and William C. Broaddus; Virginia Commonwealth University

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

CB-41. AN ANALYSIS OF THE ROLE OF MICRORNAS IN THE PHENOTYPIC EXPRESSION OF ONCOGENIC PDGF SIGNALING IN MALIGNANT GLIOMA

Joachim Silber, Tatsuya Ozawa, Edward Kastenhuber, Hakim Djaballah, Eric C. Holland, and Jason T. Huse; Memorial Sloan-Kettering Cancer Center

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

CB-42. DEVELOPMENTAL PROFILE AND REGULATION OF THE GLYCOLYTIC ENZYME HEXOKINASE 2 IN NORMAL BRAIN AND GLIOBLASTOMA MULTIFORME

Amparo Wolf, Sameer Agnihotri, Diana Munoz, Cynthia Hawkins, and Abbhijit Guha; University of Toronto

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

CB-43. STAT3 INHIBITION ALTERS THE IMMUNE PROFILE IN MURINE GLIOMAS

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Signal transducer and activator of transcription 3 (STAT3) has shown to be constitutively activated in a broad array of human and murine tumors. The role of STAT3 in cellular proliferation, angiogenesis, apoptosis, and migration has been well documented. However, STAT3 activation also induces a procarcinogenic anti-inflammatory microenvironment that antagonizes innate and adaptive antitumor immune responses. We first detected in vitro STAT3 activation on murine glioma cell lines (GL261 and GL26) and in vivo STAT3 activation on paraffin-embedded sections of basal ganglia regions of C57/BL6 mice implanted with those cell lines. We then electroporated GL261 and GL26 to transiently transfect with siRNA

anti-STAT3, resulting in substantial down-regulation of STAT3 by 80%–90% in both cell lines at the mRNA and protein level. In addition, we analyzed the effects of STAT3 suppression by measuring transcription of mRNA for selected cytokines (IFN-beta, IL-6, IL-10) and chemokines (RANTES, IP-10) using qRT-PCR. STAT3 inhibition in murine glioma cell lines resulted in enhanced expression of both pro-inflammatory cytokines and chemokines as compared to untreated controls. We found increased levels of IFN-gamma-inducible protein 10 (IP10) and IFN-beta, decreased levels of IL-6, constant levels of RANTES, and varying levels of IL-10 in both cell lines. These findings are similar to those in murine melanoma (B16) and colon carcinoma (CT26) models. However, subtle differences do exist, revealing that different types of cancers may respond in a unique manner. These results demonstrate the important role that STAT3 activation plays in suppressing the release of inflammatory chemokines and cytokines in a murine glioma model. STAT3 inhibition is a potential target for glioma therapy, as multiple upstream cytoplasmic signaling pathways converge upon it. Future in vivo studies will be conducted to determine whether STAT3 inhibition can reverse tumor-promoting inflammation and generate a potent antitumor response.

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

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

CB-45. DRR–POTENTIAL REGULATOR OF GLIOMA INVASION

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

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

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

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

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Glioblastoma (GBM) is characterized by abnormal activation of receptor tyrosine kinase (RTK) signaling pathways. As GBMs diffusely invade, growth factor availability in the tumor microenvironment could be a critical determinant of RTK signaling pathway activity. Heparan sulfate proteoglycans (HSPGs), a major component of the brain extracellular matrix, regulate the extracellular activity of diverse growth factors. As the sulfation pattern of the HS chains is a major determinant of this activity, we hypothesize that GBMs enzymatically modify HSPGs to promote growth factor signaling. We demonstrate that the extracellular sulfatase SULF2, which acts on HSPGs, is expressed in 50% of primary human GBM and in an orthotopic murine model for high-grade glioma. Knockdown of SULF2 in GBM cells resulted in decreased growth in vitro and in vivo, and revealed a striking SULF2 dependence in the activity of multiple RTKs, including PDGFR-alpha, a major signaling pathway in glioma. Furthermore, tumors generated from *Sulf2*$^{-/-}$ neural progenitor cells were smaller with decreased proliferation, were associated with prolonged survival, and had decreased PDGFR-alpha phosphorylation and decreased downstream MAPK signaling pathway activity. Interestingly, we show that GBM subtypes demonstrate dramatically different SULF2 expression. These data support a key role for SULF2 in an important subset of GBM and identify a potential upstream therapeutic target regulating RTK signaling in GBM.

CB-48. HEDGEHOG-GLI SIGNALING PATHWAY IN GLIOMAS: CORRELATION WITH HISTOLOGY, GENETIC ALTERATIONS, AND EXPRESSION OF STEMNESS MARKERS

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Brain tumors can arise following deregulation of signaling pathways normally activated during brain development. Sonic hedgehog-Gli1 (SHH-Gli1) is one such important pathway whose role has been well established in medulloblastomas; however, the few reports in gliomas have yielded conflicting results. Further, since the SHH-Gli1 pathway plays a critical role in non-neoplastic stem cells, its role in stem-like neoplastic cells needs further evaluation. Hence, in this study, we evaluated 102 gliomas for SHH-Gli pathway activity and correlated with histological type and grade, genetic alterations, and expression of stemness markers NANOG, OCT4, SOX2. The study was performed on 44 grade IV (GBM), 23 grade III (10 AA, 9 AO, 4 AOA), and 35 grade II (20 DA, 9 O, 6 OA) tumors. World Health Organization classification was done and genetic subsets defined based on TP53 mutation, EGFR amplification, and 1p/19q LOH. Real-time polymerase chain reaction was performed for expression of Gli1, SHH, PTCH, NANOG, OCT4, and SOX2. Western blot was done for SHH and Gli1 protein expression. There was inverse correlation of high Gli expression (>1.5) with histological grade (51% grade II, 22% grade III, and 16% grade IV). Two-thirds of GBMs with high Gli showed EGFR amplification versus 25% with low Gli. High Gli1 was associated with expression of SHH and PTCH in 97% and 81% cases, respectively. A significant correlation of high Gli1 with stemness markers was also noted. Thus, 93% of gliomas with high Gli1 had expression of stemness markers versus 38% with low Gli. This study confirms the presence of an active ligand-driven SHH-Gli pathway in a subset of gliomas of all types with inverse correlation to grade. The positive correlation of Gli1 with stemness markers possibly indicates that more differentiated progeny of tumor cells may be reverting to a "stem-like status" by activation of this pathway.

