Bevacizumab, a humanized monoclonal antibody developed to target and subsequently block vascular endothelial growth factor, received FDA approval for single agent treatment of recurrent glioblastoma multiforme (GBM) in 2009. Initial response rates and progression-free survival are promising for patients treated with bevacizumab, leading to its widespread adoption in both salvage therapy and as experimental initial therapy for GBM. Established bevacizumab side effects include hypertension, delayed/ impaired wound healing, gastrointestinal perforation, and proteinuria. Six cases of the MMP inhibitor clastil MG have been reported in the literature, indicating the need for greater surgical margins. These observations suggest that TLD screening may prove a useful presurgical tool to establish surgical borders and tailor-made antiprotease cocktail therapies that may be more efficacious than previous single MMP inhibitor therapies.

### AI-01. CASE REPORT: OPTIC NEUROPATHY IN A PATIENT WITH Glioblastoma RECEIVING BEVACIZUMAB

Robert A. Fishman, Erik Happ, Tracey Stevens, and Lara Kunschner; Allegheny General Hospital

Bevacizumab is a humanized monoclonal antibody developed to target and subsequently block vascular endothelial growth factor, resulting in decreased angiogenesis and improved wound healing. However, bevacizumab is associated with new onset of optic neuropathy, impaired vision, delayed wound healing, and proteinuria. Six cases of optic neuropathy have been reported in the literature, indicating the need for greater surgical margins. These observations suggest that TLD screening may prove a useful presurgical tool to establish surgical borders and tailor-made antiprotease cocktail therapies that may be more efficacious than previous single MMP inhibitor therapies.

### AI-02. DEGRADOME PROFILING FAILS TO IDENTIFY A UNIQUE PROTEASE SIGNATURE IN PRIMARY MALIGNANT BRAIN TUMORS

Diane M. Jaworski, Holly M. Stradecki, Paul L. Penar, William W. Pendlebury, Caroline J. Pennington, Dylan R. Edwards, William C. Broadus, and Helen L. Fillmore; Univ. of Vermont College of Medicine; Univ. of East Anglia; Virginia Commonwealth Univ.

The primary treatment challenge presented by glioblastoma multiforme (GBM) is the insidious propensity of glioma cells to aggressively invade normal brain tissue, making surgical resection palliative rather than curative. Much attention has been focused on the role of proteases in this process. Although the human degradome, the repertoire of proteases produced by cells, is relatively well-characterized, its contribution to gliomagenesis remains unclear. Although the human degradome, the repertoire of proteases produced by cells, is relatively well-characterized, its contribution to gliomagenesis remains unclear. The present study was designed to identify protease signatures that are unique to glioma cell lines and to evaluate the potential of these proteasome inhibitors in preclinical models of glioma.

### AI-03. ROLE OF NEUROPILIN1 AND ITS CO-FACTOR VEGF AND SEMA3A IN REGULATING HETEROGENEOUS MICRONVIRONMENT OF Glioblastoma MULTIFORME

Joydeep Mukherjee, Cynthia Hawkins, and Abhijit Guha; 1The Hospital for Sick Children; 2Department of Pathology, The Hospital for Sick Children; 3Department of Neurosurgery, Western Hospital AND The Hospital For Sick Children

INTRODUCTION: Glioblastoma multiforme (GBM) is characterized by pathological heterogeneity reflecting molecular heterogeneity in response to interactions with the tumor microenvironment and stromal cells. In this study, we focused on regional differences in the expression of Neureilin1 (Nrp1) with its co-factors to activate the vascular endothelial growth factor (VEGF) and Semaphorin3A (SEMA3A) signaling axis, thereby regulating neo-angiogenesis, migration and invasion. METHODS/RESULTS: Twenty-five paired flash-frozen and paraffin-embedded sections from the hypoxic center and non-hypoxic peripheral of GBMs were obtained on the basis of intra-operative magnetic resonance imaging–based stereotaxy and neuropathological verification. Five non-neoplastic human brain specimens were used as controls. Laser capture microdissection (LCM) was performed for sections was performed for the following degradome signatures: a) Tumor cells and endothelial cells, b) Tumor and core (102 genes), c) Tumor-stromal interface (139 genes) and d) Normal brain. The TLD screen identified 84 genes increased to at least 4 times the level of expression of control samples, indicating the need for greater surgical margins. These observations suggest that TLD screening may prove a useful presurgical tool to establish surgical borders and tailor-made antiprotease cocktail therapies that may be more efficacious than previous single MMP inhibitor therapies.

