Ikaros, a DNA-binding protein also known as Ikaros family zinc finger protein 1 (KLF1), was initially shown to function critically in hematopoietic differentiation. Deletion or mutation of this gene has been associated with lymphoblastic, as well as acute and chronic myelogenous leukemias demonstrating that Ikaros can act as a tumor suppressor. Ikaros is predominantly expressed in a variety of tissues including the brain, suggesting that Ikaros may be involved in physiological functions of glial growth. Other recent studies have shown that Ikaros can regulate the transition of neural progenitor cells to postmitotic neurons which eventually differentiate into glial cells. Deregulation of glial development and/or growth has been shown to initiate glioma (GBM) growth suggesting that Ikaros may be involved in GBM tumorigenesis. The goal of this study was to determine if Ikaros is involved in glial tumorigenesis. Analysis using the TCGA and Rembrandt databases demonstrated that decreased expression of Ikaros leads to worse prognosis in GBM patients (P < 0.01). In addition, the Ikaros promoter was found to be hypermethylated (85% of samples) in GBM samples and that inhibition of Ikaros may contribute to glial tumorigenesis. To determine if Ikaros is involved in glial tumorigenesis we generated stable GBM cell lines that were transduced with HA-Ikaros. Enhanced expression of Ikaros resulted in a 3- to 4-fold reduction in proliferation and a 2- to 3-fold reduction in colony formation. In addition, exogenous expression of Ikaros inhibited the migration of GBM cells suggesting that Ikaros may also be involved in the invasiveness of glial cancers. Together our data suggests that Ikaros may play an important role in suppressing glial tumor formation and may be a novel biomarker for GBMs.

**CB-004. TUMOR MIGRATION OF HUMAN GlioBLASTOMA IS MODULATED BY THE MOLECULE CD90 (Thy-1)**

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The molecule CD90 is a N-glycosylated, glycoprophosphatidylinositol anchored cell surface protein, originally described on thymocytes. CD90 has been considered as a surrogate marker for a variety of stem cells and has recently been reported on glioblastoma stem cells. CD90 is also expressed on lymphocytes, endothelial cells, fibroblasts and neurons. The function of CD90 is not fully elucidated. CD90 has been involved in cell-cell and cell-matrix interactions, in neurite outgrowth, T cell activation and apoptosis. In this study, we confirmed the expression of CD90 on human glioblastoma-solvent-like cells from serum-free neurosphere cultures. We also observed RNA and protein CD90 expression on primary cell lines from FSC-containing culture (adherent cell lines) and on freshly prepared glioblastoma specimen. In order to study the function of CD90 on glioblastoma cells, we used a silencing strategy to decrease the expression of CD90 on the immortalized U251 cell line. We then compared the viability, the tumor growth and the migration property of the wild-type CD90+ U251 cells and CD90 down-regulated U251 clones. The decrease of CD90 expression did not affect the viability and the tumor growth of U251 cells. In contrast, down-regulation of CD90 mediated the decreased ability of tumor cell migration using both scratch wound healing and boyle chamber migration assays. Experiments are currently on going to test the effect of CD90 expression on tumorigenicity in mice models. In total, this study might lead to better understand the role of CD90 on the pathology in particular in term of tumor migration/invasion of human glioblastoma.

**CB-005. ALK ALTERATIONS IN A SERIES OF 62 GliOBLASTOMA CASES: IS THERE A THERAPEUTIC ROLE FOR ALK INHIBITORS MOLECULES?**

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**BACKGROUND:** In the last years structural and point mutations involving ALK have been described in a number of different human cancer types. These alterations have constituted the basis for the potential use of ALK inhibitor molecules as targeted therapy in some non-small cell lung cancer, lymphoma...
Glomas are the most frequently occurring primary malignancies in the central nervous system, and glioblastoma (GBM) is the most common and aggressive of these tumors. Protein kinase CK2 is a serine/threonine kinase composed of two catalytic subunits (alpha and/or alpha') and two beta regulatory subunits, which phosphorylate over 300 substrates. CK2 is a key suppressor of apoptosis, promotes angiogenesis, and enhances activation of the NF-kappaB, PI3K/AKT, Wnt and Notch signaling pathways. Elevated CK2 expression/activity has been reported in many tumors, including GBM, and it has been suggested that CK2 is essential for cancer cell survival. With the recently discovered that CK2 is a novel interaction partner of the Janus Kinases JAK1 and JAK2, and is required for activation of the JAK/STAT-3 pathway. Aberrant activation of signaling pathways including NF-kappaB, PI3K/AKT and JAK/STAT-3, has been implicated in cancer cell survival. With the recent understanding of the biological nature of these tumors. Recent important developments include the discovery of microRNAs (miRs) and exosomes and their role in tumor microenvironment. MicroRNAs (miRs) are small, single stranded, non-coding RNAs that act as key regulators of gene expression, typically by reducing translation of target mRNAs with partial complementarity and function of CK2 in the context of this cancer. Analysis of 537 GBMs from the Cancer Genome Atlas project identified the CK2A2A1 gene, encoding for CK2alpha, as frequently amplified in GBM (33.7%), which is significantly associated with the classical subtype of GBM. Inactivation of CK2 activity by pharmacological inhibitors (CX-4945) or knockdown of CK2 expression suppresses activation of the JAK/STAT, NF-kappaB and AKT pathways in primary human GBM xenograft cells. CK2 inhibitors decrease the adhesion and migration of GBM cells, in part through inhibition of intercellular beta and integrin alpha4 expression. CK2 inhibitors also suppress growth, colony formation and cell cycle progression of GBM cells, and induce apoptosis of these cells. In vivo, CX-4945 significantly inhibits tumor growth and promotional of survival in GBM xenograft models. These results provide a basis for clinical development of CK2 inhibitors as a potential therapeutic option for GB patients.

CB-006. TARGETING THE PROTEIN KINASE CK2 SUPPRESSES PRO-SURVIVAL SIGNALING PATHWAYS AND GROWTH OF GliOBLASTOMA

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Protein kinase CK2 is a serine/threonine kinase composed of two catalytic subunits (alpha and/or alpha) and two beta regulatory subunits, which phosphorylate over 300 substrates. CK2 is a key suppressor of apoptosis, promotes angiogenesis, and enhances activation of the NF-kappaB, PI3K/AKT, Wnt and Notch signaling pathways. Elevated CK2 expression/activity has been reported in many tumors, including GBM, and it has been suggested that CK2 is essential for cancer cell survival. With the recently discovered that CK2 is a novel interaction partner of the Janus Kinases JAK1 and JAK2, and is required for activation of the JAK/STAT-3 pathway. Aberrant activation of signaling pathways including NF-kappaB, PI3K/AKT and JAK/STAT-3, has been implicated in cancer cell survival. With the recent understanding of the biological nature of these tumors. Recent important developments include the discovery of microRNAs (miRs) and exosomes and their role in tumor microenvironment. MicroRNAs (miRs) are small, single stranded, non-coding RNAs that act as key regulators of gene expression, typically by reducing translation of target mRNAs with partial complementarity and function of CK2 in the context of this cancer. Analysis of 537 GBMs from the Cancer Genome Atlas project identified the CK2A2A1 gene, encoding for CK2alpha, as frequently amplified in GBM (33.7%), which is significantly associated with the classical subtype of GBM. Inactivation of CK2 activity by pharmacological inhibitors (CX-4945) or knockdown of CK2 expression suppresses activation of the JAK/STAT, NF-kappaB and AKT pathways in primary human GBM xenograft cells. CK2 inhibitors decrease the adhesion and migration of GBM cells, in part through inhibition of intercellular beta and integrin alpha4 expression. CK2 inhibitors also suppress growth, colony formation and cell cycle progression of GBM cells, and induce apoptosis of these cells. In vivo, CX-4945 significantly inhibits tumor growth and promotional of survival in GBM xenograft models. These results provide a basis for clinical development of CK2 inhibitors as a potential therapeutic option for GB patients.

CB-007. FGFR4 INHIBITION IMPACTS ON GliOBLASTOMA AGGRESSIVENESS IN VITRO AND IN VIVO

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Fibroblast growth factors (FGF) and their high-affinity transmembrane receptors (FGFR1-FGFR4) represent a complex signal network regulating embryonic development and tissue homeostasis. In several cancer types, FGF/FGFR signal loops are deregulated by diverse genomic and epigenetic mechanisms consequently supporting cancer cell proliferation and survival. In case of glioblastoma, most cell lines (N = 8) and primary cell cultures from clinical samples (N = 26) we found a widespread expression of several FGFs (like FGF1, FGF2, and FGF5) but also a significant overexpression of FGFR1 and FGFR4. Regarding FGFR1, all glioma cell models investigated additionally expressed the mesenchymal and more oncogenic splice variant FGFR1-IIIC. Consequently, we focused in this study on the consequence of FGFR4 as compared to FGFR1 inhibition on human glioblastoma models in vitro and in vivo. Application of the FGFR inhibitors (SBFI1120, ponatinib) as well as expression of dominant-negative versions of FGFR1 and FGFR4 significantly reduced in vitro cell growth and clonogenicity in the tested glioma cell models whereby dnFGFR1 tended to be more efficient than dnFGFR4. Accordingly, both dominant-negative FGFRs induced significant apoptosis whereby the effects of dnFGFR1 were again significantly stronger. Additionally, neurosphere formation, indicative for the presence of glioma stem cells was profoundly reduced by both dnFGFRs. Interestingly, FGFR4 belonged to those genes significantly overexpressed in the cancer stem cell compartment (N = 16; mRNA expression arrays of neurosphere versus adherent cell culture). Surprisingly, the inhibitory effects on anchorage-independent growth in soft agar were opposite with significant upregulation by dnFGFR1 but almost complete blockade by dnFGFR4 in all glioblastoma models analysed. Accordingly, growth of human glioblastoma xenografts (N = 2) in SCID mice was completely inhibited by dnFGFR4 while only retarded by dnFGFR1. Summarizing our data substantiates a significant contribution of FGF/FGFR-mediated signals to different aspects of glioblastoma aggressiveness and suggests especially FGFR4 as potential target for therapeutic interventions.

CB-008. MicroRNA-1, DOWN-REGULATED IN GLIOMA, MODULATES THE CONTENT OF EXOSOMES RELEASED BY GLIOMA CELLS, MITIGATING THEIR PRO-ONCOCGENIC POTENTIAL

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Glioblastoma multiforme (GBM) is the most common and aggressive intrinsic primary brain tumor in adults. GBM presents unique challenges to therapy due to its location, aggressive biological behavior and diffuse infiltrative growth. Protein kinase CK2 is a serine/threonine kinase composed of two catalytic subunits (alpha and/or alpha') and two beta regulatory subunits, which phosphorylate over 300 substrates. CK2 is a key suppressor of apoptosis, promotes angiogenesis, and enhances activation of the NF-kappaB, PI3K/AKT, Wnt and Notch signaling pathways. Elevated CK2 expression/activity has been reported in many tumors, including GBM, and it has been suggested that CK2 is essential for cancer cell survival. With the recently discovered that CK2 is a novel interaction partner of the Janus Kinases JAK1 and JAK2, and is required for activation of the JAK/STAT-3 pathway. Aberrant activation of signaling pathways including NF-kappaB, PI3K/AKT and JAK/STAT-3, has been implicated in cancer cell survival. With the recent understanding of the biological nature of these tumors. Recent important developments include the discovery of microRNAs (miRs) and exosomes and their link with tumor microenvironment. MicroRNAs (miRs) are small, single stranded, non-coding RNAs that act as key regulators of gene expression, typically by reducing translation of target mRNAs with partial complementarity and function of CK2 in the context of this cancer. Analysis of 537 GBMs from the Cancer Genome Atlas project identified the CK2A2A1 gene, encoding for CK2alpha, as frequently amplified in GBM (33.7%), which is significantly associated with the classical subtype of GBM. Inactivation of CK2 activity by pharmacological inhibitors (CX-4945) or knockdown of CK2 expression suppresses activation of the JAK/STAT, NF-kappaB and AKT pathways in primary human GBM xenograft cells. CK2 inhibitors decrease the adhesion and migration of GBM cells, in part through inhibition of intercellular beta and integrin alpha4 expression. CK2 inhibitors also suppress growth, colony formation and cell cycle progression of GBM cells, and induce apoptosis of these cells. In vivo, CX-4945 significantly inhibits tumor growth and promotional of survival in GBM xenograft models. These results provide a basis for clinical development of CK2 inhibitors as a potential therapeutic option for GB patients.

CB-009. SUBTYPE-SPECIFIC EXPRESSION OF THE TRUNCATED NEUROKININ-1 RECEPTOR IN GliOBLASTOMA MULTIFORME

Kristine Brown,* and Madan Kwatra,‡† Duke University Medical Center, Department of Anesthesiology, Durham, NC, USA; ‡Duke University Medical Center, Department of Pharmacology and Cancer Biology, Durham, NC, USA

Considerable evidence suggests that targeting the neurokinin-1 receptor (NK1R) is a promising approach to inhibit glioblastoma multiforme (GBM). Interestingly, recent data indicate that the truncated variant of NK1R, which ends at amino acid 311 (NK1RΔ311), is oncogenic in breast and colon cell lines. However, the presence and expression pattern of NK1RΔ311 in GBMs have yet to be examined. Therefore, using a quantitative PCR
CB-010. VARIABLE EXPRESSION OF THE TRUNCATED NEUROKININ-1 RECEPTOR IN GliOBLASTOMA MULTIFORME
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While considerable evidence suggests that the neurokinin-1 receptor (NK1R) is a promising target for glioblastoma multiforme (GBM) inhibition, recent data has highlighted the role of a truncated splice variant of the receptor. This variant, which ends at amino acid 311 and is known as NK1R311, has been implicated in both breast and colon cancers. However, the presence and expression pattern of NK1R311 in GBMs have yet to be examined. Using a quantitative PCR approach, we examined the expression of NK1R311 in 25 primary GBMs. We found that NK1R311 is variably expressed in these primary tumors, with 11 samples (44%) exhibiting expression levels higher than a whole brain reference sample. Higher expression of NK1R311 in a large percentage of GBMs, in conjunction with the data on breast and colon cancers, suggests a potentially oncogenic role for the protein in a subset of GBMs. In conclusion, the data presented here provides the first demonstration of the expression of NK1R311 in GBM and underscores a potential role for this form of NK1R in GBM growth.

CB-011. GAMMA-Glutamyltransferase 7 ACTS AS A TUMOR SUPPRESSOR IN GBM AND CORRELATES WITH GOOD PROGNOSIS
Timothy Bui, Ryan Nitta, and Gordon Li; Stanford University, Stanford, CA, USA

Gamma-glutamyl transferase 7 (GGT7) is a part of a family of 13 enzymes that play a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification. The GGT family has been shown to modulate crucial redox sensitive functions such as antioxidant/antioxidative defense and cellular proliferative/apoptotic balance making it an important player in proliferation and cell maintenance. Recent reports demonstrate that the GGT family has a role in tumorigenesis by regulating cell growth, invasion, and drug resistance. GGT7 is a novel GGT family member and it is hypothesized that it plays a role in the regulation of leukotriene synthesis, glutathione metabolism or gamma-glutamyl transfer. Recent reports have demonstrated that glioblastoma (GBM) patients have decreased expression of GGT7, suggesting that loss of GGT7 may enhance glial tumor growth. Analysis of the Rembrandt database demonstrated that GBM patients with lower expression of GGT7 had a worse prognosis (P < 0.05) than patients with higher expression. To determine if GGT7 is involved in GBM tumorigenesis we modulated GGT7 expression in GBM cell lines. We determined that under normal growth conditions exogenous expression of GGT7 resulted in a 2-6 fold decrease in proliferation and 2-3 fold reduction in anchorage independent growth. Surprisingly, we observed a more pronounced reduction in GBM growth under starvation conditions, yielding a 10-fold decrease in proliferation and 3- to 4-fold in anchorage independent growth. Correspondingly, when endogenous GGT7 was reduced 5-fold using short interfering RNA (siRNAs) we found that cell growth was increased 3- to 10 fold and anchorage independent growth 2-3 fold. Together, our study suggests that GGT7 may act as a tumor suppressor in glial tumorigenesis and further analysis will be conducted to determine if GGT7 can be used as a biomarker or therapeutic target for GBM.