CB-49. MOLECULAR INTERACTION BETWEEN PTEN AND EGFRVIII/SHP-2 ACTIVATION COMPLEX AFFECTS GLIOBLASTOMA SENSITIVITY TO TYROSINE KINASE INHIBITORS (TKIS)

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Co-expression of EGFRvIII and PTEN in a small subset of recurrent glioblastoma tumors has been shown to increase sensitivity to tyrosine kinase inhibitors (TKIs). However, the exact mechanism of EGFRvIII and PTEN interaction in response to TKIs is still unresolved. Our recent work has shown that SHP-2 PTPase is required for EGFRvIII-mediated transformation. The present work was aimed at investigating the molecular interaction between the EGFRvIII/SHP-2 activation complex and PTEN in response to Tarceva treatment. We show that Tarceva treatment abolished EGFRvIII, EGFR, Gab1, SHP-2, and Erk1/2 phosphorylation in LN229.EGFRvIII cells at all time intervals. On the contrary, phosphorylation of EGFRvIII and Erk1/2 in U87MG.EGFRvIII cells was inhibited at early time points but was restored within 2 to 6 hours. Interestingly, phosphorylation of Akt, Gab1, and SHP-2 (Tyr580) was unaffected in U87MG.EGFRvIII cells, but EGFR and SHP-2 (Tyr542) phosphorylation was inhibited in a time-dependent manner. MTT proliferation and soft agar transformation assays demonstrated that U87MG.EGFRvIII cells were resistant to Tarceva treatment when compared to LN229.EGFRvIII cells. Immunofluorescent labeling of U87MG.EGFRvIII cells with an antiphospho-SHP-2 (Tyr542) antibody showed perinuclear localization of SHP-2, whereas LN229.EGFRvIII cells exhibited membrane staining of phosphorylated SHP-2. Interestingly, stable expression of PTEN in U87MG.EGFRvIII cells conferred relocalization of phospho-SHP-2 (Tyr542) to the membrane. Notably, U87MG.EGFRvIII/PTEN clones showed a partially untransformed phenotype. Furthermore, phosphorylation of SHP-2 (Tyr580) and Erk1/2 was totally abolished in U87MG.EGFRvIII/PTEN subclones. Our data indicate that the expression of PTEN in U87MG.EGFRvIII cells conferred a phenotype similar to LN229.EGFRvIII cells. Collectively, these observations allow us to infer that SHP-2 is a downstream effector of PTEN and that PTEN deficiency may lead to SHP-2 activation and perinuclear localization by EGFRvIII, which may result in increased resistance to TKIs. Future studies will be aimed at further understanding the role of SHP-2 activation and translocation in glioblastoma response to TKI treatment.

CB-50. DIFFERENTIAL EXPRESSION OF FULL-LENGTH AND TRUNCATED NEUROKININ 1 RECEPTORS IN GLIOBLASTOMA CELLS

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Neurokinin 1 receptor (NK1R) is a G protein-coupled receptor that mediates the effects of neuropeptide substance P. In 1995, NK1R was implicated in glioblastomas (GBM) when Henning et al. (1) showed that NK1R is expressed in 9 of 12 astrocytomas and 10 of 10 GBM and that the expression of NK1R correlates with degree of malignancy, with GBM expressing more receptors than grade I-III astrocytomas. At the molecular level, NK1R stimulation in U373 MG human GBM cells increases mitogenesis and cell proliferation, release of interleukin-6, activation of NF-kappaB, and transactivation of epidermal growth factor receptor (EGFR). A recent study from our laboratory further implicates NK1R by demonstrating that NK1R stimulation leads to an increase in phosphorylation and activity of Akt (2). Our study also demonstrated the presence of a constitutively active form of NK1R in GBM cells (2). We now show that the constitutively active form of NK1R arises from the truncated variant of NK1R. Further, we show that the expression of the truncated form of NK1R varies in different GBM cells. The truncated form differs from full-length NK1R in that it lacks a carboxyl tail. Apparently, the truncated NK1R is an oncogene: a study published in 2005 demonstrated that the expression of truncated NK1R but not full-length NK1R in normal breast cells resulted in a transformed phenotype (3). Taken together, these studies suggest that targeting NK1R either alone or in combination with targets such as EGFR and IGFR-1 is a potentially useful strategy for inhibiting the growth of GBM.

References: 1. Hennig et al., Int J Cancer, 1995; 61: 786–92. 2. Akazawa et al., J. Neurochem. 2009; 109: 1079–86. 3. Patel et al., Proc Natl Acad Sci U S A, 2005; 102:17436–41.

CB-51. THE USEFULNESS OF MS-MLPA FOR DETECTION OF MGMT PROMOTER METHYLATION IN THE EVALUATION OF PSEUDOPROGRESSION IN GLIOBLASTOMA PATIENTS

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Pseudoprogression is a major diagnostic dilemma in the current treatment protocols for malignant gliomas that involve concurrent chemoradiotherapy. We hypothesized that methylation-specific multiplex ligation probe amplification (MS-MLPA), an assay that permits semiquantitative evaluation of promoter methylation, may be used to diagnose pseudoprogression based on the quantification of the methylation status of the O^6-methylguanine DNA methyltransferase (MGMT) promoter. We examined the methylation ratio and copy number variation of the MGMT promoter and mismatch repair (MMR) genes with MS-MLPA in 24 samples from glioblastoma patients. The results were compared to those from methylation-specific polymerase chain reaction (MSP), and protein levels were confirmed by immunohistochemical (IHC) staining. We then evaluated the correlation between those molecular signatures and clinical outcomes. With regard to radiological progression after chemoradiotherapy, the sensitivity and specificity of the MS-MLPA were 100% and 75%, respectively. These results are better than those obtained with MSP (using cut-off values of 0.2 for the methylation ratio and 0.8 for the copy number ratio) for the identification of pseudoprogression. The results of MS-MLPA evaluation of MGMT methylation correlated well with those from MSP and IHC staining, but there was no significant correlation between MSP and IHC staining. MMR genes had little variability in promoter methylation, and their protein products demonstrated homogeneous tissue expression. We conclude that MS-MLPA is a useful method for the early detection of pseudoprogression in glioblastoma patients.

CB-52. A COMBINATORIAL ZINC-FINGER LIBRARY APPROACH TO EXPLORING TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA

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Recent therapeutic advances have improved control of glioblastomas, with a significant fraction of tumors responding to initial therapies. Unfortunately, all glioblastomas recur and lead to patient death. In an

effort to better understand therapeutic resistance, we have explored high-dimensional profiling studies to understand mechanisms of therapeutic resistance and to identify novel therapeutic targets. Temozolomide (TMZ) is an alkylating chemotherapeutic agent that is used to treat all glioblastomas. Recurrent glioblastomas, however, become resistant to TMZ through a mechanism associated with inactivation of MSH6, a member of the mismatch repair gene family; MSH6-deficient glioblastomas grow more rapidly during TMZ therapy. To explore pathways of therapeutic resistance, we have designed an unbiased drug resistance selection screen to create TMZ-resistant human glioblastoma cell lines using combinatorial zinc-finger transcription factors (ZTFs). The combinatorial ZTF library enables activation of a gene or a set of genes that will be selected for resistance to TMZ. We have isolated resistant clones in the TMZ-sensitive A172 cell line and present a molecular profile analysis of these clones. By comparative gene expression profile analysis of novel ZTF-induced, TMZ-resistant glioblastoma cell lines, we expect to identify molecular pathways that confer TMZ resistance. In doing so, we also aim to identify possible biomarkers for TMZ resistance in recurrent glioblastomas.