### AI-04. EFEMP1 ENHANCES INVASION OF U251HF HUMAN Glioma CELLS VIA MODULATION OF MMP2

Peter D. Pioli, Shadi Milani, Mark E. Linskey, and Yi-Hong Zhou; University of California-Irvine

Malignant gliomas are highly invasive primary brain tumors. The extracellular proteases and their inhibitors play a major role in defining a glioma cell’s ability to remodel its surroundings, thus allowing infiltration into surrounding normal brain tissue. One such family of proteases is the matrix metalloproteinase (MMP) family, which has demonstrated the ability to cleave various extracellular proteins and function in cell-cell signaling and also motility. MMP2 and MMP9 are the two MMP members particularly found in gliomas and can be associated with an increase of glioma cell invasiveness. Our recent study revealed a novel tumor suppression function of EGFR-containing tubulin-like extracellular matrix protein 1 (EFEMP1), which suppresses the tumorigenicity of glioma cells and inhibits proteases in vitro via suppression of angiogenesis, in s.c. xenograft models. However, we also found that EFEMP1 increased U251HF cell invasion through a Matrigel substrate. EFEMP1 has been shown to affect the tumorigenicity of multiple cancer types. Dependent upon cell type, EFEMP1 may function to suppress or enhance tumorigenic capacities. Consistent with our report of MMP2 enhancement of U251HF invasiveness, we found that MMP2 was upregulated at the protein level, along with increased activity in zymography assays of U251HF-overexpressing EFEMP1. Since real-time qRT-PCR showed that MMP2 was not increased by EFEMP1 at the mRNA level, it appears that EFEMP1 activation of MMP2 occurs post-transcriptionally. The MMP inhibitor TIMP3 was reported to be a strong binding protein of EFEMP1, which likely underlies EFEMP1’s function in activating MMP2 by sequestering MMP3 and therefore preventing its inhibition of MMP2. In conclusion, our data revealed that glioma invasiveness is also under positive regulation by EFEMP1 through modulation of MMP2 activity. The mechanism of EFEMP1 regulation of MMP2, with or without involvement of TIMP3, is under further investigation.
AI-05. USE OF HUMAN PROGENITOR CELLS FOR CELL-BASED DELIVERY OF ANGIOSTATIC GENES TO BRAIN TUMORS
Valentina Marchetti , Faith Barnett , Matthew Wang , Lea Scheppke , Javier Sanchez-Cespedes , Charles De Rossi , Glen Nemerov , Bruce Torbett and Martin Friedlander ; The Scripps Research Institute

Cell-based delivery of antiangiogenic genes, using neural and mesenchymal stem cells, has been proposed for the treatment of glioma. Previously, we have identified a rodent bone marrow population of endothelial progenitor cells (EPCs) that selectively target gliomas in vivo. These cells were transducible with lentivirus vectors (Barnett et al., CNS 2004). Previous studies have described the angiogenic molecule T2, a fragment of tryptophan-tRNA synthetase (TryptS) (Tzima et al., PNAS 2003). We have also demonstrated that T2 has a potent antiangiogenic effect in vivo (Dorrell et al., PNAS 2007).

In this study, we defined a subpopulation of the EPCs, CD44 high expressing cells, which migrate to the tumor mass as well as microsatellites in vivo in a 9L tumor model. CD44high cells were implanted into the brains of nude mice by inoculating the mice with glioblastoma cells that stably express T2 gene. We previously showed that T2 expression caused a significant reduction in tumor microvessel density and angiogenesis. In vitro, CD14+ cells targeted 9L and U87 glioma cell lines. In vivo, the activation of CD4+ cells with LPS gave similar results to those observed with murine CD44high cells, respectively. T2 gene was inserted in retroviral vectors and secreted by the same EPCs. Western blot analysis confirmed T2 expression. In vitro, CD14+ cells activated by IL1β expression. As the antiangiogenesis drug bevacizumab has come into routine use, we have identified two human cell populations useful as vehicles for delivery of antiangiogenic molecules in this cell-based tumor model are underway.

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AI-06. METASTATIC GLIOBLASTOMA (GBM): IMPLICATIONS IN THE AGE OF BEVACIZUMAB
Samuel A. Goldust , Samuel Singer , Lisa M. DeAngelis , Andrew B. Lassman , and Craig P. Nolan ; Memorial Sloan-Kettering Cancer Center

BACKGROUND: Symptomatic metastasis outside of brain parenchyma is a rare sequel of glioblastoma multiforme (GBM) usually found incidentally at autopsy. As the antiangiogenesis drug bevacizumab has come into routine use for GBM, patterns of recurrence may have changed. Bevacizumab promotes a diffuse and often multifocal nonenhancing pattern on neuroimaging.