CB-012. GENOME-WIDE shRNA SCREEN REVEALED SYNTHETIC LETHAL INTERACTION BETWEEN DOPAMINE RECEPTOR D2 (DRD2) AND EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) INHIBITION IN GliOBLASTOMA
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INTRODUCTION: Targeted therapy in the treatment of glioblastoma has achieved little therapeutic gain, in large part due to the inherently redundant and dynamic molecular circuitry that drives glioblastoma proliferation. The available data suggest that simultaneous inactivation of critical nodes within this network will be required for meaningful clinical efficacy. METHODS: To identify such critical nodes, we conducted a genome-wide shRNA screen in search of gene silencings that exhibit synthetically lethal interactions with EGFR inhibition. RESULTS: The screen revealed that genes required for Dopamine Receptor D2 (DRD2) signaling serve essential functions in glioblastoma cells. Silencing of DRD2 by independent si- and sh-RNA compromised glioblastoma survival. This effect was rescued by expression of an RNAi resistant construct of DRD2 both in vitro and in vivo. DRD2 was over-expressed in human glioblastoma specimens relative to matched normal cortex. Importantly, clinical pathologic correlation revealed that high expression of DRD2 was associated with poor overall survival, supporting the thesis that DRD2 expression enhanced glioblastoma growth. This growth effect of DRD2 was mediated through the inhibitor G protein (GNAi2) to ERK1/2, where DRD2 and EGFR signalling converge. Synergy in tumoral activity was observed when combining haloperidol, a FDA approved drug that functions as an inhibitor of DRD2, and the EGFR inhibitor, AG1478, in both in vitro and in vivo glioblastoma models. CONCLUSION: DRD2 and EGFR signalling constitute two critical nodes in driving glioblastoma proliferation through ERK1/2. Simultaneous inhibition of these pathways through combination of the FDA approved anti-psychotic agent, haloperidol, and EGFR inhibition constitute a sound strategy for glioblastoma therapy.

CB-013. CADHERIN-11 REGULATES MOTILITY IN NORMAL CORTICAL NEURAL Precursors AND GliOBLASTOMA
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Metastasizing tumor cells undergo a transformation that resembles a process in normal development when non-migratory epithelial cells modulate the expression of cytoskeletal and adhesion proteins to promote cell motility. Here we find a mesenchymal cadherin, Cadherin-11 (CDH11), is increased in cells exiting the ventricular zone (VZ) neuroepithelium during normal cerebral cortical development. When overexpressed in cortical progenitors in vivo, CDH11 causes premature exit from the neuroepithelium and increased cell migration. CDH11 expression is elevated in human brain tumors, correlating with higher tumor grade and decreased patient survival. In glioblastoma, CDH11-expressing tumor cells can be found localized near tumor vasculature. Endothelial cells stimulate TGFB signaling and CDH11 expression in glioblastoma cells. TGFB promotes glioblastoma cell motility, and knockdown of CDH11 expression in primary human glioblastoma cells inhibits TGFB-stimulated migration. Together, these findings show that Cadherin-11 can promote cell migration in neural precursors and glioblastoma cells and suggest that endothelial cells increase tumor aggressiveness by co-opting mechanisms that regulate normal neural development.

CB-014. THE COUPLING AND FUNCTION OF ADHESION RECEPTOR GPR124 IN GliOBLASTOMA MULTIFORME
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GPR124 is an orphan receptor that belongs to the Adhesion GPCR family. Members of this family are characterized by long N-terminal segments that contain many of the same functional domains found in cadherins, integrins, and tyrosine kinases, but are typically absent in GPCRs belonging to other families. While little is known about adhesion GPCRs, recent studies show
that GPR124 plays a role in endothelial cell migration and differentiation during angiogenesis. We found that GPR124 mRNA is highly expressed by various human Glioblastoma Multiforme (GBM) cell lines. Due to the high expression of GPR124 in GBM cells and its known role in cellular migration and differentiation, we hypothesized that these orphan receptors control the migration and proliferation of GBM cells through specific signal transduction pathways and thus represent promising new therapeutic targets to treat this cancer. To test our hypothesis, we measured cell migration using a modified Boyden chamber assay that was developed in our laboratory. We found that GPR124 over-expression increases the migration of U87MG cells stimulated by lysophosphatidic acid (LPA) and also increases the rate of proliferation of these cells. Specifically, stable knockdown of GPR124 expression by shRNA in U87 cells reduces their ability to migrate, and reduces their proliferation rate in vitro. To elucidate the signaling pathway involved in GPR124 biology in GBM, we used a proteomics approach to determine the effector proteins that couple to this protein. Myc-tagged GPR124 was heterologously expressed in U87MG and the proteins associated with these receptors were analyzed by Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC) mass spectrometry. We found a concise list of effector proteins that are known to control second messenger signaling and cell migration. Together, our results suggest that GPR124 is expressed by GBM and tightly regulates how these cells proliferate, and migrate.

CB-015. CADHERIN-MEDIATED CHANGES IN RADIOSENSITIVITY AND EXPRESSION OF CD133 AND LEF-1 IN U373 GBMSTOMA CELLS
Christopher P. Cifarelli1 and Robert J. Griffin2; 1University of Arkansas for Medical Sciences, Little Rock, AR, USA

BACKGROUND: Mounting pathological data exist suggesting the importance that cadherins may have in metastatic potential and prognosis of various cancers. With regard to gliomagenesis and progression, there is a paucity of mechanistic data regarding the role of members of the cadherin superfamily. Previously, we have demonstrated that a cadherin-selective adhesion substrate promotes the migratory capacity of high grade glioma cells in culture. Here, we examine the role cadherin-based adhesion and associated Wnt-signaling with respect to radiation (XRT) sensitivity. METHODS: Using the U373 (Upsalla) cells, we exposed cultures to a recombinant cadherin fusion protein, containing the full ectodomain of C-cadherin alone or with XRT. Proliferation and viability were assessed via the MTT assay, while changes in gene expression were analyzed by quantitative PCR. RESULTS: At 24 hours following radiation treatment, cultures treated with high-dose (1 µg/ml) cadherin ectodomain had increased survival compared to no treatment controls (p = 0.05), while at 72 hours, both high-dose and low-dose (100 ng/ml) groups had significant survival advantages over controls (p = 0.02, p = 0.036, respectively). Gene expression data show a two-fold decrease in CD133 expression in the cadherin-ectodomain group with a return to control baseline expression following 4 Gy. Similarly, E-cadherin gene expression was decreased two-fold in the ectodomain-only treated group, with return to baseline following XRT. Beta-catenin expression was constant, while LEF-1 expression was increased 4 Gy. CONCLUSIONS: Cadherin-mediated adhesion is capable of increasing the radioresistance of high grade glioma cells in culture and is associated with a significant decrease in CD133 expression in the absence of radiation. Increased LEF-1 expression following cadherin-ectodomain exposure alone or with radiation, implicates the Wnt signaling pathway. These data support the exploration of Wnt pathway and its components as therapeutic targets for radiosensitization in gliomas.

CB-016. ROLES OF NHE-1 ACTIVATION IN GLOBLASTOMA CANCER CELL MIGRATION AND SURVIVAL IN COMBINATION WITH TEMOZOLOMIDE
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Sodium-hydrogen exchanger isoform 1 (NHE-1) is ubiquitously expressed and regulates intracellular pH (pHi) and extracellular microdomain (pHi) homeostasis. NHE-1 was shown to play an important role in promoting survival and migration/invasion of cancer cells. However, whether NHE-1 activity affects glioma cell migration and survival, and the effects of temozolomide (TMZ) chemotherapy remain unknown. In this study, we determined increased NHE-1 protein expression in primary glioma cell lines (GC#22 and GC#999) compared to control human neural stem cells and astrocytes. NHE-1 protein was localized at the lamellipodia of GC22 via immunofluorescence staining. NHE-1 activity is involved in regulating GC resting pHi. An alkaline pHi (7.39 ± 0.04) was detected in GC cells. Pharmacological inhibition of NHE-1 with HOE 642 acidified cells (7.21 ± 0.06, p < 0.05), GC pHi was also decreased (7.15 ± 0.07, 7.26 ± 0.03, respectively) after 2 or 4 h TMZ (100 µM) treatment. But, NHE-1 blockade with HOE 642 further acidified GC (6.78 ± 0.11, and 6.88 ± 0.03, respectively), suggesting inhibition of NHE-1 function after TMZ treatment. Moreover, GC mobility and migration were increased in the presence of TMZ. HOE 642 significantly decreased the TMZ-associated glioma cell mobility and migration. Most importantly, we found that combined treatment with TMZ and HOE 642 enhanced glioma cell apoptosis (detected by cleaved caspase-3 expression) compared to TMZ treatment alone. Therefore, our study shows that 1) NHE-1 protein is important in maintaining an alkaline resting pHi in glioma cells; and 2) inhibition of NHE-1 activity suppresses glioma cell migration and augments TMZ-induced apoptosis. These findings suggest that a novel strategy of adding NHE-1 blockade may increase the efficacy of TMZ chemotherapy and improve clinical outcomes.

CB-017. pDING ENHANCES TEMOZOLOMIDE-INDUCED GLOBLASTOMA CELL GROWTH INHIBITION VIA miR-21 DOWN REGULATION
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INTRODUCTION: Introduction: pDING is a 38 kDa protein (p38J) derived from the Hypericum perforatum plant and possesses phosphatase activity known to inhibit cell cycle advancement at G1 via a variety of phosphorlated proteins, including ERK1/2 and AKT. We examined the effects of pDING exogenous treatment on Gl1 primary glioblastoma cells, a radioresistant cell line, in culture to evaluate its efficacy as an adjunct to conventional therapy in resistant glioblastoma tumors; we also examined alterations in the miRNA levels of this cell line in order to further elucidate the mode of action for pDING’s antitumor growth activity. METHODS: Gl1 primary glioma cell lines were treated with 20 µM of temozolomide, 0.6 µg/ml of pDING, 25 µJ of UV radiation, and dual and triple combinations of these therapies. Cell counts were tracked to evaluate growth. Fold regulation was calculated using quantitative RT-PCR using miScript miRNA PCR arrays and total RNA isoforms from each sample and from YFP and YFP-p38J transfected Gl1 cells. RESULTS: pDING treatment created 40-50% reductions in Gl1 cell growth in all treatment arms, in agreement with previous reports. pDING treatment increased temozolomide-induced cell growth inhibition by 6% when applied in combination to Gl1 cells. No additive effect was noted with UV radiation, and no sustained growth inhibition was noted with UV radiation alone, indicating that pDING provides some concerted antitumor abilities with alkylating chemotherapy but is insufficient to sensitize radio-resistant glioblastoma cell lines. RT-PCR demonstrated a 2.23 fold down regulation of miR-21 and a 2.21 fold down regulation of miR-125. These miRNA have been associated with AKT upregulation and miR-125 has been implicated to increase GC migration. We propose that pDING-mediated cell cycle inhibition and pro-apoptotic properties may be in part affected through miRNAs down regulation in addition to the direct phosphorylation of cell cycle-related proteins.

CB-018. TRANSCRIPTIONAL REGULATION AT IGF2 PROMOTERS AND MECHANISTIC INSIGHTS ON INDUCTION OF IGF2 DOWNSTREAM OF YAP IN Shh MEDULLOBLASTOMAS
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In mouse models for Shh-medulloblastomas, IGF2 is required for tumor formation, growth, and metastases. We showed that YAP over-expression induces IGF2 expression as a part of YAP’s radiation-resistance program in mouse Shh-medulloblastomas and in cerebellar granule neuron precursors (CGNP). We proposed cells-of-origin for the Shh subclass of medulloblastomas, IGF2 and its regulatory program may represent a therapeutic target in

Abstract
medulloblastoma, but the mechanism of IGF2 induction downstream of YAP is not well understood. The anomalous loss of IGF2 imprinting in the human fetal brain is intriguing and exemplifies the complexity of the IGF2 gene’s regulation. Although CTCF mediates allele-specific expression at the IGF2/H19-imprinted locus in both mice and humans, subsequent evidence suggests that CTCF binding at the IGF2/H19 imprinting control region is insufficient to regulate IGF2/H19 expression in human tissues. This makes a compelling case in favor of studying the transcriptional regulation at the different IGF2 promoters in order to delineate the mechanism of IGF2 induction downstream of YAP. We have employed biotinylated-DNA ‘fishing’ combined with proteomics to delineate the transcriptomes at the IGF2 promoters in medulloblastoma cell lines and directly validated them using medulloblastoma cell-derived transcriptomes. The results of transcriptome analyses revealed factors, Yb1 and Mye2, associated with IGF2 promoter Pr3 in FpZ53 cells (derived from a Ptc+/- /p53 +/- mouse medulloblastoma) and Smao1 tumor tissue. Mye2 was consistent in its association with IGF2 promoter Pr3 in mouse P5 cerebella, as in case of FpZ53 cell line and Smao1 tumor tissue. Of note, we observed increased levels of Mye2 and Yb1 in Shh-treated CGNPs. Our results indicate that association of Yb1 at IGF2 promoter Pr3 could be mediated by the induction of IGF2 downstream of YAP and the radiation-induced expression of IGF2 could be linked to DNA repair mechanisms of Yb1.

CB-019. INSIGHTS INTO THE MOLECULAR MECHANISM OF PID1 GROWTH-INHIBITORY EFFECTS IN GLIOMAS
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Phosphoryroside interaction domain containing 1 (PID1) is a recently identified gene which inhibits insulin-mediated signaling in adipocytes and muscle cells. As we reported at prior meetings, using six independent datasets we found that less favorable medulloblastomas (Group 3, Group 4, Anaplastic) and gliomas (glioblastoma multiforme, GBM) had lower PID1 mRNA compared to more favorable medulloblastomas and gliomas. Moreover, higher PID1 mRNA correlates with better patient outcome both in medulloblastoma and glioma patients. Moreover, PID1 overexpression in brain tumor cell lines exerted growth-inhibitory effect in glioblastoma and embryonal brain tumor cell lines (medulloblastomas, ATRT), suggesting a possible causal role for it in modulating tumor aggressiveness. PID1 harbors a phosphoryrosine binding (PTB) domain, hypothesized to interact with proteins harboring the consensus PTB binding sequence NPXY. A potential binding partner is LRP1 (low-density lipoprotein receptor-like protein 1), a plasma membrane receptor involved in signaling, lipid homeostasis, migration and survival, and clearance of apoptotic cells. LRP1 harbors an NPXY motif in its cytoplasmic tail. We find that immunoprecipitation of native LRP1 in three GBM cell lines using two different antibodies to LRP1 was able to communoprecipitate native PID1. To determine if the PID1 growth-inhibitory effect was indeed mediated via its PTB domain, we generated deletion mutants of PID1 that either lacked the PTB domain or expressed only it. Using colony formation assays we find that while the PID1 mutant harboring the PTB domain indeed mediated growth inhibition, the absence of this domain in other PID1 mutants, the other PID1 caused similar inhibition. Thus, we concluded that PID1 inhibition of glioma and medulloblastoma cells was mediated via several domains in PID1.