CB-53. DRR PROMOTES CELL INVASION BY REGULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION

Alix Dudley; McGill University

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

CB-54. BENEFICIAL GROWTH EFFECTS OF ACYL-COA SYNTHETASE VL3 KNOCKDOWN IN HUMAN GLIOBLASTOMA CELLS ARE NOT MEDIATED BY INCREASED AUTOPHAGY OR APOPTOSIS

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

CB-55. BIOLOGICAL BASIS FOR MULTITARGETED THERAPY IN MENINGIOMA PATIENTS

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

CB-56. DELETION OF THE SPARC ACIDIC DOMAIN OR EGF-LIKE MODULE REDUCES SPARC-INDUCED MIGRATION IN GLIOMA CELLS

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

CB-58. THE JAK2 INHIBITOR AZD1480 EFFECTIVELY BLOCKS JAK2/STAT-3 SIGNALING IN GLIOBLASTOMA TUMOR CELLS AND HUMAN XENOGRAFT GLIOBLASTOMA TUMORS

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The JAK/STAT pathway is an important signaling pathway that has been implicated in glioma progression. We have previously shown that STAT-3 is

elevated and persistently activated in human glioma samples compared to control tissues. In addition, this correlates with elevated levels of many STAT-3 target genes, which promote tumor progression. To develop a therapeutic strategy to inhibit STAT-3 signaling, we have evaluated the effects of AZD1480, a pharmacological inhibitor of JAK2. It has previously been shown that AZD1480 effectively inhibits the growth of multiple tumor types, but the effect on glioblastoma tumors has not been reported. In this study, the efficacy of AZD1480 was tested in two human glioma cell lines, U251-MG and U87-MG, as well as a mouse glioma cell line, 4C8. AZD1480 effectively blocks constitutive and stimulus-induced JAK2 and STAT-3 phosphorylation in both human and mouse glioma cells. AZD1480 also decreases cell viability of U251-MG and U87-MG cells as measured by the MTT assay. Furthermore, we used human xenograft samples as models for the study of STAT-3 signaling in vivo. Unlike human glioma cell lines passaged in vitro, human glioma samples that have been propagated as xenografts in nude mice retain both the hallmark genetic alterations and the invasive phenotype seen in vivo. In these tumors, we show that STAT-3 is constitutively phosphorylated, but the levels vary among tumors, which is consistent with human tumor samples. We found that AZD1480 inhibits the phosphorylation of STAT-3 in these xenograft tumor samples. However, more studies need to be done to validate these results and demonstrate the potential inhibition of tumor growth in vivo. Our results suggest that pharmacological inhibition of the JAK2/STAT-3 pathway by AZD1480 reveals the potential for therapeutic inhibition in malignant gliomas.

CB-59. THE PRO-CELL DEATH BCL-2 FAMILY MEMBER BNIP3 PROMOTES TUMOR CELL SURVIVAL IN GLIOBLASTOMA MULTIFORME (GBM) THROUGH THE TRAIL/DEATH RECEPTOR PATHWAY

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BNIP3 is a hypoxia-inducible pro-cell death member of the Bcl-2 family. BNIP3 is activated by the transcription factor HIF-1-alpha and mediates cell death in a caspase-independent manner. Glioblastoma (GBM) is resistant to most treatments. Response to therapy fails, in part, due to tumor hypoxia facilitating resistance to radiation and chemotherapy. BNIP3 is expressed in hypoxic regions of GBMs, but paradoxically, high BNIP3 expression does not lead to cell death. BNIP3 is primarily located in the nucleus of most GBMs, and that nuclear BNIP3 does not induce cell death in glioma cells. These observations led to the hypotheses that BNIP3-induced cell death is negatively regulated in brain tumors by nuclear localization of BNIP3. We have found that BNIP3 plays a novel role in the nucleus of glial cells by binding to a consensus sequence (CAGCCA) in the promoter regions of genes involved in induction of cell death and silencing of these genes. Previously, we determined that BNIP3 binds to the promoter of the apoptosis-inducing factor (AIF) gene. BNIP3 recruits a repressor complex to the AIF promoter, facilitating repression of transcription. Microarray analysis of glioma cells differentially expressing nuclear BNIP3 indicated that nuclear BNIP3 may down-regulate members of the TRAIL apoptotic pathway, such as DR5 (death receptor 5). We confirmed that nuclear BNIP3 down-regulates DR5 expression in glioma cells, subsequently blocking TRAIL-induced cell death. In addition, expression of nuclear BNIP3 in primary GBM tumors correlates with decreased DR5 expression. Pull-down assays confirmed that the BNIP3 protein binds to a consensus binding site in the DR5 promoter. This study provides evidence for a novel mechanism by which nuclear BNIP3 is selected for in GBM because its cell death function is impeded and its transcriptional repression function of genes such as DR5 is enhanced, thereby conferring a survival advantage to the tumor cells.

CB-60. MICRORNA-125B (MIRNA-125B; CHR 11Q24; CHR 21Q21) FUNCTIONS IN THE PROLIFERATION OF HUMAN ASTROGLIAL CELLS

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MicroRNAs (miRNAs) are post-transcriptional modulators of gene expression that regulate the stability and translation of their target messenger RNAs (mRNAs). Here we report that the levels of a human brain-enriched miRNA-125b are up-regulated in cultured human glioma cells and in interleukin-6 (IL-6)-stressed normal human astroglial (NHA) cells, the latter a treatment known to cause the proliferation of astroglial cells. An anti-miRNA-125b (AM-125b) added exogenously to IL-6-stressed NHA cultures attenuated astroglial cell proliferation and increased the expression of the cyclin-dependent kinase inhibitor 2A (CDKN2A), a known negative regulator of cell growth. With a modified gel shift assay, the 3′-untranslated region (3′-UTR) of CDKN2A was shown to strongly interact with miRNA-125b (calculated free energy of association -26.2 kcal/mole). A strong positive correlation between the astroglial cell markers glial fibrillary acidic protein (GFAP) and vimentin, miRNA-125b abundance, and CDKN2A down-regulation was noted in biopsied human glioma and glioblastoma and in several additional neurological disorders associated with astroglial cell proliferation. These results suggest that miRNA-125b contributes to the cyclin-dependent proliferation of astroglial cells and that anti-micro RNA strategies may be clinically useful in the treatment of astroglial proliferative disease. *(Support: Translational Research Initiative [LSUHSC-NO] and an Alzheimer Association IIRG Award [WJL].)*

CB-61. NF1 DEFICIENCY CONTRIBUTES TO GROWTH OF GLIOBLASTOMA CELLS AND CONFERS SENSITIVITY TO MEK INHIBITION BY PD0325901

Wendy See and Russell Pieper; UCSF

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

CB-62. DRUG SENSITIVITIES IN MOLECULAR SUBGROUPS OF GBM-DERIVED BRAIN TUMOR STEM CELLS

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Brain tumor stem cells (BTSCs) have been identified as primordial tumor cells that may initiate disease and tumor recurrence as well as confer resistance to existing treatment modalities. We have successfully isolated and propagated a large array of BTSC lines from glioblastoma multiforme (GBM) that display the fundamental cancer stem cell properties of clonogenic self-renewal, multi-lineage differentiation and aggressive tumor-initiating capacity. The frequent alteration of EGFR and PTEN in GBM suggests that these two genes are integral to the molecular pathology of a subset of GBMs and their BTSCs. We have identified subgroups with different combinations of EGFR and PTEN mutations within our BTSC lines. Here we investigate whether these subgroups display differences in growth characteristics, in vitro signaling, and drug sensitivities. Our preliminary results indicate that these mutational combinations impact diverse intracellular signaling pathways downstream of EGFR and PTEN. Furthermore, the specific subgroups have varying responses to the inhibition of single classical pathway components. We demonstrate that combinatorial inhibition of various pathway targets (EGFR, PI3K, Ras/MAPK, and PKC), predicted by the mutational status and signaling signatures of each subgroup, results in highly effective inhibition of BTSC growth in vitro. Therapies that target alterations specific to tumor cells, especially tumor-initiating BTSCs, hold the promise of providing efficacious treatment with

decreased toxicity to normal tissues. We plan to further investigate the application of targeted drug combinations in orthotopic xenograft mouse models.