Preclinical evidence in systemic malignancies suggests that drugs targeting angiogenesis may inhibit primary tumor growth while also promoting tumor invasiveness and hastening metastasis. Our objective was to determine whether the incidence of metastatic GBM has increased as bevacizumab has come into routine use.

METHODS: We identified patients with GBM and metastases between 2000 and 2005. We performed a retrospective chart review and compared the characteristics of patients with primary GBM and symptomatic metastases.

RESULTS: We identified 29 patients with metastatic GBM, 240 had received bevacizumab at any time during treatment of their GBM. In this cohort, symptomatic metastases were diagnosed in 6% of patients. Median OS for the cohort was 12 months (range 1.5–56 months). The median OS for patients with symptomatic metastases was 6 months (range 1.5–56 months) compared with 12 months (range 1.5–56 months) from diagnosis of GBM. Median OS for patients with symptomatic metastases was shorter than for patients with primary GBM (P < 0.001). Tube formation in Matrigel was also significantly reduced by two-fold (P < 0.01). Previously we have shown that antiangiogenic treatment improves outcome of oncolytic viral therapy and that in vitro combinations of SapC-DOPS and OVs have shown additive effects. This study supports the further development of SapC-DOPS as a novel antitumor and ant angiogenic agent for GBM.

AI-07. ASSESSMENT OF CETUXIMAB EFFICACY BY BIOLUMINESCENCE MONITORING OF INTRACRANIAL GLIOBLASTOMA XENOGRAFT IN MICE
Seung-Ho Yang and Sang Won Lee ; St. Vincent’s Hospital

A glioblastoma multiforme xenograft was established in athymic nude mice by inoculating the mice with glioblastoma cells that stably express luciferase (U87MG-LucNeo). The mice were monitored weekly using bioluminescence imaging (BLI). In the control groups, a steep rise in the bioluminescence signal was associated with the death of mice. As an adjuvant therapy, cetuximab (0.5 mg, 2 times) was intraperitoneally administered 4 weeks after nitrosoourea treatment. For a salvage therapy, cetuximab (0.5 mg, 2 times) was intraperitoneally administered when recovery of the bioluminescence signal was detected after nitrosoourea monotherapy. The antitumor effects of cetuximab adjuvant therapy were superior to those of cetuximab salvage therapy. This result was consistent with the results from a survival comparison among nitrosoourea monotherapy, and adjuvant and salvage therapies with cetuximab. These results suggest that BLI could be used as a simple tool for predicting the efficacy of various anticancer treatments in mouse models of brain tumors. The administration of cetuximab as an adjuvant to the conventional chemotherapeutic agent was more efficient than as a salvage therapy.

AI-08. ROLE OF VASCULOGENIC MICROMY in the DEVELOPMENT OF GLIOMAs
Zhong-Ping Chen and Xiao-Mei Liu ; Cancer Center, Sun Yat-sen University

BACKGROUND AND OBJECTIVE: Vascularogenic mimicry (VM) is defined as nonendothelial tumor cell-lined microvascular channels in aggressive solid tumors. Previous studies have demonstrated that VM enhances tumor invasion and motility by providing a unique blood supply, especially at early stages of tumor development.

METHODS: Tumor models in nude mice were established using human glioma cell line U87. To analyze the significance of VM in tumor development, we killed the mice to obtain a series of tumor specimens at different sizes. The tumor samples were dual stained for CD34 as well as PAS. The VM channels were considered present if PAS-positive channels within CD34-positive endothelial cells were found. Immunohistochemical staining (diameter about 1.0–2.0 cm), necrotic areas appeared. At the same time, VM channels were disappearing while the MVD was increasing.

CONCLUSION: These results suggest that VM channels may complement blood supply, especially at early stages of tumor development.

AI-09. NOVEL ANTIANGIOGENIC AND HYPOXIC EFFECTS OF SACP-DOPS AGAINST GLIOMA: ALONE AND IN COMBINATION WITH ONCOLYTIC VIRUSES
Jeffrey A. Worton 1, Zhengtao Zhu 1, Xiaoyang Qiu 1, and Balveen Kaur 1; 1Neuroscience Graduate Studies Program, The Ohio State University Medical Center; 2Division of Human Genetics, Children’s Hospital Research Foundation, University of Cincinnati School of Medicine; 3Department of Neurological Surgery, James Comprehensive Cancer Center and The Ohio State University Medical Center