CB-020. CDK2-MEDIATED OLG1 PHOSPHORYLATION REPRESSIONS p27 EXPRESSION AND PROMOTES BRAIN TUMOR DEVELOPMENT
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Oligodendrocyte transcription factor 2 (OLG1) is an important proneural factor regulating early neural development and promotes proliferation of neural progenitors/stem cells. However, how OLG12 is regulated, and executing its cellular functions is largely unknown. Here we identified OLG12 as a critical phosphorylation target for cyclin-dependent kinase 2 (CDK2). CDK2 directly interacts with and phosphorylates OLG12 at Ser14. The OLG12 phosphorylation by CDK2 is accompanied with cell cycle progression from late G1 through S phase and is correlated with proliferative marker Ki-67. Phosphorylated Olg1 exerts pro-proliferative effects that are reflected in glioma cells and in murine xenograft models of glioma. We show that bFGF stimulation regulates OLG12 phosphorylation and a decline of OLG12 phosphorylation was observed in differentiating neural progenitors. CDK2-mediated OLG12 phosphorylation stabilizes OLG12 protein from proteasomal degradation. We also propose p27/KIP1 as a potential gene repressed by OLG12. OLG12 phosphorylation is required for OLG12 to bind to the p27 promoter regions to repress p27 transcription thereby promoting cell cycle progression and cell proliferation. Phosphorylated OLG12 binds to the E-Box regions of p27 promoter and represses p27 gene transcription, which in turn activates CDK2 in positive feedback manner. p27 antagonizes Olg2 phosphorylation and inhibits tumor cell proliferation and tumor development. Further, The Cancer Genome Atlas (TGA) data analysis showed analogs that OLG12 co-expresses with CDK3 in GBM subclasses. Importantly, OLG2-high glioma initiating cells are highly sensitive to CDK2 inhibitor treatment, indicating that OLG12 plays an instrumental role in mediating CDK2-regulated glioma initiating cell growth. OLG2 expression levels can be a prognostic biomarker for selection of GBM patients with high OLG2 expression for CDK2 inhibitor treatment. In conclusion, our findings provides a molecular mechanism underlying the regulation of OLG2 and OLG2-regulated brain tumor development, which may improve the efficacy of treatment of GBM patient with personalized therapy by CDK inhibitors.

CB-021. MUTANT EGFGR SUPPRESSION OF microRNA-9 INDUCES FOXP1 TO ENHANCE Glioblastoma tumorigenicity
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Glioblastoma (GBM), the most common primary malignant brain tumor, frequently displays amplification and/or mutation of the epidermal growth factor receptor (EGFR) gene. Highlighting the importance of EGFR in the pathogenesis of GBM, aberrant EGFR signaling is required for GBM maintenance, growth, invasion and resistance to therapeutic agents. We hypothesized that oncogenic pathophysiology exerted by EGFR requires the modulation of microRNA (miR) activity. To test this hypothesis, we compared the miR profiles of GBM cells with activated wild type EGFR (wtEGFR) and mutant EGFR (ΔEGFR) to cells with non-activated wtEGFR or kinase dead ΔEGFR. We observed and validated that ΔEGFR suppresses miR-9. The repression of miR-9 is due to the negative regulation of the primary miR-9 encoding transcript, pri-miR-9-2, by ΔEGFR. Downstream of ΔEGFR, the Ras/PI3K/AKT axis was required to suppress miR-9. We identified the transcription factor, FOXP1, to be a bona-fide miR-9 target. Upregulation of mir-9 decreased expression of FOXP1 in ΔEGFR cells, suggesting that mir-9 and FOXP1 may regulate, in part, ΔEGFR-dependent GBM growth. Consistent with this hypothesis, mir-9 antagonized the tumor growth advantage conferred by ΔEGFR while FOXP1 knock-down inhibited the growth of ΔEGFR-driven tumors. Upregulation of FOXP1, as a consequence of inhibiting miR-9 activity, increased the tumorigenicity of GBM cells, suggesting that miR-9 is a tumor suppressor whereas FOXP1 likely functions as an oncogenic factor in GBM. Finally, high FOXP1 expression was significantly associated with poor survival in GBM patients, further supporting the notion that FOXP1 is an oncogenic factor. Collectively, these data reveal a novel regulatory mechanism by which ΔEGFR suppression of miR-9 upregulates FOXP1 to increase tumorigenicity.

CB-022. EFFECTS OF EGFR SIGNALING ON THE MODE OF DIVISION OF NEURAL PROGENITORS AND GLIOMA PRECURSORS
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Dysregulation of growth factor receptor tyrosine kinase (RTK) signaling pathways is very common in malignant glioma including glioblastoma multiforme (GBM). The intra-tumoral heterogeneity of GBM extends to RTK alterations (Smuder et al, Cancer Cell; 2011; 20; 810-817; Sherer et al, PNAS; 2012; 109; 3041-3046) and provides a rationale for multimodal therapies (Dunn et al, Genes Dev; 2012; 26: 756-784). Recent evidence suggests that oligodendrocyte precursor cells (OPCs) may act as cellular origin of astrocytomas (Chen et al, Cell; 2012; 149; 36-47). How RTK dysregulation and anti-RTK therapies affect non-neoplastic OPCs as well as astrocytoma precursors is not fully understood. OPCs self-renew and generate differentiating oligodendrocytes by undergoing asymmetric divisions. Only recently, we learned that the epidermal growth factor receptor (EGFR) participates in asymmetric OPC division (Sugarto et al, Cancer Cell; 2011; 20; 328-340). Here, we investigated in further detail the molecular and biological changes that occur as non-neoplastic OPCs turn into astrocytoma precursors due to dysregulated RTK
CB-023. ALTERNATIVE SPlicing IN Glioblastoma: A BIG NEW WORLD AHEAD

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Alternative splicing plays a key role in determining tissue-specific differentiation patterns. The emerging evidence places alternative splicing in a central position between transcription and translation, in that it can respond not only to various signaling pathways that target the splicing machinery but also to transcription factors and chromatin structure. Changes in splicing have been implicated in numerous pathologies including cancer or neurodegenerative diseases. The objective of our study was to determine whether aberrant alternative splicing could play a role in the malignant phenotype of GBM. Patients with GBM were operated with 5-aminolevulinic fluorescence guided surgery. The fluorescent was used to take biopsies from the tumor center, and from adjacent normal-looking tissue. Three paired normal/GBM samples were analyzed in a HJAY J array. We validated our results with conventional PCR and qRT-PCR in those tumor samples and in ten additional GBMs. We performed functional studies using MTT assays (proliferation) and IF qRT-PCR (differntiation). We generated a list of seven genes with differential alternative splicing between central tumor samples and the adjacent normal-looking tissue. DFP-2 (BAF45d) was one of our top candidates. Interestingly, DFP-2 is known as a tumor suppressor gene in the context of the SWI/SNF complex and plays a key role in the development of the central nervous system. The inhibition of our DFP-2 isoform resulted in a significative reduction in proliferation and in a morphology changes towards a more differentiatated phenotype in GBM cell lines. Interestingly, our DFP2 tumoral isoform was the predominant transcript in early postnatal murine neural precursors and astrocytoma precursors caused by RTK pharmacological inhibition. Our studies are expected to bring further insights into role of RTK signaling in neural progenitors and astrocytoma precursors. We expect to bridge the gap in our understanding of the effects of RTK mono- and combination therapy on neural progenitors. We may find novel defects downstream of RTK dysregulation, which when targeted pharmacologically, may effectively eliminate malignant cells.

CB-025. COORDINATE ACTIVATION OF Shh AND PI3 KINASE SIGNALING PATHWAYS IN PTEN-DEFICIENT Glioblastoma: PRELIMINARY RESULTS OF TARGETED THERAPY

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In glioblastoma, PI3K kinase signaling is frequently activated by loss of the tumor suppressor PTEN. However, it is not known whether inhibiting PI3 kinase represents a selective and effective approach for treatment. Here we interrogate large tumor databases and find that Shh signaling is activated in PTEN-deficient glioblastoma. We demonstrate that Shh and PI3K pathways synergize to promote tumor growth and viability in human PTEN-deficient glioblastomas. A combination of PI3K and Shh signaling inhibitors not only suppresses activation of both pathways, but also abrogates s6 kinase signaling. Accordingly, simultaneously targeting both pathways results in mitotic catastrophe and tumor apoptosis, and dramatically reduces growth of PTEN-deficient glioblastomas in vitro and in vivo. The drugs tested here appear safe in humans; therefore this combination may provide new targeted treatment for glioblastoma.

CB-024. AN Ehsp90-Lrp1 AXIS REGULATES EphA2 SIGNALING AND CELL MORPHOLOGY

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Our goal is to understand factors contributing to the deadly invasive and infiltrative spread of glioma. The EphA2 receptor is overexpressed in gliomas and many other solid tumors, and is required for glioma cell motility and invasion. Extracellular Ehsp90 (eHsp90) is also known to promote cell motility and invasion in tumor models. We recently demonstrated that eHsp90 plays an essential role in glioma tumor cell invasion via signaling with its receptor LRP1. We reported that eHsp90 facilitates interaction of EphA2 with LRP1, an event required for glioma cell motility and invasion. We herein investigate the mechanisms by which eHsp90 and LRP1 may regulate EphA2 signaling function. The EphA2 ligand ephrinA1 is suppressed in cancers and blocks EphA2 driven cell motility. Interestingly, ephrinA1 disrupted EphA2 interaction with its ligand and altered its interaction with LRP1. We investigated eHsp90-LRP1 internalization and subsequent Rho-mediated cell rounding, hallmarksof ephrinA1-mediated signaling events. Thus, the ability of ephrinA1 to facilitate EphA2 interaction with an LRP1-deficient protein complex coincides with its suppression of cell motility. Surprisingly, we report that eHsp90-LRP1 signaling is also required for ephrinA1-dependent signaling and internalization, indicating that eHsp90-LRP1 plays a critical role in coordinating both surface and internalized receptor populations. This is the first report to demonstrate a role for eHsp90-LRP1 as regulators of receptor internalization, and may point to a broader role for these proteins in regulating glioma receptor signaling, invasion, and dissemination.
A NOVEL AGENT FOR THE TREATMENT OF GLIOBLASTOMA: CB-028. THE IMMUNOMODULATORY EFFECTS OF DECORIN - PLEDGE FOR TREATING GBM.

CONCLUSIONS: Ad-REIC gene therapy may have upstream effects functional molecules downstream, thereby exerting anti-tumor effects in GBM.

CB-028. THE IMMUNOMODULATORY EFFECTS OF DECORIN - A NOVEL AGENT FOR THE TREATMENT OF GLIOBLASTOMA

BACKGROUND: Current treatments for Glioblastoma (GBM) are inadequate and immunotherapies may provide better outcomes for patients. Human recombinant Decorin is a glycoprotein that modulates inflammation & GBM growth by inhibiting Transforming Growth Factor, Vascular Endothelial Growth Factor and Epidermal Growth Factor Receptor. As Decorin is found in human tissue rich in fibrillar collagen (e.g. skin, heart, bone lung & liver) it is a potentially safe drug to be trialled in humans. METHODS: U87MG, U251MG, and GB-111 cells and a murine U87MG cell xenograft model to compare the anti-tumor effects of Ad-REIC and Ad-LacZ. We analysed the expression of the Wnt proteins and their co-receptors by western blot and quantitative RT-PCR. Their interaction was examined by the immunoprecipitate and the activities of downstream cascade.

RESULTS: There was no inflammatory reaction or cavity formation of Decorin and change in inflammatory markers. Immunohistochemistry was performed in three glioma cell lines (U251, LN229, and U87). Consistent with repeated observations of Decorin was investigated. Rats (n = 6 for each group) received either an injection of PBS (control) or 5 μl 5mg/ml Decorin and were sacrificed at 30min, 3 & 24 hrs or after continuous infusion of either PBS (control) or Decorin for 7 days. Macroscopic evaluation of brain, lung, heart, liver, spleen, intestine and kidney was performed for signs of abnormalities. Blood and CSF samples were taken and analysed by ELISA or Luminex for presence of Decorin and change in inflammatory markers. Immunohistochemistry was also performed to investigate the effects of Decorin on microglia using OX42 and ED1 antibodies. RESULTS: There was no inflammatory reaction or cavity formation in brain. No end-organ damage was visible in brain, heart, lungs, kidney and intestines. Compared to PBS, Decorin suppressed the microglial response, after direct injections into the brain. At 30 mins, low levels of decorin were detected, with ELISA in CSF and serum but none was detected at 3hrs and 24hrs after injection. CONCLUSIONS: Decorin showed no dose limiting toxicity, whilst maintaining its immunomodulatory effects in brain. These findings show a safe pharmacokinetic and toxicological profile of Decorin.

CB-029. MUTANT IDH1 SUPPRESSES PROSTATE APOPTOSIS: RESPONSE-4 IN GLIOMAS

BACKGROUND AND PURPOSES: Glioblastoma multiforme (GBM) is a lethal primary brain tumor. Whether adenovirus-mediated REIC/Dkk-3 (Ad-REIC) inhibits the proliferation of these cells and how REIC/Dkk-3 protein regulates the Wnt signaling pathway remain to be elucidated. To verify the mechanisms underlying these effects we focused on the regulation by REIC/Dkk-3 protein on the interaction between the Wnt proteins Wnt5a and Wnt3a and their co-receptors LRPS and ROR2. In this study we used a pair of human GBM cell lines expressing high level of EGFR, while lacking a tumor suppression effect in xenograft, and transgenic models. This downregulation occurs via Par-4-mediated degradation, not promoter methylation, in U87MG glioma cells.

IDH1-mutant glioblastomas (GBMs) from the Cancer Genome Atlas have lower Par-4 mRNA relative to wild-type tumors (P < 0.01). Par-4 expression is significantly lower in IDH1-mutant high grade gliomas via immunohistchemistry (P = 0.003). Although IDH1 mutations are a well-known favorable prognostic marker in gliomas, among high-grade gliomas that are IDH1 wild-type, those that express Par-4 on initial resection correlate with significantly longer survival (median survival 18.4 versus 8.0 months, P = 0.002). These data suggest that a.) the effects of mutant IDH1 and D-2-HG on gliomas extend beyond direct gene hypermethylation, b.) expression of Par-4 might contribute to IDH1-mutant tumorigenesis, c.) regardless of IDH1 status, Par-4 may be an effective sensitizer of gliomas to apoptosis.

CB-030. EFEMP1 ATTENUATES EGFR SIGNALING ACTIVITIES AND THE PROGNOSTIC EFFECT OF EFEMP1 DEPENDS ON THE LEVEL OF EGFR EXPRESSION IN GLIOMAS

BACKGROUND: Current treatments for GBM are inadequate and immunotherapies may provide better outcomes for patients. Human recombinant Decorin is a glycoprotein that modulates inflammation & GBM growth by inhibiting Transforming Growth Factor, Vascular Endothelial Growth Factor and Epidermal Growth Factor Receptor. As Decorin is found in human tissue rich in fibrillar collagen (e.g. skin, heart, bone lung & liver) it is a potentially safe drug to be trialled in humans. METHODS: We used U87MG, U251MG, and GB-111 cells and a murine U87MG cell xenograft model to compare the anti-tumor effects of Ad-REIC and Ad-LacZ. We analysed the expression of the Wnt proteins and their co-receptors by western blot and quantitative RT-PCR. Their interaction was examined by the immunoprecipitate and the activities of downstream cascade.