CB-63. ABERRATIONS IN HIPPO SIGNALING IN GBMS—A POSSIBLE CONNECTION TO MESENCHYMAL PHENOTYPE

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INTRODUCTION: Developmentally, Hippo pathway controls organ size by regulating cell proliferation and apoptosis. Upon activation of this pathway, a signaling cascade ensues that results in the inactivation of YAP, a transcriptional co-activator of this pathway. Over-expression of YAP has been implicated in several cancers. Research indicates that changes in Hippo signaling induces epithelial to mesenchymal transformation, promoting a more aggressive tumor. Recent studies identified a molecular subclass of glioblastomas (GBMs) with a mesenchymal phenotype that is synergistically driven by STAT3 and C/EBP-beta. Thus, we hypothesize that possible aberrations in Hippo signaling may give rise to this aggressive subclass of GBMs. METHODS: Western blot, immunocytochemistry, qRT-PCR, Kaplan-Meier analysis. RESULTS: Supporting possible aberrant Hippo signaling, 78% of GBMs exhibit over-expression of YAP, which correlates with poor patient survival ($p \leq 0.05$). Furthermore, we demonstrate YAP nuclear localization and activation of downstream targets, including Mcl-1 and CTGF. In addition, 87% of GBMs that exhibit a mesenchymal phenotype show increased expression of YAP. Thus, a high correlation ($p \leq 0.05$) is observed between YAP over-expression and co-expression of STAT3 and C/EBP-beta. Contrary to previous reports, expression of merlin, a negative regulator of YAP, is lost in only 21% of GBMs. Thus, other upstream regulators, such as FAT3, must be investigated to account for increased YAP expression in GBMs. Based on Kaplan-Meier analysis, GBMs with 2-fold down-regulation of FAT3 are associated with poor clinical outcome ($p \leq 0.05$). Interestingly, YAP is over-expressed in some gliosarcomas and metastatic tumors to the brain. CONCLUSION: We show pronounced expression of YAP in GBMs, gliosarcomas, and metastatic tumors to the CNS. Furthermore, we demonstrate that in addition to merlin, down-regulation of FAT3 may account for this ectopic YAP expression. Moreover, this aberrant expression promotes a mesenchymal phenotype. Understanding the role of Hippo pathway in these tumors will have important implications in the management of these malignant diseases.

CB-64. ABERRANT LOCALIZATION OF THE GUANINE NUCLEOTIDE EXCHANGE FACTOR, ECT2: IMPLICATIONS FOR INVASION AND MIGRATION

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Despite concerted efforts in the field of neuro-oncology, malignant gliomas remain a treatment challenge even with the best medical and surgical management. We have found the cytokinetic protein ECT2 to be elevated in gliomas in a progression- and prognosis-dependent manner. In contrast to normal human astrocytes, which maintain nuclear ECT2 expression, primary human GBM and GBM cell lines exhibit expression of ECT2 in the cytoplasm and the leading edge of migrating cells. This ectopic expression results in activation of the pro-migratory small cytoskeletal GTPases RAC1 and CDC42. Using a mouse xenograft model, we implanted glioma cells expressing an inducible shRNA to ECT2. Mice with diminished ECT2 expression, as compared with controls, had a dramatic increase in survival and a decreased incidence of tumor formation. Only 6/15 of ECT2 experimental mice formed tumors, despite evidence of implantation of human cells, whereas all controls (25/25) formed tumors. Interestingly, staining of normal glial and neuronal progenitors also revealed a cytoplasmic expression pattern of ECT2, suggesting that in gliomas, ECT2 re-acquires a progenitor phenotype. Using mass spectrometry to discover candidate proteins that may be responsible for ECT2 cytoplasmic localization, we have identified the RNA processing/cytoplasmic RNA granule protein HnRNPA2/B1 and the polarity protein ZO-1 as novel ECT2 interactors. The potential functional relevance of these interactions in the context of cytoplasmic ECT2 are currently being explored to reveal a novel regulatory paradigm of migration and invasion in gliomas.

CB-65. SONIC HEDGEHOG ACTIVITY IS REQUIRED FOR CXCR4 SIGNALING IN CEREBELLAR GRANULE PRECURSOR NEURONS AND MEDULLOBLASTOMA

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Intracellular signaling mediated by the chemokine CXCL12 and its G-protein coupled receptor CXCR4 plays a crucial role in central nervous system development as well as the genesis of the most common malignant brain tumor of childhood, medulloblastoma. In each case, CXCL12 works together with sonic hedgehog (Shh) to regulate proliferation. Evidence in the literature suggests that CXCL12 modulates Shh-induced cerebellar granule neuron precursor cell (GNP) proliferation through the inhibition of intracellular cAMP levels. The aim of the current study was to determine whether activation of the Shh pathway can reciprocally regulate CXCR4 expression and/or function. Notably, previous work from our lab has demonstrated potential cross-talk between CXCR4 and other signaling systems in the brain, such as opioids (μ opioid receptor) and neurofibromin (NF). The effect of Shh on CXCR4 function was examined in GNPs as well as in Daoy medulloblastoma cells, which exhibit constitutive activation of the Shh pathway. We found that in GNPs, Shh can strengthen coupling of CXCR4 to activation of Gi and its downstream pathways, including CXCL12-induced cAMP suppression, calcium flux, and ERK/Akt activation. Interestingly, in the Daoy cells, treatment with the Shh inhibitor cyclopamine blocked CXCR4-induced Gαi activation and downstream signaling. In either case, changes in CXCR4 activity required long-term (12 hours) treatment with either Shh or cyclopamine and involved alterations in the cell surface levels of the receptor. Overall, this study demonstrates a potential positive feedback mechanism whereby Shh enhances surface levels and hence overall signaling through CXCR4, which in turn amplifies Shh function through suppression of cAMP. Such interactions could result in novel signaling functions relevant to the regulation of proliferation, survival, and cellular migration in those circumstances during normal development and tumorigenesis when these pathways are co-activated.