Saposin C (SapC) is a sphingolipid activating protein found ubiquitously throughout the body, and it functions to catalyze glycosphingolipids. SapC preferentially fuses with negatively charged phospholipids at an acidic pH. When SapC is coupled with dioleoylphosphatidylserine (DOPS), stable nanovesicles are formed, which can fuse with- and lead to the apoptosis of—cancer cells. We hypothesized that SapC-DOPS nanovesicles could also have an effect on rapidly proliferative and migratory tumor endothelial cells. This was investigated using several in vitro and ex vivo angiogenic assays involving HUVEC and HDMEC. Treatment of HUVECs and HDMECs with SapC-DOPS yielded a significant two-fold reduction in cell migration at 6 hours (P < 0.01). Tube formation in Matrigel was also significantly reduced at 6 hours, and completely abolished at 15 hours, with a sublethal treatment of SapC-DOPS (P < 0.01). Further antiangiogenic effects were displayed in an ex vivo rat aortic ring assay, where vascular outgrowths were reduced by two-fold (P < 0.01). Intravenous delivery of fluorescence-labeled SapC-DOPS revealed specific targeting and antitumor efficacy of intracranial glioblastoma (GBM) tumors in vivo. Through live animal imaging, these nanovesicles were shown to specifically target phosphatidylserine (PS), a phospholipid normally sequestered on the inner leaflet of the cell membrane but frequently exposed on the cell surface of many cancer cells. As numerous stressors have been shown to increase the PS exposure of tumor cells, SapC-DOPS sensitivity was investigated in a hypoxic environment. Glioma cell killing in a hypoxic environment (1% O2) was significantly improved (P < 0.001) at several dosages at several dosages at several dosages at several dosages at several dosages at several dosages at several dosages at several dosages at several dosages at several dosages.
AI-10. EFEMP1 EXPRESSION IN GLIOMA: FRIEND OR FOE?
Yi-Hong Zhou1, Yuanjie Hu1, Peter D. Pohl1, Eric Siegel1, Daniel I. Ro1, S. Marlon 1, Nelson Hsu1, Shadi N. Milani1, Siddharth Mohan1, Liping Yu1, Kenneth R. Hess1, and Mark E. Liskey1; University of California, Irvine; 2University of Arkansas for Medical Sciences; 3Ziren Research LLC; 4University of Texas MD Anderson Cancer Center

EGF-containing fibrulin-like extracellular matrix protein 1 (EFEMP1) has demonstrated pro- and antitumorigenic roles dependent on cancer type. Our recent study revealed that EFEMP1 expression has a protective effect in prognosis for the most malignant glioma, glioblastoma multiforme (GBM), which is consistent with our data showing EFEMP1 suppression of tumorigenicity by a GBM cell line U251HF. Specifically, EFEMP1 overexpression led to the suppression of angiogenesis, partially via downregulation of proangiogenic gene VEGFA. We found that EFEMP1 expression was co-activated by PAX6 and PTEN—the two tumor suppression pathways found to be co-inactivated in GBM with poor prognosis by our prior prognosis study on PAX6 and PTEN expression. In contrast to GBM, the sign of the regression coefficient on EFEMP1 expression for patients with lower grade gliomas showed a deleterious effect on prognosis, which is in agreement with a recent report of EFEMP1 function in promoting human glioma cell migration. We studied the invasiveness by the same EFEMP1-transfected U251HCF cells with suppressed tumorigenicity and angiogenic ability and found that EFEMP1 overexpression increased cell invasiveness through Matrigel substrate via modulation of CXCR4 receptor protein level and activity but not MMP transcription. Our overall data from the study of EFEMP1 function in human glioma cell behaviors in vitro and in vivo, and in prognosis of patient survival, demonstrated content-dependent roles of EFEMP1, with its function in promoting invasion or suppression of angiogenesis, which could be dependent on or responsible for the malignant stage of gliomas. In summary, our data showed that maintaining or restoring EFEMP1 may have a therapeutic benefit against GBM by suppressing angiogenesis; however, the effect on enhancing cell invasion must be taken into account with anti-MMP agents.

AI-11. INHIBITION OF CXCR7 PREVENTS GLIOMA CELL PROLIFERATION AND MOBILITY AND TUMOR GROWTH
Yang Liu, Eleanor Carson-Walter, and Kevin Walter; University of Rochester