RESULTS: There was no inflammatory reaction or cavity formation of Decorin and change in inflammatory markers. Immunohistochemistry was performed in three glioma cell lines (U251, LN229, and U87). Consistent with repeated observations of Decorin was investigated. Rats (n = 6 for each group) received either an injection of PBS (control) or 5 μl 5mg/ml Decorin and were sacrificed at 30min, 3 & 24 hrs or after continuous infusion of either PBS (control) or Decorin for 7 days. Macroscopic evaluation of brain, lung, heart, liver, spleen, intestine and kidney was performed for signs of abnormalities. Blood and CSF samples were taken and analysed by ELISA or Luminex for presence of Decorin and change in inflammatory markers. Immunohistochemistry was also performed to investigate the effects of Decorin on microglia using OX42 and ED1 antibodies. RESULTS: There was no inflammatory reaction or cavity formation in brain. No end-organ damage was visible in brain, heart, lungs, kidney and intestines. Compared to PBS, Decorin suppressed the microglial response, after direct injections into the brain. At 30 mins, low levels of decorin were detected, with ELISA in CSF and serum but none was detected at 3hrs and 24hrs after injection. CONCLUSIONS: Decorin showed no dose limiting toxicity, whilst maintaining its immunomodulatory effects in brain. These findings show a safe pharmacokinetic and toxicological profile of Decorin.

CB-027. REIC/Dkk-3, ONE OF DICKKOPF (Dkk) FAMILY MEMBERS, CONTRIBUTES TO THE ANTI-TUMOR EFFECTS IN GLIOBLASTOMA THROUGH REGULATION OF BOTH Wnt SIGNAL PATHWAYS

CB-031. MICROGLIAL/Brain Macrophage Toll-Like-Receiver 2 Signaling Promotes glioma Expansion

Malignant gliomas are the most frequent primary tumors of the brain with poor clinical prognosis. Infiltrating peripheral macrophages and resident microglia as the intrinsic immune competent brain cell contribute significantly to the tumor mass. We have previously shown that microglia/macroglia promote glioma expansion by up-regulating metalloproteinase MT1-MMP through Toll-like receptor (TLR) and its adaptor protein MyD88. In this study we identified TLR2, as the main TLR controlling MT1-MMP expression and pro-tumorigenic signaling in microglia. Glioma-derived soluble factors and synthetic TLR specific ligands induced MT1-MMP expression in microglia from wild-type (WT) mice but not TLR2−/− mice. By using this novel brain slice, we found that tumor expansion depended on both parenchymal TLR2 expression and the
presence of microglia. The implantation of mouse GL261 glioma cells into TLR2−/- mice resulted in significantly smaller tumors, reduced MT1-MMP expression, and enhanced survival rates as compared to WT control mice. TLR2 is also highly expressed in human gliomas (which contains microglia-brain macrophages) and inversely correlates with patient survival. In search for an endogenous TLR2 ligand released from glioma cells, we screened glioma conditioned medium by mass spectrometry and found versican, an extracellular matrix proteoglycan and a reported ligand of TLR2. Versican is highly up-regulated in gliomas but not in microglial cells. Versican silenced gliomas induced less MT1-MMP expression on microglia both in vitro and in vivo. Implanting versican silenced GL261 cells into mouse brain resulted in smaller tumors compared to the controls. Our results show that glioma released factors convert microglia to brain macrophages into a pro-tumorigenic phenotype through TLR2 signaling and thus TLR2 might be a novel target for glioma therapies.

CB-032. THE ROLE OF ARHGAP36 AS A NOVEL DRIVER IN HIGH-RISK HUMAN MEDULLOBLASTOMA
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INTRODUCTION: Medulloblastoma (MB) is the most frequent childhood malignancy of the CNS. Current trials aim to identify effective treatments with reduced side effects, but lack of knowledge regarding drivers prevents development of more precise therapies. MB is divided into four molecular subgroups based on gene expression, histology and developmental origin: Shh, Wnt, 3 and 4. Our lab identified a novel, non-Shh, non-Wnt driver of MB using Sleeping Beauty insertional mutagenesis. Rho GTPase Activating Protein 36 (Arhgap36) was the top candidate to emerge from this screen, showing insertion and overexpression in 14/22 MBs harvested. METHODS: Preliminary studies with qRT-PCR, tissue microarray and immunohistochemistry showed Arhgap36 overexpression in Group 3 and 4 human MB. We are employing multiple genetic techniques to establish the role of Arhgap36 in medulloblastogenesis. We will overexpress Arhgap36 in NIH 3T3 cells and monitor the effects on cell signaling and Rho GTPase activation. We will also use TALENs to knockout Arhgap36 in human MB cell lines and assess the effects of Arhgap36 on cellular signaling through mass spectrometry and micro Western analysis. RESULTS: Constructs for Arhgap36 overexpression have been synthesized and transfected into NIH 3T3 cells for analysis. One human Group 3 MB line has shown an “oncogene-addiction” phenotype, as it was resistant to TALEN knockout of Arhgap36. Knockout experiments have been repeated in the presence of a rescue, FRET-regulated DNA to conditionally complement ARHGAP36 expression. CONCLUSIONS: Arhgap36 overexpression is strongly associated with MB, and our findings indicate that it may play a driving role in Group 3 and Group 4 medulloblastogenesis. Furthermore, Arhgap36 may represent a novel target for therapeutic efforts aimed at treating patients with MB.

CB-033. A ROLE FOR MATRIX METALLOPROTEINASES IN INVASIVE AND MALIGNANT MENINGIOMAS
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INTRODUCTION: Invasive and malignant meningiomas present a significant therapeutic challenge due to high recurrence rates and invasion into surrounding bone, brain, neural and soft tissues. Understanding the mechanism of invasion could help in designing novel therapeutic approaches in order to prevent the need for repeat surgery, decrease morbidity and improve patient survival. The aim of this study was to identify the differential gene and protein expression profile in bone-invasive and malignant versus non-invasive meningiomas, focusing on factors that regulate invasion and identifying molecular mechanism of invasion. METHODS: Samples from bone-invasive, non-invasive and malignant meningiomas were used for RNA microarray, quantitative real-time PCR and Western blot analyses. Malignant meningioma cell lines (F5) were used for in vitro and in vivo functional assays. RESULTS: RNA microarray data analysis identified over 300 differentially expressed invasive versus non-invasive meningiomas. Ingenuity pathway analysis showed cell movement and invasion pathway among the significantly enriched networks, with an increased expression of its molecules including ADAMTS4, MMP19 and MMP6. Increased expression of these molecules was also identified in malignant meningiomas. Among these proteins, only MMP16 was identified as a secreted form in the conditioned media of the F5 cells. Knock down of MMP16 resulted in reduced MMP9 and MMP3 activity as determined by zymography and decreased invasion and migration of tumor cells both in vitro and in vivo. CONCLUSION: Our data identifies MMP16 as a novel factor involved in regulating the invasive phenotype of meningiomas and may represent new avenues for future studies with the aim to translate to clinical studies.

CB-034. HYPOXIA INDUCED ROS PRODUCTION REGULATES STAT3 ACTIVATION FOLLOWED BY ANGIOGENESIS IN HUMAN GliOBLASTOMA
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OBJECTIVE: Glioblastoma has historically histopathologic findings, pseudopalisading necrosis and microvascular proliferation, all of which are associated with hypoxia. In a previous report, we found that Stat3 plays an important role in glioblastoma angiogenesis and migration by hypoxia. However, it is poorly understood for underlying mechanism of Stat3 activation in hypoxic condition. In this study, we examined reactive oxygen species (ROS) for hypoxia-induced Stat3 activation followed by angiogenesis in human glioblastoma. METHODS: To determine reactive oxygen species (ROS) production in hypoxic human glioblastoma cells, we examined ROS by flow cytometry in 1% O2 hypoxic condition. For the intracellular localization of ROS, we examined nuclear and cytoplasmic ROS by DAPI and Mitosox fluorescence respectively. To determine involvement of ROS on Stat3 activation and its inhibition, we determined ROS production in the presence of antioxidants DPI, NAC, Stat3 siRNA, and NOX inhibition. We also determined the role of ROS on angiogenesis by measuring VEGF expression and tube formation in hypoxic glioblastoma. RESULTS: In 1% O2 condition, ROS was increased in glioblastoma cells compared to normoxia. Using antioxidants treatments such as DPI, NAC, Stat3 siRNA, hypoxia induced VEGF expression and tube formation was decreased in hypoxic glioblastoma. Further, we determined the role of NOX in hypoxia induced VEGF expression and tube formation in hypoxic glioblastoma. CONCLUSION: Hypoxia induced ROS production may regulate Stat3 activation followed by angiogenesis in glioblastoma. Considering that the hypoxia is associated with poor prognostic, inhibition of ROS induced Stat3 activation can be a new therapeutic target for human glioblastoma and induce longer survival.

CB-035. HOMEBOX GENE HOXA10 IS RELATED WITH TEMOZOLOMIDE RESISTANCE BY REGULATING HOMOLOGOUS RECOMBINATION DNA REPAIR PATHWAY IN GliOBlastOMA CELL LINES
Sung Kwon Kim1, Jin Wook Kim1, Ji Young Kim1, Ja Eun Kim1, Seung Hong Choi1, Tae Min Kim1, Se-Heon Lee1, Seung-Ki Kim1, Sung-Hye Park1, Ji Han Kim2, Chul-Kee Park1, and Hee Won Jung1; 1Departments of Neurosurgery, Seoul National University Hospital, Seoul, Republic of Korea; 2Departments of Radiation Oncology, Seoul National University Hospital, Seoul, Republic of Korea; 3Radiology, Seoul National University Hospital, Seoul, Republic of Korea

O6-methylguanine DNA methyltransferase (MGMT), mismatch repair (MMR), and homologous recombinant (HR) pathways are three DNA repair pathways involved in anticancer mechanism of temozolomide. We found that there are relatively less investigated for temozolomide resistance, and homeobox gene HOXA10 for its candidate regulator. Homeobox genes play essential role in embryonic development, but are known to be aberrantly expressed in glioblastoma. In this study, we used two glioblastoma cell lines with different MGMT status; LN18 (unmethylated MGMT promoter) and LN229 (methylated MGMT promoter). Synergistic anticancer effect of HOXA10 inhibition with temozolomide was observed regardless of MGMT status. We found that HOXA10 inhibition is related with impaired double strand DNA breakage repair and decreased expression of Rad51 gene. Screening of differential gene expression between cell lines with or without HOXA10 inhibition using mRNA microarray and further
CB-036. S-NITROSYLATION OF THE p33 TUMOR SUPPRESSOR PROTEIN IN MEDULLOBLASTOMA
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p33 dysfunction plays a major role in the pathology of malignant brain tumors, and numerous post-translational modifications can regulate p33 activity. Nitric oxide, a gaseous signaling molecule, contributes to both physiologic and pathologic states through S-nitrosylation of protein cysteine residues, thereby altering protein function. Signaling associated with nitric oxide has increasingly been implicated in the biology of malignant brain tumors, and so we hypothesized that p33 might be regulated by S-nitrosylation. p33 contains ten cysteine residues, several of which are essential for zinc coordination and DNA binding. Using the biotin switch method, we detect S-nitrosylation of p33 in cells exposed to nitric oxide donors, as well as under physiologic conditions in mouse brain. We demonstrate p33 S-nitrosylation in ONS76 medulloblastoma cells which express endogenous neuronal nitric oxide synthase (nNOS). Manipulation of nitric oxide levels in cell lines suggests nitric oxide-dependent regulation of p33-mediated transcriptional activity and cell death. S-nitrosylation is a novel post-translational modification of p33 that may regulate p33 response to cell stressors and contribute to the pathogenesis of medulloblastoma and other malignant brain tumors in which nitric oxide is generated.

CB-037. GENE-EXPRESSION PROFILING ELUCIDATES MOLECULAR SIGNALING NETWORKS THAT CAN BE THERAPEUTICALLY TARGETED IN VESTIBULAR SCHWANNOMA
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Vestibular schwannomas (VS) are common benign tumors of the vestibular nerve that cause significant morbidity. The current treatment options for VS include surgery or radiation with each treatment option having associated complications and morbidities. The transcriptome of schwannoma is still under investigation. In this study we performed gene expression profiling of 49 schwannomas and 7 normal control vestibular nerves to identify differentially expressed genes. We identified over 4000 differentially expressed genes between control and schwannoma with network analysis uncovering proliferative and anti-apoptotic pathways previously not implicated in VS. Furthermore, using several distinct clustering technologies, we could not reproducibly identify subtypes of schwannomas suggesting that our schwannoma cohort was molecularly distinct from normal tissue yet highly similar among themselves. At the molecular level the PI3K/AKT/mTOR signaling network was overexpressed in our schwannoma cohort and evaluated for therapeutic targeting. Testing compounds BEZ235 and PKI-587 both novel dual inhibitors of PI3K and mTOR attenuated tumour growth in a preclinical cell line model of schwannoma (HEI-293). In vitro findings demonstrated that ablation of the PI3K/AKT/mTOR pathway with next generation inhibitors lead to decreased cell viability and increased cell death. Elucidation of novel molecular targets in vestibular schwannoma by transcriptional profiling versus appropriate controls may lead to promising effective therapeutic strategies and shed insight into the molecular ontology of this tumour.

CB-038. ERLOTINIB RESISTANCE IN EGFR-AMPLIFIED GLOBLASTOMA CELLS IS MEDIATED BY UP-REGULATION OF EGFRVIII AND PI3K p110d
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BACKGROUND: Treatment efficacy of EGFR tyrosine-kinase inhibitors like Erlotinib has not met expectations for glioblastoma therapy in clinical trials, even for EGFR-overexpressing tumors. We determined possible mechanisms of therapy resistance using the unique BS153 GBM cell line that has retained amplification of the egfr gene and expression of the constitutively active receptor variant EGFRVIII. METHODS: Functional effects of Erlotinib, Gefitinib and Cetuximab on BS153 proliferation, migration and EGFR-dependent signal transduction were systematically compared in vitro. Tumor-initiating capacity of parental and treatment-resistant BS153 was studied in NMR1/Foxn1nu mice. Potential mediators of resistance were knocked down using siRNA. RESULTS: Erlotinib and Gefitinib inhibited proliferation and migration of BS153 in a dose-dependent manner, whereas Cetuximab had no effect. BS153 developed resistance to Erlotinib (BS153resE) but not to Gefitinib. Resistant cells displayed significantly decreased phosphorylation of all major receptor tyrosine kinases (Met, PDGFRα, HER2) except for EGFR. Resistance was associated with strong upregulation of EGFRVIII and subsequent activation of the phosphatidylinositol-3-OH kinase (PI3K)-pathway in BS153resE and an increased expression of the regulatory 110kd delta subunit of PI3K (p110δ). Knockdown of EGFRVIII in BS153resE largely restored sensitivity to Erlotinib. Targeting PI3K pharmacologically caused a significant decrease in cell viability, and specifically targeting p110δ by siRNA partially restored Erlotinib-sensitivity in BS153resE. In vivo, BS153 formed highly invasive tumors with an unusual growth pattern, displaying numerous satellites distant from the initial injection site. Erlotinib resistance led to delayed onset of tumor growth as well as prolonged overall survival of mice without changing tumor morphology. CONCLUSION: EGFRVIII can mediate resistance to Erlotinib in EGFR-amplified glioblastoma via an increase in P3Kp110δ. Interfering with P3Kp110δ can restore sensitivity towards the TKI.