CB-66. RESISTANCE TO EGFR INHIBITION VIA THE EMERGENCE OF EGFRVIII-INDEPENDENT TUMOR GROWTH-PROMOTING MECHANISMS IN GLIOBLASTOMA

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The exact mechanisms of resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) remain largely unknown in glioblastoma (GBM), despite the fact that amplification of EGFR and/or the expression of constitutively active EGFRvIII are among the most frequent molecular alterations in this disease. Recently, we showed that U373 cells expressing doxycycline (dox)-repressible EGFRvIII were dependent upon expression of this receptor for tumor initiation and maintenance in a xenograft mouse model. EGFRvIII silencing caused an extended period of growth stasis, after which some tumors regained the ability to grow rapidly, in striking similarity to the clinical scenario of resistance. These breakthrough tumors, termed "escapers," remained silenced for EGFRvIII and employed distinctly different signaling pathways when collectively compared to EGFRvIII-dependent tumors. Escapers exhibited elevated activity of the MAPK pathway, and cells derived from the tumors were more sensitive to Mek inhibition than cells from EGFRvIII-dependent tumors. Re-expression of EGFRvIII in escapers markedly suppressed tumor growth, suggesting that the unique signaling preferences of escapers are not compatible with those pathways preferentially utilized by EGFRvIII. Genes were identified by microarray analysis that were specifically expressed at higher levels in the escapers. These genes represent candidates for involvement in the process of overcoming EGFRvIII dependence and thus may contribute to resistance to EGFR inhibition. Significantly, escaper cells were resistant to the in vitro growth-inhibitory effects of the TKI gefitinib as measured by colony formation in soft agar. Thus, the emergence of EGFRvIII-independent tumor growth-promoting mechanisms in escapers is characterized by a switch in pathway signaling preferences and the up-regulation of novel genes and confers resistance to EGFR inhibition. The genes and pathways identified may shed light on novel mechanisms of TKI resistance and point to potential targets for the development of agents that could be used in combination with TKIs to circumvent resistance while improving clinical response.

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

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Resident progenitor cells of the adult white matter are a potential source of primary glial tumors of the forebrain. The adult human brain contains a population of glial progenitor cells that can be isolated on the basis of ganglioside epitopes recognized by the A2B5 antibody (Nature Med 9:239, 2003; Ann Neurol 59:763, 2006). In this study, we used A2B5-based selection to isolate a population of tumor-initiating progenitor cells from human gliomas and then used a substractive genomic strategy to identify the transcriptional events associated with their oncogenesis. A2B5+ cells were abundant in human gliomas at all stages of tumor progression. Glioblastoma-derived A2B5+ cells demonstrated self-renewal and multilineage differentiation potential in vitro and were tumorigenic after transplantation into the brain of immunodeficient mice in vivo. We further compared the gene expression profiles of A2B5+ cells isolated from low-grade ($n = 10$) and high-grade gliomas ($n = 10$) to those of their non-neoplastic adult A2B5+ counterparts ($n = 8$). While most of the genes that were differentially expressed by at least 3-fold (1% FDR) by glioma-derived A2B5+ tumor cells varied as a function of tumor stage, our analysis identified a discrete cohort that was differentially expressed at all stages of gliomagenesis as well as a select group that was differentially regulated in low-grade gliomas. Real-time qPCR and immunolabeling confirmed the differential expression of these genes. Pathway analysis revealed a major dysregulation of the TGF-beta and Wnt/beta-catenin pathways, suggesting a key role of these pathways in the transformation of glial progenitors as well as in the pathogenesis of both low- and high-grade gliomas. By comparing the gene expression profiles of glial tumor progenitor-like cells isolated from low- and high-grade gliomas to their non-neoplastic homologues, we have identified a discrete set of genes and pathways by which glial tumorigenesis may be both better understood and more efficiently targeted.

CB-68. ELUCIDATION OF THE RELEASE AND FUNCTION OF MONOMERIC EPHRINA1 IN GLIOBLASTOMA MULTIFORME

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INTRODUCTION: A monomeric functional form of ephrinA1 is released by proteolytic cleavage from glioblastoma multiforme (GBM) cells. Activation of the EphA2 receptor by ephrinA1 leads to a decrease in the oncogenic properties of GBM cells. However, the exact mechanism of release of ephrinA1 from the cell membrane is unknown, as are the exact downstream effects of EphA2 receptor activation in GBM by both the soluble and membrane-bound forms of ephrinA1. METHODS: We used broad-spectrum inhibitors of serine proteases and matrix metalloproteinases (MMP) as well as specific inhibitors of MMPs in addition to generating ephrinA1 mutants interfering with the potential mechanism of its release. Specific recombinant MMPs were also used to treat ephrinA1-transfected U-251 MG GBM cells and to treat a recombinant ephrinA1 protein to determine which MMPs could lead to the cleavage of ephrinA1. Additionally, a phosphorylation antibody array was used to identify candidates mediating the effects of ephrinA1 on EphA2-expressing GBM cells. RESULTS: GM-6001, an MMP inhibitor, and AEBSF, a serine protease inhibitor, prominently decreased the release of ephrinA1 from GBM cells. An ephrinA1 mutant that is truncated upstream of the membrane anchorage site did not demonstrate any decrease in the release from GBM cells. Additionally, ephrinA1 treatment (10 minutes) caused the phosphorylation of genes such as Fgr, a negative regulator of cell migration and adhesion, and FRK, a positive regulator of PTEN. EphrinA1 also led to an increase in the phosphorylation of FGRFs. CONCLUSIONS: The proteolytic cleavage releasing ephrinA1 takes place in response to not only an MMP but also to serine protease(s), and the exact nature of the proteases involved is under further investigation. Moreover, monomeric ephrinA1 is able to induce significant changes downstream of EphA2 activation within the GBM cell that could be involved in the overall tumor-suppressing properties of ephrinA1.

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

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Autophagy is a cellular stress response that protects cells from harmful conditions. Emerging evidence suggests that this cellular process is also a tumor suppressor pathway. Previous studies showed that cyclin-dependent kinase inhibitors (CDKIs) induce autophagy. Whether retinoblastoma protein (RB), a key tumor suppressor and downstream target of CDKIs, induces autophagy is not clear. Here we first demonstrate that RB triggers autophagy in human sarcoma osteogenic (Saos-2) cells, hepatoma (Hep3B) cells, and brain tumor stem cells (MDNSC23), as indicated by accumulation of LC3B, a punctate pattern of GFP-LC3 cellular localization, and the double-membrane trimmed vacuole formation in the cytoplasm shown by transmission electron microscopy. Autophagy flux study with double-labeled EGFP-mRFP-LC3 fusion protein reveals that RB induces complete maturation of autophagosome into autolysosome. In addition, RB activators p16INK4a and p27/kip1 induce autophagy in RB-expressing sarcoma osteogenic (U2OS) cells and malignant glioma (U-87 MG) cells in an RB-dependent manner. Furthermore, RB with deletions in the E2F1 binding region fails to induce autophagy, and overexpression of E2F1 antagonizes RB-induced autophagy, leading to apoptosis. Consistently, down-regulation of E2F1 in U-87 MG cells results in high levels of autophagy. Overall, our data reveal that RB induces autophagy by repressing E2F1 activity. We speculate that this newly discovered aspect of RB function provides one of the mechanisms by which RB acts as a tumor suppressor in cancer development, and resistance to cancer therapy is correlated with RB status.