INTRODUCTION: The chemokine CXCL12/SDF-1 and its receptor, CXCR4, are well known for their critical roles in tumor invasion, proliferation, and metastasis. Recently, it has been reported that CXCR7 serves as second receptor for CXCL12, exerting an important role in tumor vascular formation and promoting the growth of many types of tumors. Elevated CXCR7 mRNA levels are associated with poor survival in glioma and GBM patients. However, the functional contribution of CXCR7 to malignant brain tumors remains largely unknown. METHODS: We used immunohistochemistry staining to identify cellular localization of CXCR7 in human glioblastoma (GBM) specimens. We suppressed expression of CXCR7 in glioma cell lines using small interfering RNA (siRNA) and measured the results using XTT proliferation assays, migration assays, and Transwell invasion assays. We examined the effects of CXCR7 inhibition on in vivo growth using U251MG intracranial xenografts in mice. RESULTS: CXCR7 protein was expressed in tumor cells as well as tumor vasculature. CXCR7 was expressed in multiple glioma cell lines. siRNA mediated suppression of CXCR7 significantly impeded cell proliferation and invasion in U87MG, U251MG, and U373MG glioma cells and reduced migration in U251MG and U373MG cells. Treatment of glioma cells with CXCR7 siRNA reduced the proliferation rate and the viability of glioma cells. siRNA-mediated knockdown of CXCR7 mRNA expression in U251MG cells significantly inhibited intracranial xenograft growth and improved mouse survival. CONCLUSIONS: CXCR7 contributes to glioblastoma proliferation and progression and can be regulated by HIF-2α. Targeting CXCR7 and HIF-2α might provide novel opportunities for improving brain tumor therapy.

AI-12. SPECIFIC KNOCKDOWN OF UPA/UPAR ATTENUATES INVASION IN GlioBLASTOMA CELLS AND XENOGRAPHS BY INHIBITION OF CLEAVAGE AND TRAFFICKING OF NOTCH 1 IN R127 TUMOR
HariRaghu, Christopher S. Gondi, Meena Gujrati, Dzung H. Dinh, and Jasti S. Rao; University of Illinois College of Medicine

uPA/UPAR is a multifunctional system overexpressed in many cancers and plays a critical role in glioblastoma (GBM) invasion. Studies have shown that Notch 1 is overexpressed and promotes invasion in glioblastoma. We tested whether downregulation of uPA/UPAR singly or in tandem attenuates GBM invasion via Notch 1 receptor. Targeted downregulation of uPA/UPAR, independently or simultaneously, inhibited the angiogenesis-independent growth of U251 and GBM xenograft cell lines by solid colony formation assay. All the four Notch receptors and the Notch ligands, Delta and Jagged, were expressed in U251 cells and in the GBM xenograft cell lines examined. Among them, Notch 1 mRNA was significantly downregulated by uPA/UPAR and U2 siRNA treatments in U251 and xenograft cells. In U251 cells and tumor xenografts, downregulation of uPA/UPAR, singly or simultaneously, inhibited the cleavage of Notch receptor between Gly 1743 and Val 1744 position, suggesting inhibition of activated cytosolic fragment release and downregulation of Notch gene transcription. Morphological analysis revealed inhibition of NICD in uPA/UPAR downregulated cells. Nuclear lysate analysis by Western blot revealed that uPA/UPAR siRNA treatment inhibited the cleavage of Notch 1 receptor in a RBP-J-dependent manner. uPA/UPAR siRNA downregulated Notch-mediated nuclear activation of NFκB/Rel subunits and the P3F-K/akt/mTOR pathway in U251 and GBM xenograft cells. Fibrinogen plasminogen zymography and Western blot analysis revealed that downregulation of Notch 1 by siRNA inhibited uPA activity and expression. Notch 1 receptor co-localized with LAMP-1, a lysosomal marker, in uPA/UPAR downregulated U251 and xenograft cells, indicating that trafficking of Notch 1 was altered. Notch 1 expression was significantly inhibited in uPA-, uPAR-, and uP2-treated pre-established intracranial tumors in nude mice. Taken together, we conclude that downregulation of uPA, uPAR, and U2 could be an effective approach to attenuate Notch 1 receptor cleavage, signaling, and endosomal trafficking in U251 and xenograft cells, thereby resulting in inhibition of invasion and metastasis.

AI-13. INVASION AS A DOMINANT FEATURE OF FAILURE PATTERN IN HIGH-GRADE GLIOMAS FOLLOWING BEVACIZUMAB THERAPY
Ashwatha Narayanan, Saroj D. Kummakatt, Praveen Medaballi, John Goflinos, Erik Parker, Edward Knopp, David Zloza, Deborah Gruber, and Michael L. Gruber; New York University Langone Medical Center