CB-039. NUCLEAR FACTOR IA (NFIA) NEGATIVELY REGULATES p53, p21, AND PAI1 TO PROMOTE THE MALIGNANT BEHAVIOR OF GLOBLASTOMAS
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Glioblastoma (GBM) represents the most common primary malignant brain cancer and carries a dismal prognosis. Recent evidence suggests that Nuclear Factor I A (NFIA), a transcription factor and essential regulator in embryonic glial development, is highly expressed in human GBM compared to normal brain, suggesting that NFIA may have a role in astrocytoma biology. However, the contribution of NFIA to GBM pathogenesis remained unknown. Here, we show that NFIA promotes growth and migration of GBM and establish the molecular mechanisms mediating these functions. NFIA overexpression accelerated growth, proliferation and migration of GBM in cell culture and in mouse brains whereas knockdown of native NFIA blocked tumor growth and induced cell death and apoptosis. These NFIA tumor-promoting effects were mediated by transcriptional repression of p53, p21, and plasminogen activator inhibitor 1 (PAI1) through specific NFIA-recognition sequences in their promoters. Importantly for GBM, in which TP53 is frequently mutated, the effects of NFIA on proliferation and apoptosis were independent of TP53 mutation status. Thus, NFIA is a previously unrecognized modulator of GBM growth and migration, which functions by distinct regulation of critical oncogenic pathways that govern the malignant behavior of GBM.

CB-040. OCT 7 IS EXPRESSED IN HUMAN GLIOMAS AND CORRELATES WITH MALIGNANT GRADE
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Glioblastoma (GBM) represents the most common primary malignant brain cancer and carries a dismal prognosis. Recent evidence suggests that Nuclear Factor I A (NFIA), a transcription factor and essential regulator in embryonic glial development, is highly expressed in human GBM compared to normal brain, suggesting that NFIA may have a role in astrocytoma biology. However, the contribution of NFIA to GBM pathogenesis remained unknown. Here, we show that NFIA promotes growth and migration of GBM and establish the molecular mechanisms mediating these functions. NFIA overexpression accelerated growth, proliferation and migration of GBM in cell culture and in mouse brains whereas knockdown of native NFIA blocked tumor growth and induced cell death and apoptosis. These NFIA tumor-promoting effects were mediated by transcriptional repression of p53, p21, and plasminogen activator inhibitor 1 (PAI1) through specific NFIA-recognition sequences in their promoters. Importantly for GBM, in which TP53 is frequently mutated, the effects of NFIA on proliferation and apoptosis were independent of TP53 mutation status. Thus, NFIA is a previously unrecognized modulator of GBM growth and migration, which functions by distinct regulation of critical oncogenic pathways that govern the malignant behavior of GBM.
INTRODUCTION: The transcription factor Oct7 (also called POU3F2/BM2) is expressed during neurogenesis and constitutes the CNS equivalent of Oct4, a critical regulator of induced pluripotent stem cells. Moreover, data suggest its expression is regulated by hypoxia, and Oct7 expression has been reported in malignant melanoma of the skin. Since both melanomas and CNS-malignancies arise in organs of neuroectodermal origin, we investigat- ed the expression of Oct7 in human gliomas and compared the expression patterns with that of Oct4. MATERIAL AND METHODS: We performed immunohistochemistry of 150 grade II-IV gliomas from our tumor bank, and subsequently performed Western blots of 20 glioma samples. In addition, we performed flow cytometry analysis of Oct7 expression in 3 acutely dissociated tumors. Using lentiviral transfection we established Oct7 overexpression in a panel of constitutively negative glioma cell lines, as well as knock-down in an Oct7 positive glioma cell line, to investigate the effect of Oct7 expression on proliferation, migration and differentiation. RESULTS: Immunohistochemistry showed that Oct7 was almost uniformly expressed in human gliomas, although at a varying degree. Microscopy revealed a predomi- nantly nuclear staining pattern, but cytoplasmic immunopositivity for Oct7 could also be detected in some tumors. Both Western blot and flow cytometry confirmed expression of Oct7 in human gliomas. With two independent observ- ers we obtained a nuclear staining index for all tumors, with 61% positive nuclei in GBM specimens, which was significantly higher than for grade II (37%) and grade III (36%) tumors (p = 0.0001). Moreover, ongoing studies suggest that overexpression of Oct7 increases the proportion of cells in G2/M phase of the cell cycle, suggesting that this transcription factor has a role in regulating tumor cell proliferation, and hence possibly overall tumor aggressiveness. Ongoing studies aim at further elucidating the multiple roles of Oct7 in brain tumor progression.

CB-041. PATTERNS OF MOLECULAR HETEROGENEITY IN RODENT MODELS OF Glioblastoma
Richard Leung, Orlando Gil, Liang Lei, and Peter Canoll; Columbia University Medical Center, New York, NY, USA
INTRODUCTION: Gliomas are the most malignant brain tumors with a dismal survival rate and no effective treatment. Recruitment of glial progeni- tor by platelet-derived growth factor (PDGF) signaling has been implicated in glioma progression and invasion accompanied by cytoplasmic and nuclear activation of EGFR, which is constitutively active in glioblastoma. Various mathematical models have correlated levels of PDGF to re- cruitment of healthy oligodendrocyte progenitor cells (OPCs) into the tumor. We sought to determine whether PDGF is uniformly distributed through the tumor or whether it is expressed heterogeneously within the tumor, and to correlate those levels with changes in global gene expression in heterogeneous regions of the tumor. METHODS: By stereotactically inject- ing PDGF overexpressing retrovirus into subcortical white matter of rodent brains we generated tumors with the histological and molecular hallmarks of prouneural glioblastoma. After isolating coronal sections harboring the tumor focal core biopsies were taken from the center of the tumor continuously to the invasive border and beyond, as well as in a grid pattern encompassing the entire tumor and peripheral tissue. Using sandwich ELISA, the PDGF levels within each biopsy were measured. Subsequently, high throughput RNA se- quencing was employed to quantify global gene expression within regions of the tumor. RESULTS: PDGF concentrations at the core of the tumor exist at levels 10 times higher than at the invasive border (p < 0.0001). Concentration of PDGF declines sharply to undetectable levels in adjacent biopsies, and global expression patterns show high degree of heterogeneity. CONCLUSION: Our results are consistent with the established histological heterogeneity of glioblastoma, which may in part, be due to a heterogeneous molecular microenvironment. Furthermore, findings of molecular heterogene- ity can guide the treatment of glioblastoma in the clinical setting.

CB-042. UP-REGULATION OF THE CHAPERONE PROTEIN PROLYL 4-HYDROXYLASE, Beta POLYPEPTIDE (P4HB), PROMOTES TUMOUR INVASION, ANGIogenesis AND GROWTH VIA EGFR/MAPK (ERK) SIGNALING
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INTRODUCTION: Endoplasmic reticulum (ER) chaperones have received considerable attention as emerging therapeutic targets. We have recently demon- strated that knock-down of the chaperones, prolyl 4-hydroxylase, beta polypeptide (P4HB), may play a role in determining chemodrug sensitiv- ity through the unfolded protein response (UPR) in glioblastoma multiforme (GBM). OBJECTIVE: To investigate the role of P4HB in gliomagenesis. The hypothesis was that P4HB could promote tumour growth and invasion in glioma. MATERIALS AND METHODS: We examined correlations between P4HB expression with clinical and pathological parameters using clinical specimens and publicly available database (GEO). Gene clustering and pathway analysis were performed using GSE16011 database. Cell prolif- eration and invasion were assessed by in vitro assays using GBM cells with (U87- and U251-P4HB) and forced expression of P4HB (U87- and U251-P4HB). Western blotting was employed to confirm phenotypes and ac- tivation of signaling pathways. We examined in vivo tumour growth by biolu- minescence imaging of luciferase-tagged (U87- and U251-VEC); (U87- and U251-P4HB) GBM cells in an orthotopic nude mouse model. H&E and IHC staining of tumor tissue was performed to examine angiogenic markers on xenografts. RESULTS: High-grade gliomas (grade III and IV) had significantly higher P4HB expressions than low-grade gliomas and control tissue brains. High P4HB expression was significantly associated with upregulated expres- sions of both invasive (vimentin, MMP2 and MMP14) and angiogenic (CD31 and VEGF-A) markers. Pathway scan showed that P4HB was involved in the activation of EGFR/MAPK (ERK) signaling. GBM cells with enhanced P4HB expression exhibited a greater ability to proliferate and invade com- pared to the parental cells and cells with shP4HB. Moreover, U87- and U251-P4HB cell implants showed greater tumourigenicity in vivo. CONCLUSION: P4HB promotes tumour invasion, angiogenesis and growth via EGFR/MAPK (ERK) signaling. Targeting P4HB may serve as a novel approach in retarding critical pathways in the treatment of GBM.

CB-043. THE MAJOR VAPOR PROTEIN MEDIATES SURVIVAL AND MIGRATION COMPETENCE OF HUMAN Glioblastoma CELLS VIA STABILIZATION OF THE EGFR/PI3K SIGNALING
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Although vaults are ubiquitously expressed and highly conserved ribonu- cleoparticles, their precise cellular functions are still enigmatic. Vaults are pre- dominantly built of multiple copies of the major vault protein (MVP) shown to be upregulated during glioma progression and drug resistance development. Such we demonstrated that human astrocytic brain tumors including glioblasto- ma are generally high in vault levels while the normal brain is widely devoid of MVP. Consequently, the aim of our study was to investigate whether MVP itself has a pro-tumorigenic function and how it supports glioblastoma aggres- siveness. Based on a large tissue collection we reconfirm MVP overexpression in gliomas as compared to healthy brain. The impact of vaults on human glioblas- toma cell survival and migration competence was further analyzed using MVP knock-down and dominant-negative genetic approaches. Finally, the role of MVP promoting subcutaneous and orthotopic tumor growth in SCID mice was tested. Our results demonstrate that MVP/vaults significantly support glioblastoma cell migration and invasion as well as starvation resis- tance. The enhanced aggressiveness was based on MVP-mediated stabilization of the epidermal growth factor receptor (EGFR)/phosphatidylinositol-3-kinase (PI3K) signaling axis supported by PTEN hyper-phosphorylation and translocation into the nucleus. Accordingly, MVP overexpression led to enhanced tumor growth and brain invasion in mouse subcutaneous and ortho- topic xenograft models. Overall our data elucidate for the first time a direct tumor-promoting function of vaults based on EGFR/PI3K-pathway stabiliza- tion in human glioblastoma.

CB-044. REGULATION OF PRIMARY HUMAN ASTROGLIAL (HAG) CELL PROLIFERATION VIA A BRAIN-ENRICHED, NF-kB-SENSITIVE micro-RNA-125b (miRNA-125b) Walter J. Lukic1,2, Brandon M. Jones1,2, Yuhan Zhao1,3; 1Suryaajipta Bhattacharjee1,2, and Frank Culicchia1,3; 1LSU Neuroscience Center, New Orleans, LA, USA; 2LSU Departments of Neurology and Neurosurgery, New Orleans, LA, USA; 3LSU Department of Ophthalmology, New Orleans, LA, USA
Micro RNAs (miRNAs) constitute a class of small, single-stranded, non- coding RNAs (sncRNAs) that bind, via base-pair complementarity, to the 3′...
CB-045. SUBTYPE-SPECIFIC DIFFERENCES IN THE COAGULOME OF GLOBLASTOMA SUGGEST A LINK BETWEEN EGFR, TISSUE FACTOR AND REGULATION OF TUMOR ANGIOGENESIS, INFLAMMATION AND DORMANCY
Nathalie Magnin, Delphine Garner, Brian Mehan, Serge McGraw,* Meinam Hashemi, Tae Hoon Lee,† Chloé Milon, Noha Gerges,† Nada Jabado,† Jacquetta Trasler, Rafael Pawlinski, Nigel Mackman, and Janusz Kak,† – Montreal Children’s Hospital Research Institute, Montreal, QC, Canada;† Sunnybrook Research Institute, Toronto, ON, Canada;‡ University of North Carolina, Chapel Hill, NC, USA

INTRODUCTION: Glioblastoma (GBM) is associated with florid angiogenesis, recruitment of inflammatory cells and perivascular neovascularization. EGFR and its downstream mitogenic and pro-angiogenic signals are critical for glioblastoma proliferation and angiogenesis. It is now recognized that the expression of tissue factor (TF), which is expressed in tumour cells and stromal myeloid cells, contributes to the formation of perivascular endothelial tubes and to endothelial cell proliferation, recruitment of inflammatory cells and angiogenesis. Using biological models of glioblastoma, we have previously shown that TF is up-regulated in glioblastoma and that anti-TF strategies are able to inhibit tumour cell proliferation, migration and angiogenesis. Here we studied the expression of TF in GBM and its role in glioblastoma progression.

METHODS: We used various biological models of GBM and non-tumoural human brain tissue, including co-culture models of GBM and normal human brain endothelial cells, transwell models of GBM cell migration and invasion, and ex vivo xenograft models of GBM. We assessed TF expression using immunohistochemistry, immunoblotting and real-time PCR. We also assessed the effects of anti-TF strategies on glioblastoma proliferation and migration.

RESULTS: We found that TF is up-regulated in glioblastoma and that anti-TF strategies are able to inhibit tumour cell proliferation, migration and angiogenesis. Our results suggest that TF plays a critical role in glioblastoma progression and that targeting TF may be a promising strategy for the treatment of glioblastoma.

CONCLUSION: Our results suggest that TF plays a critical role in glioblastoma progression and that targeting TF may be a promising strategy for the treatment of glioblastoma.
Cholesterol ester accumulation. Cholesterol ester synthesis from free cholesterol is catalyzed by the enzyme Sterol O-acetyltransferase 1 (SOAT1). To determine whether YAP regulates SOAT and other metabolic enzymes, we performed a genome-wide expression analysis of YAP knockdown followed by mass spectrometry and analyzed the results with Ingenuity Pathway Analysis (IPA). We found that predicted direct and indirect targets of YAP comprised multiple metabolic pathways, including cholesterol esterification and FAS, consistent with our experimental results. The present study has attempted to dissect the mechanism by which YAP regulates SOAT expression and activity and also confirm the IPA-predicted metabolic targets of YAP. The result of disrupting these pathways through YAP ablation and pharmacological means, on CGNP and medulloblastoma cell proliferation, is also presented. The study investigates YAP-predicted metabolic pathways as potential targets for novel medulloblastoma therapies that may reduce or eliminate the requirement for high dose radiation.

CB-049. HCMV GLYCOPROTEIN B IS EXPRESSED IN PRIMARY HUMAN GlioBLASTOMAS AND ENHANCES GROWTH AND INVASIVENESS VIA PDGFRα ACTIVATION
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Our laboratory was the first to demonstrate that the ubiquitous human pathogen cytomegalovirus (HCMV) is highly associated with the most common and deadly form of primary brain tumor, glioblastoma (GBM). We believe that HCMV can act as an oncomodulator of glioblastoma by driving cellular signaling pathways essential to the neoplastic process. Our previous work demonstrated that the viral surface glycoprotein B (gB) mediates viral attachment, penetration, and cell-to-cell spread via the cellular receptor tyrosine kinase PDGFRα which results in activation of the PI3K/Akt pathway. Because this signaling pathway is implicated in gliomagenesis, we hypothesized that persistent expression of gB in tumor cells could enhance glioma cell invasiveness and growth by activating PDGFRα and downstream oncogenic pathways that regulate cells survival and invasion. Using RT-PCR, immunofluorescence, and western blot approaches we identified gB expression in several primary GBM tissue samples and demonstrated that expression of gB in glioma cells resulted in constitutive phosphorylation of PDGFRα, Akt, Src, and FAK. Incubation of U87 or primary GBM cells with recombinant gB or whole virus resulted in increased migration in transwell, Matrigel and brain slice invasion assays, which was specifically inhibited with neutralizing antibodies to gB or PDGFRα. Likewise, infection of U87 cells with HCMV stimulated migration in a wound healing scratch assay in a PDGFRα and integrin αvβ3-dependent manner. Stable expression of gB in U87 cells enhanced in vitro migration and proliferation as well as in vivo tumor growth in an intracranial xenograft mouse model. Immunohistochemical staining of xenograft tissue sections demonstrated sustained expression of gB in vivo in an associated increase in phospho-Akt and tumor cell dispersal relative to controls. Our results suggest that HCMV gB can induce key hallmarks of glioma, i.e., tumor cell proliferation and invasiveness which could lead to a more aggressive glioblastoma phenotype.