CB-70. TNF-ALPHA-INDUCED CELL DEATH OF PRIMARY HUMAN BRAIN MICROVESSEL ENDOTHELIAL CELLS: ROLE OF P38 MAP KINASE AND REGULATION BY THE MATRIX/INTEGRINS

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TNF-alpha induces the death of primary human brain microvessel endothelial cells (MvEC) in a dose-dependent manner through TNF-R1 activation. To determine whether TNF-R1 could be therapeutically manipulated on tumor-associated endothelial cells in glioblastoma tumors, we evaluated the expression of TNF-R1 on the endothelial cells in 31 human glioblastoma tumor biopsy samples and 30 normal brain samples by immunohistochemistry. We found a significantly increased expression of TNF-R1 and TNF-alpha on the endothelial cells in the tumor biopsies as compared to the normal brain samples. We then investigated the regulation of TNF-R1 pro-apoptotic signaling in normal brain MvEC and found that the extracellular matrix (ECM) protein that the MvEC are adherent to or the integrin receptor that is engaged regulates the pro-death signal on TNF-alpha stimulation. TNF-alpha stimulation readily induced cell death of normal brain MvEC plated on collagen, but a significantly reduced amount of cell death was seen when normal brain MvEC were plated on vitronectin. We investigated whether different downstream signals were activated when the normal brain MvEC were plated on the different matrices and stimulated with TNF-alpha and found a time-dependent phosphorylation of p38 MAP kinase in the cells plated on collagen, suggesting that p38 MAP kinase is a downstream effector for TNF-alpha-induced apoptosis. No phosphorylation of p38 MAP kinase was observed in the MvEC plated on vitronectin. To substantiate the clinical relevance of these findings, we examined the effect of TNF-alpha stimulation on endothelial cells isolated from two different glioblastoma tumors and found that TNF-alpha-induced cell death was similarly regulated by the ECM or integrins. Our data suggest that TNF-R1 on tumor-associated endothelial cells can be therapeutically manipulated as part of an antiangiogenic therapy for glioblastoma tumors, and that the effectiveness of such a therapy maybe in part dependent on the activity of specific integrins expressed on the tumor endothelial cells.

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

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The endoplasmic reticulum (ER) is an organelle critically involved in protein folding and lipid and steroid biosynthesis as well as intracellular Ca^{2+} storage in eukaryotic cells. Various physiological or pathological stimuli cause disruption to these physiological functions of the ER, namely ER stress, and unfolded protein response (UPR) is subsequently activated to restore the ER homeostasis. However, prolonged and unresolved ER stress will ultimately lead to autophagy and apoptosis. Induction of ER stress has thus emerged as a new anticancer strategy based on the premises that UPR is often elevated in tumor cells compared normal cells owing to a harsh microenvironment, such as hypoxia, glucose deprivation, or misfolded mutant proteins. Tumor cells will be more sensitive to small-molecule inhibitors that target components of UPR or inducers that "super"-upload UPR. Several studies on ER stress in glioma cells have shown promising results. In this report, we found that ER stress inducers triggered autophagy much more prominently than PI3K/mTOR inhibitors and AMPK activators did in U251MG cells. Interestingly, we also discovered that an exogenous nuclear PTEN tumor suppressor could induce autophagy in U251MG cells independent of its inhibition of mTOR and/or activation of AMPK. Further analyses showed that UPR was enhanced, including up-regulation of BiP/GRP78 and PERK-mediated inaction/phosphorylation of eIEF2. Nuclear PTEN-induced autophagy could be attenuated only by inhibitors of PERK but not GCN2 or PKR. In addition, nuclear PTEN could induce autophagy and suppress self-renewal substantially in several glioma stem cell lines. Whether this is mediated through induction of ER stress requires further characterization. We propose that identification of the molecular targets specifically involved in nuclear PTEN-mediated, ER stress-induced autophagy will provide a new therapeutic regimen for treating GBM more effectively.

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

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Gene expression profiling studies have revealed that the mesenchymal signature (MES) is a predictor of poor survival and treatment resistance in glioblastoma (GBM) patients, whereas patients with a proneural (PN) signature perform better in the clinic. Using bioinformatic approaches as well as experimental testing of glioma stem cells (GSCs) and murine primary neural stem cells (NSCs), we have found that the transcription co-factor WWTR1/TAZ acts as a master regulator of PN to MES differentiation in GBM. TAZ is epigenetically silenced in lower-grade/PN tumors, whereas its promoter is hypomethylated in GBM/MES tumors. Treatment with a demethylation agent (2'-deoxy-5-azacytidine) induced dramatic increases in TAZ mRNA levels. Silencing TAZ in GSCs decreased invasion and expression of multiple MES genes and blocked tumorigenesis when implanted intracranially into mice. Conversely, over-expression of TAZ in GSCs that lack this protein increased MES gene expression and suppressed PN gene expression. Notably, these cells showed increased invasion, differentiation toward an osteogenic lineage, and tumorigenesis. In primary NSCs, over-expressing TAZ increased expression of fibronectin and smooth muscle actin, both mesenchymal markers, whereas astrocytic or neuronal differentiation was suppressed in response to serum. All aforementioned phenotypes were abolished when over-expressing a single point mutant version of TAZ (S51A) that lacks binding to its bona fide transcription partner, TEAD. Intriguingly, when tested in chromatin immunoprecipitation experiments, both PN and MES genes were direct targets of the TAZ-TEAD4 complex. Currently, we are testing whether this complex delineates specific epigenetic modifications at target gene loci, directly up-regulating MES genes while down-regulating PN genes, by recruiting histone modifiers to their promoters. Our studies have identified TAZ as an important master regulator of PN to MES transition in gliomas and as an attractive therapeutic target.

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

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Translation initiation is a highly regulated step in protein synthesis. Inhibition of translation initiation impedes cell growth and proliferation. Several translation factors are implicated in tumorigenesis. We recently showed that DHX29 is a novel translation initiation factor required for tumorigenesis. DHX29 is a member of the DEAD/DExH-box protein family. It functions as a helicase and stimulates translation initiation of mRNAs with a structured 5′ untranslated region (5′UTR). The vast majority of mammalian mRNAs contain some form of secondary structures at their 5″UTR and as such might be a target of DHX29 regulation. We found that DHX29 depletion impedes cancer cell growth in culture and in xenografts. We queried the ONCOMINE™ database and found that DHX29 mRNA is significantly over-expressed in brain cancers. We validated the data by showing that DHX29 protein is differentially over-expressed in various brain cancer cells as assessed by immunoblotting. Depletion of DHX29 from U87, U118, U251, U343, and U373 glioma cells resulted in a substantial decrease in cell proliferation rate and cell viability. DHX29 silencing caused a cell cycle arrest in U87 and U251 cells, with an increased number of cells in the subG0 phase. DHX29 silencing caused a dramatic decrease in the number of colonies formed by the U87 glioma cell line as assessed by a focus formation assay. This indicates that depletion of DHX29 leads to a reduction of the malignant and proliferative properties of the brain cancer cells and that silencing of DHX29 is sufficient to impair tumorigenicity. Therefore, translational inhibition through DHX29 depletion or pharmacologic targeting might represent a novel means by which tumorigenesis can be repressed.