PURPOSE: With conventional therapy, local recurrence (LR) accounts for > 90% of all recurrences in high-grade glioma (HGG). Recent preclinical data have suggested that bevacizumab therapy can promote invasion and result in diffuse invasive recurrence (DIR). This study was done to see whether bevacizumab treatment has resulted in a change in recurrence pattern in HGG. METHODS: One hundred sixty-four consecutive patients with HGG, either newly diagnosed (n = 58) or recurrent (n = 106), were treated with bevacizumab at 10 mg/kg every 2 weeks along with convention al chemotherapy ± involved field radiotherapy. The treatment was continued until disease progression or development of dose-limiting toxicity. Determining pattern of recurrence and time to relapse were the primary aims of the study. DIR was defined as involvement of multiple lobes ± crossing the midline. RESULTS: With a median follow-up of 7 months (range 1–90), 100 patients experienced recurrence, with 77 patients experiencing DIR. Of all patients assessed, 29 newly-diagnosed patients (50%) and 47 recurrent patients (44%) experienced DIR. The median PFS for all patients was 6.8 months. No significant difference was observed between those who experienced LR versus DIR (6.2 vs. 6.4 months, respectively; P = 0.19). Overall survival was not affected by the pattern of recurrence (P = 0.104). CONCLUSION: While a propensity to invade brain parenchyma is inherent in HGGs, the occurrence of DIR increases significantly following treatment with bevacizumab. A modest improvement in survival and a shift in recurrence pattern with bevacizumab need to be confirmed in future clinical trials.

AI-14. VASCULAR RESPONSE TO IONIZING RADIATION IN NORMAL BRAIN AND HIGH-GRADE GLIOMAS
Kelly Batrull, Salomeh Jelveh, Patricia Lindsey, Richard Hill, and Gelareh Zadeh; University of Toronto

INTRODUCTION: Recent interest is focused on the role of bone marrow–derived precursor cells (BMDCs) in tumor neovascularization; however, very little is known about its role in glioblastoma multiforme (GBM). In this study, we investigated the role of BMDC in GBM neovascularization. METHODS: Animal Models: Bone marrow (BM) of NOD/SCID mice was subcutaneously injected into BM-depleted SCID mice at 9 months of age. Treatment: Mice were treated with/following 60 Gy whole brain irradiation. Tumor xenografts were generated using U87 human glioma cell line. Flow cytometry was used for BMDC quantification. RESULTS: BMDC infiltration was significantly higher in tumors from irradiated U87 xenografts compared to non-irradiated controls (P < 0.05). CONCLUSION: Exposure to ionizing radiation increases BMDC infiltration in glioma xenografts. Future studies will determine the role BMDCs play in glioma neovascularization.
in-vivo longitudinal images of the tumor cells, tumor vasculature, and tracing of the circulating GFP+ BMDC. Mice were imaged daily following cell implantation between 1 and 30 days. Histology: Mice were euthanized in accordance with our local animal care protocol and brains were analyzed by immunohistochemistry. RESULTS: One day following tumor cell implantation, BMDC were circulating intra-vascularily and lining the abluminal vessel wall. At 7 days, there was a significant migration of BMDC within the tumor periphery, they demonstrated clear incorporation of BMDC into vessel endothelial cells, supporting the process of de novo vessel formation or vasculogenesis, while tumor vessels within the central mass had no contribution by BMDC, supporting a role for the process of angiogenesis. CONCLUSIONS: Our results were the first to examine the dynamic contribution of BMDC to GBM neovascularization in real-time. We demonstrated that both vasculogenesis and angiogenesis contribute to GBM neovascularization in a tumor growth-dependent manner.

AI-15. DIRECT INHIBITION OF NON-MUSCLE MYOSIN II EFFECTIVELY BLOCKS GLIOMA INVASION IN THE PRESENCE OF MULTIPLE MOTOGENS
Sanja Iko,1 Christopher Beadle,1 Susan C. Massey+,1 Kristen R. Swanson+,1 Peter Carroll+1, and Steven S. Rosenfeld1; 1Columbia University; University of Washington

INTRODUCTION: Glioblastoma invasion is stimulated by dysregulated signaling pathways that are functionally redundant. We propose that targeting points where these redundant pathways converge will consistently block glioblastoma invasion, and that nonmuscle myosin II (NMII) is an example of such a point of convergence. We systematically examined how stimulation of two signal transduction pathways relevant to glioma invasion affects the need for NMII. METHODS: We have examined the effects of EGF- and PDGF-mediated stimulation of glioblastoma invasion using: 1) in vitro migration through Transwell membranes of C6 glioma cells expressing PDGFR and EGFR; 2) migration of glial progenitors that overexpress EGFR through brain slice, using a rodent model of gliomatosis cerebri; and 3) migration of EGFR-expressing tumor cells in a rodent model of glioblastoma that expresses PDGF and EGFR. RESULTS: EGF and PDGF each stimulated Transwell migration in C6 cells, and the effects of these two ligands were additive. While the EGF inhibitor gefitinib blocked EGF-stimulated Transwell migration, it had no effect on PDGF-stimulated migration. In contrast, the promigratory effects of these ligands could be completely blocked by blebbistatin, a direct inhibitor of NMII. In our model of gliomatosis cerebri, gefitinib blocked EGF-stimulated brain slice invasion, but the addition of exogenous PDGF could completely overcome this inhibition. However, blebbistatin was able to block invasion in the presence of high concentrations of both ligands. Finally, in our glioblastoma model, gefitinib was ineffective in blocking invasion, while blebbistatin remained completely effective. CONCLUSIONS: Our results confirm two key features of our underlying hypothesis. First, stimulating one promigratory signal transduction pathway can overcome the effects of blocking another. Second, a direct inhibitor of NMII blocks glioma invasion, while gefitinib does not, even with activation of two promigratory signal transduction cascades. Taken together, our results highlight the potential value of targeting NMII to block glioblastoma dispersion.