CB-050. NF-κB INDUCED IL-6 ENSURES STAT3 ACTIVATION AND TUMOR AGGRESSIVENESS IN GlioBLASTOMA
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Glioblastoma (GBM) is the most aggressive and neurologically destructive tumor of the central nervous system (CNS). In GBM, the transcription factors NF-κB and STAT3 are aberrantly activated and associated with tumor cell proliferation, survival, invasion and chemoresistance. In addition, common activators of NF-κB and STAT3, including TNF-α and IL-6, respectively, are abundantly expressed in GBM tumors. Herein, we sought to determine the signaling crosstalk that occurs between the NF-κB and STAT3 pathways in GBM tumors. We demonstrate that TNF-α induced activation of NF-κB is sufficient to induce IL-6 expression, activate STAT3, and elevate STAT3 phosphorylation in GBM tumors. Herein, we show that the combination of NF-κB and/or STAT3 signaling significantly increases survival of mice bearing intracranial tumors in vivo. We determined that the combined inhibition of NF-κB and STAT3 signaling significantly increases survival of mice bearing intracranial tumors when compared to single agent therapy. Furthermore, the NF-κB and STAT3 pathways contribute to cellular communication within the tumor environment, activating additional non-tumor cells that then suppress immune defense mechanisms and allow continued growth of the tumor. We are currently by activating the effect of NF-κB and STAT3 inhibition on tumor infiltrating macrophages/microglia in a variety of orthotopic tumor models, including a syngeneic model which lacks SOCS3, the negative regulator of STAT3, in myeloid lineage cells. We believe that these studies will verify that pharmacological interventions to effectively inhibit the activity of both NF-κB and STAT3 will correlate with reduced GBM size and aggressiveness, and are imperative to more accurately develop pharmacological clinical interventions for patients with GBM tumors.

CB-052. JAKe-2-STAT3 ACTIVATION IS ASSOCIATED WITH CEREBROSPINAL FLUID INTERLEUKIN-10 (IL-10) IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA
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Objective: The Janus kinase 2/JAK2-signal transducers and activators of transcription 3(STAT3) signaling pathway is constitutively activated in various cancers. In the present study, we examined JAK2-STAT3 activation in primary central nervous system lymphoma (PCNSL) and analyzed a relationship between the activation of the JAK2-STAT3 pathway and the CSF levels of IL-10 and IL-6. METHODS: Thirty-five adult patients with PCNSL newly diagnosed from January 2004 to September 2012 were analyzed retrospectively. In all patients, IL-10 and IL-6 levels of CSF were measured prospectively. In 35 patients, we examined activation levels of JAK2 and STAT3 in surgically obtained PCNSL tissues by immunohistochemistry. The relationship between JAK2 or STAT3 activation levels and CSF IL-10 or IL-6 levels was analyzed. The pJAK2 and pSTAT3 expression was scored semi-quantitatively (score 1-6). STAT3 activation was also analyzed in 17 frozen samples by western blot. RESULTS: The expression level of the pSTAT3 was correlated with the expression level of the pJAK2 (r=0.613, p<0.001). CSF IL-10 level was statistically correlated with pJAK2 expression (Student’s t-test; p=0.02) and pSTAT3 expression (Student’s t-test; p=0.027) in the immunohistochemical examination. However, there was no association between the CSF IL-6 levels and the pJAK2 or pSTAT3 expression levels. Western blot examination revealed that pSTAT3 expression levels were relatively increased in the high CSF IL-10 group compared with the levels in the low CSF IL-10 group.
CB-053. GENE EXPRESSION PROFILES OF SUNITINIB-TREATED BUT NOT UNTREATED SHORT-TERM SERUM-FREE CULTURES PREDICT TREATMENT RESPONSE OF HIGH-GRADE GLIOMAS IN VITRO
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The increasing knowledge of the pathogenesis and progression of high-grade gliomas has led to the development of a novel group of therapeutics which includes small molecule kinase inhibitors. These agents directly interfere with growth factor receptor signaling pathways which are upregulated in brain tumors and are supposed to impact oncogenesis. Despite promising preclinical results, the clinical benefit of these agents in human gliomas is uncertain. Nevertheless, a subgroup of patients responds to therapy, often with long-term disease stabilization. The molecular mechanisms which would explain the heterogeneity of response have not been understood yet. Diagnostic strategies to identify those patients that will profit from a specific targeted therapy are urgently needed. The most common approach for biomarker identification is the retrospective supervised analysis contrasting the expression profiles of responders with those of non-responders. We here suggest a novel strategy for developing signatures predictive of drug response. We postulate that for certain therapeutic agents predictive gene expression patterns emerge only after tumor cells have been treated with the respective agent in vitro beforehand. As a proof-of-principle we analyzed the global gene expression profiles in 18 short-term serum-free cultures of high-grade gliomas before and after in vitro treatment with the tyrosine kinase inhibitor Sunthitinib. Furthermore, we examined the modulation of mitogenic signaling after treatment and analyzed the impairment of proliferation and migration capacity in vitro. In every aspect, glioma cultures exhibited extremely individual responses. Signaling analysis by Western-blot did not predict in vitro response. However, genetic profiles evaluated by microarray from treated but not from untreated glioma cultures allowed to predict therapy induced impairment of proliferation in vitro. Prediction can be achieved with as little as 6 genes possibly allowing for a straightforward translation into the clinic once the predictive power of the signature is shown in vivo in the context of a clinical study.

CB-054. TUMOR SUPPRESSIVE ROLE OF DACH1 IN GlioBLASTOMA STEM-LIKE CELL
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DACH1 gene encodes a chromatin-associated protein that associates with other DNA-binding transcription factors and is reported to regulate gene expression and cell fate determination during development. Decreased expression of DACH1 is reported in several types of cancers, and loss of its expression is suspected to contribute to tumorgenesis. Recently by allelic DNA copy number analysis using single-nucleotide polymorphism genotyping array and mass spectrometry, we identified homozygous deletions in glioblastomas at chromosome 13q21, where DACH1 gene is located. In addition, we observed frequent promoter methylation of DACH1 gene, which potentially contribute to tumorgenesis. Here, we hypothesize that DACH1 has tumor suppressive activity in glioblastoma. Indeed, forced DACH1 expression decreased cell proliferation of glioma cell lines in vitro and growth of engrafted tumors in mice. In glioblastoma stem-like cells cultured in serum-free NBE medium supplemented with EGF and bFGF, the ability of sphere formation was markedly disrupted by increased DACH1 expression. We also reported that fibroblast growth factor 2 (FGF2/bFGF) is transcriptionally repressed by DACH1, in these cell lines. When we xenografted sphere-forming cells that expressed DACH1 into the mouse brain, treatment with DACH1 was considerably superior to the control group which could be rescued by bFGF expression. From these results, we think that DACH1 suppress growth of glioblastoma stem-like cells through the regulation of bFGF-mediated cell proliferation mechanisms. Here we further analyzed the expression levels of DACH1 and bFGF in several glioblastomas stem-like cell lines established from surgical specimens and demonstrated their role for glioma development.

CB-055. A NEW ROLE FOR PKM2 AS A DIRECT REGULATOR OF THE CELL CYCLE MACHINERY CONTROLLING MITOTIC PROGRESSION
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BACKGROUND AND PURPOSE: Multidrug resistance (MDR) is a well-known drug efflux pump whose overexpression has been primarily observed in human cancer cells that are resistant to chemotherapy. MDR encodes for a P-glycoprotein and has been found to be expressed in majority of brain tumors, including glioblastomas. It has been reported that MDR activity was inhibited via JNK activation. We previously reported that the overexpression of REIC-Dkk3 exerts anti-tumor effects in a glioblastoma cell line and it was associated with JNK activation. However, the relationship between REIC-Dkk3 and MDR remains to be elucidated. In this study, we examined whether adenovirus vector REIC-Dkk3 (Ad-REIC) augments the anti-tumor effects of temozolomide by down-regulating MDR in glioblastoma cells.

METHODS: We treated glioblastoma cells (U87MG) with temozolomide or Ad-REIC alone or in combination and compared to the cells treated with Ad-LacZ. The gene and protein expression of MDR, JNK, pJNK, c-JUN and p-c-JUN was analyzed by western blot, immunohistochemistry and quantification of RT-PCR.

RESULTS: Effects of Ad-REIC or temozolomide alone in cell survival were limited in the U87MG cells. However, the combination of Ad-REIC and temozolomide induced significant cell death. In cells treated with Ad-REIC the expression of MDR1 was significantly reduced compared to cells treated with Ad-LacZ or temozolomide alone. Furthermore, Ad-REIC enhanced the anti-proliferative activity of pJNK and p-c-JUN. Applications of a JNK inhibitor reverse the effects of Ad-REIC on MDR...
expression and cell death. These results suggest that Ad-REIC contributes to the improvement of chemosensitivity to temozolomide therapy by downregulation of MDR through activation of JNK. CONCLUSION: Ad-REIC gene therapy may be a potential therapy for glioblastoma.

CB-057. THE microRNA-31-TRADD/NF-κB CIRCUITRY IS DYSREGULATED IN GliOBLASTOMA
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GBM are the common and deadliest tumors of the CNS. Despite aggressive therapeutic approaches, these tumors remain incurable. The NF-κB family of transcription factors mediates immune and inflammatory signaling. Normally, NF-κB is inhibited by interactions with IκBκ. However, in response to a stimulus such as TNFα, NF-κB becomes activated. Specifically, TNFα binds to its receptor, TNFR1, which promotes oligomerization and TRADD recruitment. Next, TRADD initiates a signaling cascade that ultimately activates NF-κB. Normally, the activity of NF-κB is tightly regulated. However, in many tumors, including GBM, NF-κB is constitutively activated and its target genes are overexpressed. MicroRNAs (miRs) are a class of short, endogenous, single-stranded RNA molecules that bind specific mRNA to inhibit their translation. Genes encoding miRs are often found in Cancer Associated Genomic Regions (CAGRs) and are usually dysregulated in cancer. This leads to disrupted mRNA and protein expression, and contributes to tumor development and/or progression. miR-31 is a microRNA with pleiotropic properties. In many cancers, miR-31 expression is reduced or absent. Here, we demonstrate that homozygous deletion of miR-31 is most pronounced in GBM compared to other cancers analyzed, and its loss occurs in >35% of all GBMs. We find that miR-31 regulates TRADD expression and show that in response to TNFα stimulation, miR-31 is sequestered in the nucleus, thus stabilizing TRADD and allowing NF-κB activation. However once activated, NF-κB increases miR-31 levels, which reduce TRADD levels and limit NF-κB signaling. In vivo, the loss of miR-31 correlates with increased NF-κB activity and tumor size. Conversely, miR-31 restoration reduces NF-κB activity and limits tumor growth. Finally, in patients, the loss of miR-31 translates to significantly shorter survival rates. Collectively, these data underscore the important role of miR-31 in glioma biology, and highlight its attractive therapeutic potential in treating GBM.

CB-058. REGULATION OF CANCER PROMOTING TRYPTOPHAN CATABOLISM IN GliOBLASTOMA BY THE GLUCOCORTICOID RECEPTOR
Martina Ott1,2, Ulrike Litzenburger1,2, Katharina Raaschuschenbach1,2, Lukas Bunscher1,2, Stefan Pusch3,4, Katharina Ochs1,2, Felix Sahm1,2, Christiane Opitz1,2, Andreas von Deimling3,4, and Christiane Opitz2,5, Andreas von Deimling3,4, and Wolfgang Wick2,6; a Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany; bDepartment of Neurooncology, University Hospital Heidelberg and National Center for Tumor Diseases, Heidelberg, Germany; cClinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany; dInstitute for Neuropathology, University Hospital Heidelberg and National Center for Tumor Diseases, Heidelberg, Germany; eJunior Research Group Brain Tumor Metabolism, German Cancer Research Center (DKFZ), Heidelberg, Germany; fClinical Cooperation Unit Neuromediated TDO downregulation was abolished. Most important, this interaction also takes place in vivo as demonstrated by in situ proximity ligation assay on human glioblastoma tissue. Accordingly, gene expression profile analyses revealed negative correlations of the GR and FKBP52 with TDO in glioma and neural tumors. In summary, we identify a novel steroid-responsive FKBP52-dependent pathway suppressing the expression and activity of TDO, a central enzyme in human brain tumor immunobiology.

CB-059. Mir-128 CONTROLS THE ACTIVITY OF EPIGENETIC REGULATORS PRC1 AND PRC2 IN NEURAL STEM CELLS: IMPLICATIONS OF ITS LOSS IN GLIOMAGENESIS
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Polycrumb Repressor Complexes (PRC) 1 and 2 are major chromatin modulators whose coordinated activity determines histone modification of H2A and H3, respectively. This results in transcriptional repression of genes involved in cellular differentiation, maintaining cellular stemness. In glioblastoma, PRC activity has oncogenic functions and is involved in radio-resistance by favoring DNA Double Strand Break repair. While it is known that PRC1 and PRC2 are functionally related and partially redundant in their repressive action, little is known on the mechanisms that control their activity. Here we show that miR-128, downregulated in glioblastoma, directly targets BMI1 and SUZ12, key components of PRC1 and PRC2 respectively, thus exerting a fundamental role in PRC repression and epigenetic silencing. Loss of function of miR-128 is inversely correlated to that of BMI1/SUZ12 in operative specimens of glioblastomas versus normal brain and in glioma stem cells versus neural stem cells. Gain of function of miR-128 results in decrease CD133 stem cell marker, reduced proliferation and impaired clonal ability of glioma initiating cells. Knock down of miR-128 in neural stem cells results in increase BMI1 and SUZ12 levels, associated to greater cell clonogenicity. In a genetic mouse model of glioblastoma, we found that miR-128 expression is blocked in young, pre-symptomatic mice, suggesting that loss of miR-128 expression is an early event in gliomagenesis. Furthermore, as BMI1 and SUZ12 take part in the cellular response to DNA double strand break repair, here we show that miR-128 prevents BMI1 and SUZ12 upregulation after irradiation resulting in an impaired capacity of DNA repair and consequent cell death. Finally, miR-128, although virtually undetectable, is not loss in glioblastoma initiating cells, and its expression can be stimulated by pharmacologic treatment with cyclic AMP (cAMP). This is of great importance as it lays the bases for a technically achievable, miR-128-based therapy for glioblastoma multiforme.

CB-060. A KINOME-WIDE RNAi SCREEN IN DROSOPHILA GLIA REVEALS THAT THE RIO KINASES MEDIATE CELL PROLIFERATION AND SURVIVAL THROUGH TORC2-AKT SIGNALING IN GliOBLASTOMA
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Glioblastomas frequently display mutations that activate receptor tyrosine kinase (RTK) and PI3 kinase (PI3K) signaling pathways. Yet, therapeutic targeting these pathways in glioblastomas have proven ineffective in the clinic. In Drosophila melanogaster, activation of RTK and PI3K pathways in glial progenitor cells creates malignant glial tumors that display many features of human glioblastoma. We used this Drosophila glioblastoma model to perform genetic screens for new genes required for RTK- and PI3K- dependent glioblastoma progression. From this screen, we isolated orthologs of the RIO kinases, which, upon knockdown in Drosophila, caused a synthetic growth reduction and cell lethality in the context of oncogenic RTK-PI3K signaling. Our results from human glioblastoma cells and tumors revealed that RIO1 and RIO2 are overexpressed in tumor cells in response to Akt signaling downstream of oncogenic RTK-PI3K activity. Little is known about the functional role of RIO in glioblastoma. Here, we show that RIO expression is reduced in normal neurons and glia, suggesting that upregulation of RIO expression is tumor specific. Knockdown of
of bromodouronydine-positive (S-phase) and -negative (G1 and G2 phase) cells showed that carnosine treatments resulted in a significant increase in the percentage of G2 cells, 37 ± 0.4% compared to 17 ± 0.3% in controls. Carnosine-induced G2-accumulation was associated with 2.5- to 4-fold increase in cyclin B1 mRNA and protein levels. Among the carnosine derivatives tested, anserine (methylated carnosine) exhibited the most potent antiproliferative effect. These results demonstrate that the antiproliferative property of carnosine is due to its ability to enhance MnSOD expression and to induce G2-delay. These results will be of significance in the potential clinical application of carnosine and its derivatives in glioblastoma therapy.