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

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The malignant progression of many tumors, including malignant gliomas, involves the loss of the p14ARF tumor suppressor gene. This genetic alteration occurs with the transition to high-grade glioma and precedes the associated pathological features of intravascular thrombosis, the formation of hypoxic regions and dramatically increased neovascularization. The mechanisms underlying and possibly connecting these biological features are poorly understood. The tissue factor pathway inhibitor-2 (TFPI2) is an extracellular serine protease inhibitor that prevents initial blood coagulation reactions. Given the high frequency of ARF gene deletions and the decreased expression of TFPI2 with astrocytomas progression, ARF loss might be one of the genetic events that dysregulates TFPI2 and promotes plasma clotting and vascular thrombosis/hypoxia within gliomas. To examine novel functions of ARF, we expressed the ARF gene in ARF-deficient glioma cells and examined the expression level of TFPI2. Pharmacological inhibitors, RNA interference, and CHIP assays were used to examine ARF effects on TFPI2 transcription and plasma clotting triggered by gliomas. Our findings show that ARF up-regulates TFPI2 at the transcriptional level and significantly reduces the ability of glioma cells to promote plasma clotting as demonstrated by the increase in coagulation time of plasma in contact with ARF-induced versus ARF-uninduced glioma cells. ARF increases c-jun phosphorylation as an immediate response to JNK activation and subsequently the binding of the AP-1 transcription factor to its specific sites located on the TFPI2 gene promoter. Furthermore, this new ARF's signaling axis is p53-independent. In summary, these data present the first evidence of p14 ARF-mediated genetic control of plasma coagulation, initiated by glioma cells through AP1-mediated activation of TFPI2 expression, and suggest that therapies directed toward restoring ARF activity, TFPI2 expression, or TFPI2 activity could reverse the intratumoral thrombotic cascade that may initiate hypoxia-driven malignant progression.

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

George B. Twitty, Susan E. Nozell, Suk W. Hong, and Etty N. Benveniste; UAB

Glioblastomas (GBMs) are among the most common and deadly tumors of the central nervous system. NF-kappa-B and STAT3 are transcription factors that regulate the expression of genes that promote cell proliferation, apoptotic resistance, and angiogenesis. In gliomas, both NF-kappa-B and STAT3 are constitutively activated and may contribute to the processes of gliomagenesis. While the precise mechanisms underlying constitutive NF-kappa-B and STAT3 activation in GBMs are largely undefined, there are numerous proteins and pathways dysregulated in GBMs that may cause NF-kappa-B activation. Also, NF-kappa-B can induce the expression of IL-6, an activator of STAT3. Therefore, we hypothesize that in gliomas, the aberrant activation of NF-kappa-B may lead to and promote STAT3 signaling. To test this hypothesis, we evaluated human glioma cells grown in the absence or

presence of TNF-alpha, which activates NF-kappa-B. We found that NF-kappa-B was activated and correlated with enhanced IL-6 mRNA and protein expression, followed by STAT3 activation and the expression of *SOCS-3*, a STAT3-dependent gene. Using cell lines that inducibly down-regulate endogenous NF-kappa-B activity, we determined that the levels of *IL-6* and *SOCS-3* were significantly reduced in the absence of NF-kappa-B. These data suggest that NF-kappa-B, via the production of IL-6, can activate the STAT3 signaling pathway. Moreover, in gliomas, the dysregulation of NF-kappa-B signaling may promote STAT3 activation and signaling. Therefore, this potential crosstalk between the NF-kappa-B and STAT3 pathways may promote cellular processes that lead to the development and/or progression of gliomas and thus may represent important targets for therapeutic interventions.

CB-76. RASGRP3 INTERACTS WITH THE ACTIN-RELATED PROTEIN ARP3 AND REGULATES THE MIGRATION OF GLIOMA CELLS

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Gliomas, the most frequent primary brain tumor, are characterized by increased invasion into the surrounding normal brain tissue. Signaling pathways coupled to DAG production are highly active in glioma cells, mainly downstream of the growth factor receptors EGFR and PDGFR. Similarly, Ras proteins are activated in gliomas and play a role in their malignant phenotype. Although gliomas express high Ras activity, Ras-activating mutations have not been identified in these tumors. RasGRP represents a new family of GEFs that belong to the DAG/phorbol ester receptor family and that act as Ras activators by promoting the acquisition of GTP to maintain the active GTP-bound form. We found that RasGRP3 regulates glioma cell migration and invasion and that it activates the Ras and Rap1 proteins in these cells. In addition, RasGRP3 activates the Erk and AKT pathways, and AKT is partially involved in the effect of RasGRP3 on glioma cell migration. To further delineate the molecular mechanisms underlying RasGRP3 effects, we performed a pull-down assay followed by mass spectroscopy and identified the actin-related protein, Arp3, as a novel interacting protein of RasGRP3. Using immunofluorescence staining and co-immunoprecipitation, we validated the interaction of RasGRP3 and Arp3. We further found that PMA, which induces the translocation of RasGRP3 to the peri-nuclear region, increased the association of RasGRP3 and Arp3. Moreover, silencing of Arp3 modulated the subcellular localization of RasGRP3 in control and PMA-treated cells and partially decreased the effect of RasGRP3 on the spreading and migration of glioma cells. These results implicate RasGRP3 as an important regulator of glioma cell migration and indicate that RasGRP3 acts in a Ras/AKT-dependent and Ras-independent, Arp3-dependent manner to regulate this process. RasGRP3 protein is an important link between the DAG, Ras signaling pathways and actin polymerization and may represent an important therapeutic target in the treatment of gliomas.

CB-77. RAL SIGNALING IN MEDULLOBLASTOMA: BIOLOGICAL AND THERAPEUTIC OUTCOMES

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Medulloblastoma is one of the most common malignant central nervous system tumors in children. Treatment is often associated with untoward long-term effects, and new targeted therapies are needed for this devastating tumor. Ras, one of the most important proto-oncogenes involved in human cancers, has been shown to be involved in the development of neurological malignancies. We studied Ral (Ras-like) activation as a novel downstream effector of Ras, with a goal of targeting this pathway as a potential therapeutic strategy. Affinity precipitation analysis of active RalA (RalA-GTP) in eight medulloblastoma cell lines revealed the majority have some level of RalA-GTP. Six cell lines (DAOY, RES256, RES262, UW228-1, UW426, and UW473) had significantly higher RalA-GTP than the other two (D283 and D425). To further evaluate the Ral pathway, we investigated the levels of RalBP1 and PP2A.In this scenario, RalBP1 was of special interest owing to its reported effects of conferring chemotherapy resistance in other malignancies. RalBP1 was expressed in all eight medulloblastoma cell lines. PP2A is a negative regulator of Ral, but no inverse correlation was observed in our analysis. Further analysis of the Ral pathway revealed strong expression of phospho-aurora kinase, an activator of Ral. We then selected DAOY as a model cell line for further evaluation of the outcome of inhibiting Ral expression. Using a lentivirus expressing anti-RalA shRNA, we successfully inhibited Ral expression as compared to a negative control virus. Upon treatment, we observed a greater than 65% reduction in proliferation by day three post-infection. We concluded that high levels of active RalA are needed for cell survival in the DAOY cell line and may represent a new therapeutic target for medulloblastoma. Our future work will focus on the evaluation of the effects of inhibition of Ral signaling on the invasiveness and in vivo tumorigenicity of medulloblastoma cells.