AI-16. A KEY TRANSCRIPTIONAL REGULATOR OF BRAIN TUMOR CELL INVASION AS A NOVEL THERAPEUTIC TARGET
Sean M. McAllister, Liliana Soraceano, Arash Pakdel, Chandani Limbad, Isabel Adrados, and Pierre-Yves Desprez; California Pacific Medical Center, San Francisco

The ability of gliomas to disperse from the primary tumor and invade into healthy brain tissue is driven by alterations in the regulatory mechanisms of cellular growth, migration, invasion and differentiation. The expression of Id proteins (inhibitor of basic helix-loop-helix transcription factors) has been reported to be dysregulated in many different types of cancer and to be a key determinant of tumor invasiveness in a wide range of tissues. We recently determined that one of these factors, Id-1, plays a critical role in the modulation of the dispersed and invasiveness of high grade gliomas (glioblastoma multiforme, or GBM). Id-1 expression correlates with human glioma cell invasiveness and proliferation in biopsies. Moreover, its specific knockdown inhibits cell invasion, induces profound morphological changes (i.e., redifferentiation), and induces glioma cell death. Finally, targeting Id-1 expression using compounds isolated from natural products may provide a new therapeutic strategy for GBM invasiveness and progression. Particularly, the nonpsychoactive and nontoxic cannabidiol significantly downregulates Id-1 gene expression and associated glioma cell invasiveness. Our ongoing studies aimed at assessing the efficiency of this compound and its analogues in culture and in animal models will shed light on potential clinical applications.

AI-17. INTEGRIN ALPHA3 IS INVOLVED IN THE INVASIVE BEHAVIOR OF GLIOMA STEM-CELL-LIKE CELLS
Mitsutoshi Nakada, Emi Nambo, Natsuki Furuyama, Yuya Yoshida, Daisuke Kita, Yutaka Hayashi, Yasuhiko Hayashi, and Jun-ichiro Hamada; Kanazawa University

INTRODUCTION: Accumulated evidence has shown that glioma stem-like cell properties are responsible for the gliomagenesis and are the source of recurrence. Glioma stem-like cells are shown to be invasive, but the mechanism of invasion remains to be elucidated. In this study we demonstrate the function of integrin in glioma stem-like cell invasion. MATERIALS AND METHODS: From glioblastoma specimens and glioma cell lines, neurospheres and CD133+ cells were collected by the sphere formation method and magnetic affinity cell sorting, respectively. Based on the adhesion assay, differential expression of the integrin family in glioma stem-like cells was analyzed by quantitative PCR, and the role in migration was further evaluated in glioma cell lines. RESULTS: Neurospheres from surgical specimens attached to both fibronectin and laminin, which are receptors in the integrin family. The expression profile of the integrin family in CD133+ cells was analyzed by quantitative PCR, and the role of integrin family in cell invasion was evaluated in glioma cell lines. CONCLUSION: Our results suggest that endogenous integrin alpha3 contributes to the invasive nature of glioma stem-like cells, which indicates integrin alpha3 as a prime candidate for anti-invasion therapy for glioblastoma.

AI-18. SLIT2 INHIBITS BRAIN TUMOR INVASION BY DOWNREGULATING MMP14
Mohamad Seyed Sadri1, Deborah Maret1, Emad Seyed Sadri1, Vincent Su1, Jad Alodhani1, Jean-Sebastien Denault2, Damien Faury3, Nada Jabado3, Andre Nantel3, and Rolando Del Maestro1; 1MNI - McGill University; 2IRB - NRC; 3MCH - McGill University