CB-063. GLIOMA-ASSOCIATED IDH MUTATION INDUCES miR-34A REPRESSION AND SCREW-CELL-LIKE PHYSIOLOGY THROUGH ENHANCED PDGF SIGNALING
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Point mutations in isocitrate dehydrogenase enzymes (IDH1 and IDH2) have been identified in 70-90% of lower-grade gliomas (LGGs); WHO grade II and III) as foundational genomic alterations. Glioma-associated IDH mutations also occur and confer therapy resistance in active site arginine residues and confer a neomorphic activity resulting in overproduction of the oncometabolite R(+)-2-hydroxyglutarate (2HG). The effects of 2HG on cellular physiology are widespread and include global shifts in epigenomic profiles. LGGs have also been repeatedly linked with dysregulated platelet-derived growth factor (PDGF) signaling. We have recently demonstrated that miR-34a is downregulated in gliomas in response to dysregulated PDGF signaling and that miR-34a directly targets the PDGFR receptor (PDGFRα) in high-grade gliomas. Analyzing a panel of diffuse gliomas, we find that miR-34a is specifically downregulated in LGG in response to IDH mutation. Similarly, in multiple isogenic cell line systems, IDH mutation induces repression of miR-34a. Also in these contexts, IDH mutation induces neurosphere formation and the expression of stem cell-associated genes. miR-34a has been reported to target pluripotency factors such as LIN28, OCT4, and NANOG. Accordingly, we find that murine neural progenitor cells expressing IDH mutant isoform express higher levels of these factors than isogenic controls. The stem cell-like phenotype of IDH mutant-expressing cells is morphologically reversed by restoring miR-34a expression in the cells. Despite findings that IDH mutation correlates with hypermethylation at the MIR34a locus in LGG tumors, we have not been able to demonstrate the functional relevance of this. Instead, we find that IDH mutation is associated with enhanced PDGF signaling, and we demonstrate that inhibition or silencing of PDGF signaling in IDH mutant cells increases or decreases miR-34a expression, respectively. Our studies support a model in which glioma-associated IDH mutation induces miR-34a repression and stem cell-like physiology through enhanced PDGF signaling.

CB-064. PRONEURAL PHENOTYPE AND TRANSCRIPTIONAL NETWORK PRECEDES AND SELECTS FOR GENETIC ALTERATIONS DURING GLIOMA PROGRESSION
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Glioblastoma is a diverse disease that can be divided into distinct subtypes based on the basis of molecular and genetic profiling. The Proneural subtype has an expression profile, which resembles that of oligodendrocyte progenitor cells (OPCs), and is associated with a specific set of genetic alterations. However the functional relationships between the Proneural phenotype and the associated genetic alterations have not been resolved. To address this issue, we used a mouse model of Proneural glioma and long-term tracked alterations in gene copy number (by array CGH) and expression profiles (by RNA-Seq) at multiple time points during tumor progression. We
showed that murine gliomas induced by retrovirus delivery of PDGF and Crt-mediated deletion of Pten, acquired a specific set of genetic deletions with remarkable consistency as they progressed from low to high grade tumors. Cross-species analyses revealed that subsets of these genes are also selectively deleted in human Proneural glioblastoma. Expression analysis at early time points showed that mouse tumors had a Proneural expression pattern, characterized by high levels of OPC genes, prior to the acquisition of the recurrent gene deletions. Further analysis identified transcription factors that act as master regulators of the Proneural phenotype, revealing a transcriptional network that was highly connected to p53 as one of the key master regulators at early and late stages of glioma progression. Experimental alteration of this regulatory network by upfront deletion of Trp53, led to accelerated glioma formation and obviated the acquisition of subsequent deletions. These results show the interplay between the pre-existing regulatory network, which defines cellular phenotype, and the genetic alterations that accumulate during progression of Proneural glioma.

CB-065. DIFFERENTIATION OF GliOBLASTOMA MULTIFORME STEM-CELL-LIKE CELLS LEADS TO DOWN REGULATION OF EGFR AND EGFRvIII AND DECREASED TUMORAL EXTRASTENTENTIAL

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Glioblastoma multiforme (GBM) is the most common and devastating primary brain tumor among adults. Despite recent treatment progress, most patients succumb to their disease within 2 years from diagnosis. Current research have highlighted the importance of a subpopulation of cells, assigned brain stem-like cells (bCSC), to play a crucial role in GBM. bCSC are identified by their resemblance to normal neural stem cells (NSC), and it is speculated that the bCSC have to be targeted in order to improve treatment outcome for GBM patients. One hallmark of GBM is aberrant expression and activation of the epidermal growth factor receptor (EGFR) and expression of a deletion variant EGFRvIII. In the normal brain, EGFR is expressed in neurogenic areas where also NSC are located and it has been shown that EGFR is involved in regulation of NSC proliferation, migration and differentiation. Thus, it is intriguing to speculate if EGFR and EGFRvIII are involved in the regulation of bCSC. In this study we use GBM neurosphere cultures, known to preserve bCSC features. We demonstrate that EGFR and EGFRvIII are down regulated upon differentiation and moreover that when EGFR signaling is abrogated, differentiation is induced. Furthermore, we show that differentiation leads to decreased tumorigenic and stem cell-like potential of the neurosphere cultures and that by specifically inhibiting EGFR signaling it is possible to target the bCSC population. Our results suggest that differentiation therapy, possibly along with anti-EGFR treatment would be a feasible treatment option for patients with GBM, by targeting the bCSC population.

CB-066. THE GliAL DIFFERENTIATION FACTOR NUCLEAR FACTOR ONE B (NFIB) INDUCES DIFFERENTIATION AND INHIBITS GROWTH OF GliOBLASTOMA

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The phylogenetically-conserved vertebrate transcription factor, NFIB, is an orchestrator of glial differentiation in the developing mammalian central nervous system. We found NFIB expression to be reduced in glioblastoma (GBM), the commonest and most lethal primary adult brain cancer, so investigated what effect increased expression of NFIB had on GBM. Increased expression of NFIB in primary GBM cell lines induced expression of markers of glial differentiation, inhibited cell proliferation, reduced stem/progenitor cell growth, altered cell cycle progression and inhibited tumor growth in murine models of GBM. We thus identified NFIB to be a novel tumor suppressor gene in GBM.

CB-067. AUTOPHAGY BLOCKADE IN GBM IMPROVES SENSITIVITY TO TYROSINE KINASE INHIBITION

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Gliomas are the most common type of brain tumor and occur in both adults and children. Current treatments, radiation and cytotoxic chemotherapy (temozolomide), are ineffective, with an average survival of 14 months and a treatment response rate of approximately 10%, indicating that new therapeutic agents need to be developed. The TAM family of receptor tyrosine kinases (RTKs), represented by Tyro-3, Axl, and Mer-TK, is ectopically expressed in glioma tumors, resulting in anti-apoptotic signaling, facilitating tumor cell survival. My preliminary data shows treatment of cells with radiation upregulates MerTK expression. Inhibition of TAM RTKs decreases cell survival, proliferation and migration. Previous studies in the lab have shown shTAM inhibition sensitizes cells to temozolomide. Radiation and temozolomide therapy increases autophagy, suggesting autophagy is a cytoprotective mechanism and therefore an attractive target. I hypothesize TAM inhibition and autophagy blockade will increase efficacy of glioma treatment. To conduct our experiments we utilized two primary patient glioblastoma cell lines and two commercially available glioblastoma cell lines. Using GFP-m-Cherry tagged LC3 protein along with western blot analysis we determined that inhibition of TAM, both genetically (shRNA) and pharmacologically (TKIs), leads to increased autophagic flux. Inhibition of autophagy flux, using chloroquine, in combination with TAM inhibition results in decreased proliferation compared to either agent alone. Additionally, we have confirmed that in our cell lines autophagy is increased post radiation treatment. These results suggest that TAM inhibition in combination with autophagy blockade results in more efficacious therapy for glioblastoma.

CB-068. BRAIN-SPECIFIC GENE SIGNATURE OF MELANOMA METASTASIS

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Melanoma brain metastases are highly resistant to therapy and carry a dismal prognosis. In this work, we sought to identify genetic drivers of melanoma brain metastases using high throughput gene expression and copy number analysis. We used a composite model of human melanoma (Cancer Res 2013; 73: 2445–56). Immunodeficient mice were injected intracardially with a cell line developed in our laboratory from a resected tumor of a patient with multiple brain metastases. Bioinformatic analysis was used to identify the most dysregulated gene families and to track brain metastasis formation. The animals consistently developed metastases in their brains and frequently in the adrenals, ovaries, and femurs. These four organs were enzymatically dissociated and a supervised rank product analysis comparing brain samples with all the other organ samples (percentage of false positives cut-off < 0.001). Five brain-specific genes were significantly over-expressed in both analyses: CB544, MX1, XAF1, and AC021615.4 – all previously implicated in cancer, several in melanomas and gliomas, but none in brain metastasis. Each gene could also be validated in a rank product-based meta-analysis comparing the brain sample set with each of the other sample sets. At present, we are correlating the brain metastasis gene expression signature with protein expression from immunohistochemistry of human TMA’s of brain metastases, and mouse brain and peripheral metastases at different developmental stages. These results will be linked, through pathway and drug sensitivity analyses, to clinical data. Furthermore, each gene will be functionally validated in vivo by screening a shRNA library targeting all candidate genes.

CB-069. EXPRESSION OF NDRG2 SENSITIZES GliOBLASTOMA CELLS TO TEMOZOLOMIDE VIA Akt INACTIVATION

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The N-Myc down-stream-regulated gene 2 (NDRG2) has been identified as a potential tumor suppressor. Its expression was negatively correlated with the pathological grade of gliomas. The relationship between NDRG2 expression and the effect of temozolomide (TMZ) has not been reported. In the present

Abstract

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CB-070. MicroRNA-183 UPRREGULATES HIF-1α BY TARGETING ISOCITRATE DEHYDROGENASE 2 (IDH2) IN GLIOMA CELLS
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INTRODUCTION: Our previous study revealed extensive modulation of a set of microRNAs (miRs) in malignant glioma. In that study, miR microarray analysis demonstrated the upregulation of microRNA-183 (miR-183) in glioblastomas. Therefore, we examined the expression levels of miR-183 in various types of glomas and the association of miR-183 with isocitrate dehydrogenase 2 (IDH2), which has complementary sequences of miR-183 in the 3'-untranslated region (3'UTR). MATERIALS AND METHODS: We searched for the target of miR-183 using the “miRanda”, “TargetScan”, “PicTar” website, and found that miR-183 contains complementary sequences to the 3'UTR of IDH2 mRNA. We transfected glioma cell lines with miR-183 mimic RNA and analyzed the expression levels of IDH2 and HIF-1α using real-time RT-PCR and western blotting. We also determined the association between miR-183 and vascular endothelial growth factor (VEGF) or glucose transporter 1 (GLUT1), which are downstream molecules of HIF-1α. Furthermore, we generated a luciferase reporter plasmid containing the wild-type or mutant IDH2 3'UTR, and performed luciferase assays by co-transfecting the miR-183 and plasmid into the glioma cells.

RESULTS: We demonstrated that miR-183 is upregulated in the majority of high-grade gliomas and glioma cell lines compared with peripheral, non-tumorous brain tissues by the real-time PCR analysis. The expression levels of IDH2 are downregulated via the overexpression of miR-183 mimic RNA in glioma cells. We also verified that miR-183 directly affects the level of IDH2 mRNA in glioma cells using luciferase assays. Furthermore, we showed that expression levels of HIF-1α, VEGF, and GLUT1 are upregulated in glioma cells following transfection with miR-183 mimic RNA by real-time RT-PCR. CONCLUSION: Upregulation of miR-183 in malignant glioma induces HIF-1α expression by targeting IDH2 and may play a role in glioma metabolism.

CB-071. INTEGRATIVE miRNA-mRNA GENOMIC ANALYSIS IDENTIFIES DYSREGULATED miRNAs IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)
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Epigenetic dysregulation has been implicated in many cancers. Recently, a specific histone mutation was identified in ~80% of DIPG tumors, suggesting a critical role of epigenetic dysregulation in DIPG pathogenesis. MicroRNAs (miRs) are another important component of the epigenetic regulatory system. Aberrant expression of microRNAs has been shown to play important roles in the oncogenesis of many cancers. To understand the role of microRNAs in DIPG pathogenesis, we performed an integrative miRNA-mRNA genomic analysis of post-mortem DIPG tumors in comparison to normal brain tissues from the same subjects together with an analysis of miRNA-mRNA profiles from RNA tumorsphere cell lines, normal brain cell lines, and normal cerebellar tissue. In this study, we performed integrative genomic analyses of DIPG cases demonstrating a significant enrichment of differentially expressed miRNAs and mRNAs in DIPG. We also identified a set of differentially expressed miRNAs and mRNAs in DIPG tumors. These results suggest that microRNAs may play a critical role in the pathogenesis of DIPG and provide new insights into the molecular mechanisms underlying this disease.

CB-072. MUTATIONS OF CD79B ARE COMMON EVENT IN PRIMARY CENTRAL NERVOUS SYSTEM B-CELL LYMPHOMA PATIENTS AND ARE ASSOCIATED WITH UNFAVORABLE PROGNOSIS
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PURPOSE: Constitutive activation of the nuclear factor-kappa B (NF-kB), a transcription factor that regulates cell proliferation, differentiation, and apoptosis, is crucial for cell survival of systemic activated B-cell like diffuse large B-cell lymphoma (ABC-DLBCL) as well as primary central nervous system lymphoma (PCNSL). CD79B mutations occur in a small subset of ABC-DLBCL and trigger chronic B-cell receptor signalling, resulting in NF-kB pathway activation. In this study, we set out to examine the incidence and prognostic impact of CD79B mutation in PCNSL patients. MATERIALS AND METHODS: 61 immunocompetent PCNSL patients with tissue samples available for analysis were included in the study. Exon 5 of CD79B, which contains the sequence encoding the first tyrosine (Y196) of the ITAM domain, was amplified and sequenced. RESULTS: CD79B mutations were identified in 26.2% (16/61) of the cases, confirming the somatic origin of the mutations. Of these, missense mutations affecting the first ITAM tyrosine (Y196) were observed in 87.5% (14/16). The PS in patients with CD79B-mutated tumors was significantly shorter than those with wild-type tumors (P = 0.049, Wilcoxon test). Patients with CD79B wild-type tumors showed a tendency to have longer overall survival, however the difference did not reach statistical significance (P = 0.13, Wilcoxon test). CONCLUSIONS: Mutations of CD79B frequently occur and predominantly target the ITAM domain in PCNSL. We propose that the CD79B ITAM domain status is a clinically applicable candidate prognostic marker for PCNSL patients.