CB-78. THE ING4 TUMOR SUPPRESSOR INDIRECTLY SUPPRESSES STAT3 SIGNALING BY REDUCING IL-6 EXPRESSION

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ING4 is a tumor suppressor that is absent or mutated in gliomas. Previously, we showed that ING4 attenuates the transcriptional activity of NF-kappa-B. STAT3 is a transcription factor activated by IL-6. Both NF-kappa-B and STAT3 regulate the expression of genes that promote cell proliferation, apoptotic resistance, and angiogenesis. In gliomas, both NF-kappa-B and STAT3 are constitutively activated and may contribute to gliomagenesis. Because ING4 can attenuate the expression of NF-kappa-B regulated genes, we hypothesized ING4 would indirectly inhibit the activation and/or transcriptional activity of STAT3 by reducing the levels of IL-6, an NF-kappa-B regulated gene. To test this hypothesis, we evaluated human glioma cells grown in the absence or presence of TNF-alpha, which activates NF-kappa-B. We found that NF-kappa-B was activated within 15 minutes and IL-6 mRNA and protein were expressed within 30 minutes and 1 hour, respectively, of TNF-\alpha stimulation. STAT3 was activated and coincided with the expression of SOCS-3, a STAT3-dependent gene, at 2 hours post-TNF-alpha. Moreover, we determined that the levels of IL-6 and SOCS-3 were significantly reduced in the absence of NF-kappa-B p65. These data suggest that NF-kappa-B, via the production of IL-6, can activate the STAT3 signaling pathway. Next, we assessed the impact of ING4 on STAT3 signaling using U251-TR/F-ING4 cells, which inducibly express exogenous ING4 expression in an ING4-null background. In these cells, TNF-alpha induced NF-kappa-B activation, and IL-6 and SOCS-3 mRNA expression in the absence of ING4. However, the levels of IL-6 and SOCS-3 were significantly reduced when ING4 was co-expressed in these cells. Together, these data indicate that ING4 attenuates NF-kappa-B activity and reduces the levels of IL-6, and thus ING4 indirectly inhibits the activity of STAT3. Therefore, the loss of ING4 expression/activity in gliomas may explain why both the NF-kappa-B and STAT3 signaling pathways are constitutively activated.

CB-80. DIVERSITY OF ENGRAFTMENT BEHAVIORS AMONG CD133-EXPRESSING PATIENT-DERIVED BRAIN TUMOR CELL LINES

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

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

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

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

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Medulloblastoma (MB) is a primitive neuroectodermal tumor of the cerebellum. HGF/cMET signaling plays a role in cerebellar development, and the overactivation of this pathway has been implicated in several human malignancies. Our genome-wide epigenetic screens on human MB cell lines and primary tumor specimens have identified SPINT2/HAI-2, an HGF/cMET signaling inhibitor, as a novel tumor suppressor gene that is frequently silenced by promoter hypermethylation in MB. Furthermore, the aberrant activation of the HGF receptor cMET tyrosine kinase has been associated with the pathogenesis of MB. To determine whether mutation contributes to aberrant HGF/cMET signaling in MB, we will perform a mutational analysis of *SPINT1*, *SPINT2*, and *cMET* in a large cohort of primary human MB specimens. This can provide direct genetic evidence implicating HGF/cMET signaling in MB tumorigenesis. Furthermore, to assess the role of aberrant HGF/cMET signaling on cerebellar development and MB pathogenesis, we will construct a transgenic mouse overexpressing a mutant, constitutively active form of cMET specifically in the granule precursor cells of the developing cerebellum, which are the suspected cells of origin for MB. Mice will be examined at various ages to characterize the effects of upregulated cMET activity on cerebellar development and will be observed for evidence of tumor formation. This work will help provide greater insight into the involvement of HGF/cMET signaling in the genesis of MB and will provide a valuable preclinical model for testing the existing and novel HGF/cMET targeted antitumor agents.

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

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

CB-84. PDGF-DRIVEN GLIOMA MODEL REVEALS THAT ENVIRONMENTALLY TARGETED THERAPIES MAY BE MORE EFFECTIVE AT ALTERING RECURRENT DISEASE KINETICS

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Glioblastoma multiforme is widely noted for its heterogeneous cellular make-up, with greater heterogeneity correlated with higher levels of biological aggressiveness. To better understand this correlation, we examined the microenvironmental relationships between cell populations in a mathematical model for a platelet-derived growth factor (PDGF)-driven experimental glioma model. Through the creation and parametrization of a mathematical model of this experimental rodent model, we examined the effect of PDGF signaling between three cell populations within the tumor: glial progenitor cells that are infected with PDGF-expressing retrovirus, glial progenitors that are not infected, and other normally present cells in the brain. By changing cell numbers or PDGF levels after the tumor has reached a magnetic resonance imaging (MRI)-detectable size in simulation, we examined the effects of treatment on tumor recurrence patterns. Pre-operatively, our model shows that tumors with high levels of recruitment are faster growing; post-operatively, however, these tumors recur more quickly and continue a growth pattern quantitatively similar to the pre-operative pattern. Blocking recruitment as treatment leads to a delay in tumor growth and also to recurrence with a pattern of radial growth that is slower than pre-treatment growth. Thus, the mathematical model for the PDGF tumor ecology suggests traditional therapies such as resection that target nodular growth do not significantly affect the tumor phenotype (in this mathematical experimental model) that will appear on recurrence, whereas therapies that target the tumor ecology (e.g., paracrine signaling via small molecular inhibitors like bevacizumab) may affect the pattern of tumor growth upon recurrence. This is consistent with recent reports that GBM patients treated with bevacizumab have recurrent tumors that exhibit a more diffuse growth pattern.

EPIDEMIOLOGY

EP-01. RETROSPECTIVE STUDY OF THE GEOGRAPHIC EFFECTS ON SURVIVABILITY OF POST-OPERATIVE MALIGNANT PRIMARY CNS TUMORS REPORTED IN THE SEER DATABASE

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OBJECTIVE: Our objective was to explore the geographic effects on the postoperative survivability of primary malignant central nervous system (CNS) tumors reported in the Surveillance, Epidemiology, and End Results (SEER) database. METHODS: Using the SEER*Stat software available at www.seer.cancer.gov/seerstat version 6.6.1 and the Incidence - SEER 17 Regs Limited-Use + Hurricane Katrina Impacted Louisiana Cases, Nov 2008 Sub (1973–2006 varying) database, survival rates of 22,380 patients diagnosed with a malignant primary CNS tumor and who underwent