CB-073. ELUCIDATING THE MECHANISM OF RECEPTOR TYROSINE KINASE INHIBITION BY SOLUBLE LRIG1 IN GBM TUMORS
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In Glioblastomas (GBM) aberrant receptor tyrosine kinase signaling is found in up to 90% of all cases. A majority of GBMs display gene amplification and overexpression of epidermal growth factor receptor (EGFR) and co-express a mutant form of the receptor, often displaying a truncation in the extracellular domain. The most prominent of the truncated receptors is EGFRVIII which is constitutively active and therefore highly oncogenic. We showed recently that the soluble, extracellular domain of LRIG1, a tumor suppressor and stem cell regulator, strongly inhibits glioma growth in vitro and in vivo in patient-derived GBM xenografts. Local delivery of soluble LRIG1 in pre-established tumors significantly increased survival of tumor bearing mice. LRIG1 belongs to the family of Leucine-rich Repeats and Immunoglobulin-like domain proteins and is a negative regulator of EGFR signaling by inducing receptor degradation. Interestingly we found that the tumor growth inhibiting effect of secreted LRIG1 is independent of EGFR expression level and was effective on wildtype and mutant EGFR expressing tumors. Moreover the phosphorylation of EGFR was not affected by exposure to soluble LRIG1. However we observed reduced phosphorylation of its downstream effector MAPK, while AKT signaling was not altered. In order to unravel the mechanism of action of soluble LRIG1 we currently analyze the signaling pathways involved in the response and determined the minimal part of soluble LRIG1 responsible for the tumor growth inhibitory effect in GBM. Using co-immunoprecipitation and gene expression studies, we further address if the effect of secreted LRIG1 involves interaction of other receptor tyrosine kinases and its signaling partners in glioma cells. The results from our ongoing studies addressing the mechanism

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The dioxin/aryl hydrocarbon receptor (Ahr) is a transcription factor, which has been attributed a role in human carcinogenesis, cell cycle progression and transformation growing factor-b (TGF-β) signaling. The endogenous tumor-promoting ligand of the human Ahr kynurenine was discovered recently. It is constitutively generated by tumor cells via tryptophan-2,3-dioxygenase (TDO), a neuron-derived tryptophan-degrading enzyme. The TDO-Ahr pathway is active in human brain tumors and is associated with poor survival. We used real-time RT-PCR to quantify the expression of Ahr, TDO, the Ahr nuclear translocator (ARNt), and the repressor of Ahr (AHRR). We examined a set of 70 human glioma samples representing the differences between the three degrees of malignancy (WHO grade II-IV), primary and recurrent gliomas, pure and mixed astrocytic tumors, and with and without chemotherapy. We also studied whether the expression profile changes as tumor progression progresses in 20 individual patients. Both, the expression of Ahr and ARNT were significantly increased after chemotherapy (CTX). The Ahr was upregulated from 0.69 before CTX to 3.49 after CTX in secondary GBM and from 0.76 to 1.51 in primary GBM respectively (p = 0.003). The mRNA expression of ARNT was increased from 1.37 before CTX to 2.7 after CTX in secondary- and from 0.86 to 1.68 in primary GBM (p = 0.009). In contrast, TDO expression was downregulated during temozolomide CTX in GBM from 0.77 to 0.35 (p = 0.03). Moreover, ARNT was significantly upregulated in grade II and grade III astrocytomas compared to peritumoral tissue (p = 0.0001). TDO expression was significantly higher in GBMs compared to lower grade astrocytomas (p = 0.001). Ahr and ARNT were significantly upregulated after chemotherapy in glioblastoma. In contrast, TDO was significantly downregulated. These results provide evidence for an important pathophysiological role of the Ahr with profound implications for cancer and immune biology and could have broad implications for potential targeted therapies.

The glutathione S-transferase pi gene (GSTP1) is a polymorphic gene encoding a drug-metabolizing gene product on chromosome 1q21. Four different GSTP1 genotypes exist, which have different roles in xenobiotic metabolism and might play a role in susceptibility to cancer, and other diseases. This placental form of GST is the predominant form in human brain tissue and an important drug-detoxifying enzyme. Our hypothesis was a correlation of this protein to the grade of malignancy and chemotherapy resistance in glioma. Therefore, we set out to measure the GSTP1 expression at the mRNA and protein level in order to elucidate the potential role of GSTP1 expression as a biomarker for disease progression and predictor of chemosensitivity in human glioma. We quantified the expression of the drug-metabolizing gene GSTP1 using real-time RT-PCR. More than 70 astrocytic tumor samples were used including all three degrees of malignancy (grade II-IV), primary and recurrent gliomas, pure and mixed astrocytic tumors, with and without chemotherapy. Moreover, we analyzed longitudinally tumor recurrence and disease progression in individual patients (n = 13). In addition, protein expression and localization was evaluated by Western Blotting and immunohistochemistry. There were no changes in GSTP1 mRNA expression, determined by quantitative RT-PCR, between diffuse astrocytomas (1.80 ± 0.85), anaplastic astrocytomas (2.53 ± 2.13), secondary GBM without (2.48 ± 1.02) and with chemotherapy (2.57 ± 1.40), primary GBM (1.95 ± 1.14) and recurrent primary GBM after Stupp protocol (3.13 ± 1.70). The latter difference concerning the expression of GSTP1 after radiochemotherapy in primary GBM, however not a significant one (p = 0.08). The expression of GSTP1 was highly heterogeneous within the surgical specimens. No significant differences in gene expression were detected between primary or recurrent gliomas, suggesting that glioma chemoresistance either intrinsic or might be multifactorial and GSTP1 independent.

CB-075. HETEROGENEITY OF HUMAN GLIOMAS: GLUTATHIONE S-TRANSFERASE PI EXPRESSION PROFILE DURING DISEASE PROGRESSION

CB-076. THE ROLE OF TWIST1 IN GLIOMA FORMATION AND PROGRESSION

CB-078. MODULATING PDGF LIGAND AND RECEPTOR SIGNALING IN GLIOBLASTOMA
Platelet-derived growth factor receptor A (PDGFRA) signaling is an important driver of oncogenesis in GBM. Amplification of the receptor is common, including in 22–38% of adult and pediatric GBM, and activating mutations in PDGFRA occur. In many cases, however, PDGFRA signaling is thought to be ligand dependent, suggesting the importance of the extracellular milieu. Heparan sulfate proteoglycans (HSPGs) regulate cell signaling by interacting with morphogens, growth factors, extracellular matrix and growth factor receptors. A major determinant of the affinity and specificity of HSPG-ligand interactions is the sulfation status of 6-O-sulfate (6OS) on heparan sulfate (HS).

We recently identified a novel role for the extracellular sulfatase, SULF2, in regulating PDGFRA signaling in GBM, and we demonstrated that ablation of SULF2 confers prolonged survival in murine glioma. Here we elucidate how SULF2 alters PDGFRA signaling in GBM and the factors associated with SULF2 upregulation that could drive oncogenesis. Consistent with our findings on SULF2 in PDGFRA signaling, we found that SULF2 was significantly upregulated (p = 0.0033) in GBM compared to non-amplified tumors (n = 40 amplified, 332 non-amplified). In contrast, SULF2 was downregulated (p = 0.0006) in EGFR-amplified GBM (n = 167 amplified, 205 non-amplified). As SULF regulates extracellular 6OS sulfation, SULF2 could potentially alter binding of PDGF ligands to HS or their release from sequestration. We found that SULF2 treatment of heparan sulfate did not alter levels of HS-bound PDGF or PDGFB, suggesting SULF2 does not affect binding of PDGF to HS. Studies addressing modulation of ligand availability by SULF2 and PDGFRA localization will also be presented. Interestingly, SULF2 knockdown or genetic ablation caused decreased expression of PDGF and PDGFB while SULF2 overexpression resulted in increased expression of PDGFs, suggesting multiple mechanisms of signaling modulation.

Thus, SULF2 may promote oncogenic PDGFRA signaling by influencing both ligand and receptor levels in GBM.

CB-079. REDUNDANT INSULIN RECEPTOR AND IGF-1R SIGNALING IN Glioblastoma
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Increased cancer risks and poor prognosis has been found in patients with obesity, 22–38% of adult and pediatric GBM, and activating mutations in PDGFRA occur. In particular, 2 diabetes is associated with poor prognosis in high-grade glioma. These observations can be attributed to, at least in part, increased levels of insulin, IGF1 and IGF2 in circulation and activation of corresponding signaling pathways in cancer. Aberrant activated IGF1R pathway induces downstream oncogenic signaling, such as MAPK and PI3K/AKT, and consequently promotes proliferation, survival, and therapeutic resistance in a range of human cancers. Loss of IGF1R signaling can be compensated by activation of the closely related insulin receptor (InsR). In this study, we interrogate the functions of the InsR and IGF1R pathway in glioblastoma. We identified a large subset of glioblastoma tumors that were highly sensitive to the dual InsR/IGF1R inhibitor, OSI-906. We further demonstrated that the InsR/IGF1R pathway represented the major activator of the PI3K/AKT signaling axis in these tumors. The majority of these tumors expressed both InsR and IGF1R. Knockdown of either InsR or IGF1R significantly impaired IGF1R dependent signaling can be compensated by activation of the closely related insulin receptor (InsR).

CB-080. EGFR PHOSPHORYLATES TUMOR-DERIVED EGF-RVIII, DRIVING STAT3/S AND PROGRESSION IN Glioblastoma
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A frequently occurring mutation in primary glioblastoma, EGF-RVIII (vIII), deletes ligand binding and signals constitutively. How this EGFR allele causes transformation has been elusive because of potential autocrine, paracrine loops triggered by vIII alone or heterodimers of vIII with EGFR. Our inability to fully elucidate and target this signaling has contributed to failed clinical trials in patients with few options for therapy. Here, we document co-expression of EGFR and vIII in primary human glioblastoma, with co-expression driving transformation in a cell intrinsic manner. We demonstrate a unique downstream pathway of STAT signaling triggered by EGFR-catalyzed phosphorylation of vIII, and tumorigenesis in vitro and in vivo. We show that EGFR promotes unidirectional phosphorylation of vIII, phosphorylating STAT even in the setting of kinase-dead vIII, implicating vIII as a substrate for EGFR. Phosphorylation of STAT3 required an allele of vIII competent for nuclear entry and with a nuclear component between vIII and STAT3. Our findings elucidate signaling interactions between EGFR and vIII, and suggest combinatorial targeting of the EGFR-vIII-STAT axis as a therapeutic approach to vIII-mutant glioblastoma.

CB-081. IDENTIFICATION OF MOLECULAR MECHANISMS OF RESISTANCE TO PI3K PATHWAY INHIBITION TO IMPROVE THERAPEUTIC TARGETING OF Glioblastoma
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Over 50% of human glioblastomas have PIK3R1/PIK3CA/PTEN mutations. PI3 kinase inhibitors produce a partial response, but complete response is rare. In the preclinical experimental models, about 50% of glioblastoma patients who benefit from PI3 kinase treatment eventually develop drug resistance after transient response. We propose that, as a result of selective pressure by PI3 kinase inhibitors, glioblastoma: 1) up regulate compensatory survival signaling pathways that, in some cases, may be ‘‘etable’’ with an existing drug or combination of drugs, and 2) utilize complimentary pathways as predominating mechanism of escape from anti-PI3K drugs that can be eliminated by concomitant use of complimentary inhibitors. To test these hypotheses, a large panel of glioma cells and glioma initiating stem cells (GICs) lines were treated with PI3K inhibitor BKM120 or PIK/mTOR dual inhibitor BEZ235. A differential response to PI3K pathway inhibitors BKM120, BEZ235 divided GICs into responder and non-responder with an aim to identify the molecular profiles that distinguishes the two groups. Gene expression profiling before and after PI3K inhibitor treatment was analyzed by Affymetrix microarrays to identify molecular targets/signatures contributing to resistance to PI3K inhibitors. We identified top 10 up-regulated candidate genes and validated these genes/proteins by quantitative PCR and western blot analysis. Disruption of the PI3K and AKT signaling pathway signatures before and after PI3K inhibitor treatment were analyzed by antibody-based proteomics (RPMA). ERK-b-catenin pathway was identified as pathways activated in the non-responders that may lead to resistance to PI3K inhibition. Therefore, our study suggested that targeting essential drug-resistance-related proteins and signaling pathways might allow successful GIC targeting therapy and improve glioblastoma treatment outcome.

CB-082. ACQUIRED RESISTANCE TO EGFR INHIBITORS IN Glioblastoma IS ASSOCIATED WITH UROKINASE-TYPE PLASMINOGEN ACTIVATOR-MEDIATED SUPPRESSION OF BIM
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The EGFR (EGFR) is the most commonly amplified or mutated gene in glioblastoma (GBM), but therapeutic targeting with small molecular tyrosine kinase inhibitors (TKIs) is limited by inherent and acquired resistance. Moreover, GBM is a highly heterogeneous disease and as a result, mechanisms of resistance may be equally heterogeneous from patient to patient. In an effort to model and characterize mechanisms of resistance to EGFR TKIs in GBM we developed vIII-hos, and in vivo model systems of acquired resistance to the EGFR TKIs gefitinib and erlotinib using TKI-sensitive mbv4a/arf−/− or mbv4a/arf−/− astrocytes expressing the constitutively active mutant AEGFR (or EGFRVIII). Clonal TKI-resistant cell lines were generated by chronic drug exposure in two systems: cells grown in vitro in colony growth assays or as xenograft tumors. Characterization of TKI-resistant cell lines revealed that although heterogeneous mechanisms do exist, a common feature was the
growth of MB in mouse, suggesting that Nestin is important for MB progression. We have further revealed that Nestin can stabilize the zinc finger protein Gli3, and subsequently augments Shh pathway activation in MB cells, indicating the important role of Nestin in regulating the output of Shh signal activation. Moreover, Nestin expression in MB cells depends on enhanced synthesis of leukotriene, a lipid derivative. Leukotriene inhibition abolishes Nestin expression in MB cells and simultaneously decreases their proliferation, but has no effect on normal GNP proliferation. These data suggest that inhibition of leukotriene synthesis may represent promising new treatments for human MB. This study sheds light on the essential role of Nestin in Shh signaling activation and the associated medulloblastoma tumorigenesis. Given that leukotriene synthesis is an established drug target, our work paves the road to develop improved approaches to treat medulloblastoma through targeting leukotriene synthesis.

CB-085. BAII IS A NOVEL TUMOR SUPPRESSOR IN MEDULLOBLASTOMA
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Brain-specific Angiogenesis Inhibitor 1 (BAII) is a seven transmembrane G protein-coupled receptor (GPCR) with potent anti-angiogenic and anti-tumorigenic properties. Recent studies have shown that the genes encoding BAII family proteins are lost and/or undergo somatic mutations in several cancers, including lung, breast, ovarian and brain, suggesting that BAII proteins might be tumor suppressors. In the present study we provide evidence that BAII expression is substantially downregulated in patient-derived samples of medulloblastoma, the most malignant brain tumor in children. Our molecular analyses provide evidence that the gene is epigenetically silenced and we identified several molecular mediators involved in its downregulation. We demonstrate that BAII expression can be reactivated by RNA interference against EZH2, a histone methyltransferase overexpressed in MB and MB2D, a DNA methyltransferase. Small molecule epigenetic modulators were also able to reactivate gene expression. As an independent means to study the role of BAII in tumor suppression we generated BAII knockout mice and crossed them with MB-pros transgenic mice. Deletion of BAII in mice induced aberrant proliferation of granule neuron precursors and accelerated tumorigenesis in mouse models. Taken together, our novel findings provide insight into the neurobiological mechanisms underlying cerebellar development and its susceptibility to neoplastic transformation.