INTRODUCTION: Chemotropic cues such as Slit guide the migration of neuronal and glial cell precursors during development. Slit and its receptor Roundabout (Robo) have been implicated in tumor angiogenesis and leukocyte migration. Recent studies placing brain tumors in the context of neurodevelopment have led to the recognition of new tumor suppressors and oncogenes involved in tumor progression. Using methods such as sodium alginate bead technology, we have shown that SLIT2-ROBO1 signaling inhibits medulloblastoma cell invasion (Werbowskig-Oglivie et al., Oncogene 2006). We are expanding our observations by characterizing the SLIT-ROBO signaling pathways in tumor cell invasion. METHODS: We tested the transcriptional response of medulloblastoma and glioma cell lines to exogenous SLIT by DNA microarray analysis. We validated the top ten transcriptional targets of SLIT proteins, and we assessed the biochemical interaction of one of the candidates, MMP14, with ROBO1. RESULTS: We demonstrated that over 200 transcripts are altered in medulloblastoma cells treated by SLIT2, while the glioma cells tested had negligible transcriptional response. We selected ten candidates (MMP14, CathD, ColIVa1, Iga3, Arhgdia, Vim, App, p8, PI3KR3, AAMP) for qPCR validation. Our results demonstrated that MMP14 expression is strongly induced in glioma cell lines but not in medulloblastoma cells. CONCLUSIONS: This study demonstrated that medulloblastoma and glioma cell lines respond specifically to SLIT by modulating transcripts necessary for cell migration and invasion. Downregulating MMP14 expression in medulloblastoma cells decreases invasion and proliferation, rendering them more susceptible to temozolomide. Our results suggest the possibility that altering known neurodevelopmental pathways may be useful in modifying the invasive paradigm of brain tumor cells.
AI-19. INVASION IS AN IMPORTANT PROGNOSTIC FACTOR IN NEWLY DIAGNOSED HIGH-GRADE GLIOMAS
Saroj D. Kunakakkat, Donato Perretta, Praveen Medabalmi, Michael L. Gruber, Debbech Gruber, John Golfinos, Erik Parker, and Ashwatha Narayana; New York University Langone Medical Center

PURPOSE: While the importance of mitosis and angiogenesis is well appreciated in the prognostication of newly diagnosed high-grade gliomas (HGG), the role of invasion remains controversial. An apparent increase in invasiveness following antiangiogenic therapy makes this question clinically relevant. Our goal was to assess survival differences in patients with newly diagnosed HGG who presented with invasion as a dominant factor compared with those who did not but went on to experience disease progression following treatment. METHODS: Thirty-eight patients with diffuse invasive pattern either at initial diagnosis or at recurrence were analyzed. Twenty-one of these patients presented with newly diagnosed multifocal HGG. All patients underwent maximal surgical resection of contrast-enhancing disease when possible, followed by treatment with involved-field radiation therapy or whole-brain radiation therapy to 54–59.4 Gy with concomitant temozolomide that was titrated up to 360 mg/m2. In November 2005, magnetic resonance imaging (MRI) of the brain showed increasing the duration between bevacizumab treatments.

AI-20. EXTRACELLULAR EFFEMP1 BLOCKS AKT PHOSPHORYLATION AND DECREASES EGFR IN GLIOMA CELLS
Peter D. Piodi, Mark E. Linskey, and Yi-Hong Zhou; University of California-Irvine

Previous studies in our lab have shown that EGF-containing fibulin-like extracellular matrix protein 1 (EFFEMP1) demonstrates an antiangiogenic effect. In particular, this partially occurs through the downregulation of VEGFA expression. The classical pathway for VEGFA regulation is through HIF1A. However, an alternative mechanism through the EGF receptor has also been reported. The EGF receptor signaling pathway plays a major role in the malignant behavior of glioma. EGFR alterations, from gene amplification or mutation, were found in 45% of primary glioblastoma multiforme (GBM) cases. Additionally, aberrant EGFR signaling plays a role in the progression of many other tumor types. Downstream effectors of EGFR include Ras, which acts to induce proliferation, and also Akt, which functions in a prosurvival/antiapoptotic fashion. Our data show that signaling through Akt is abrogated via EFFEMP1. We treated LN229 and U251HF cells with 50 ng/mL of a human recombinant EFFEMP1 and observed 90% and 40% decreases in phosphorylated Akt, respectively. Interestingly, this effect was accompanied by a reduction of the EGFR protein levels. Further studies are needed to determine whether the effect of EFFEMP1 on Akt is a consequence of downregulation of EGFR at either the mRNA or protein level. Given our previous findings connecting EFFEMP1 to the downregulation of VEGFA gene expression, it appears that EFFEMP1 may downregulate VEGFA through the suppression of EGFR signaling. This may provide an alternative patient care strategy in which EGF and VEGFA can be simultaneously targeted by treatment.

AI-21. PROLONGED REMISSION OF GliOBLASTOMA MULTIFORME WITH BEVACIZUMAB AND IRINOTECAN TREATMENT
Govardhanan Nagaiah, Mohammed Almubarak, Alejandro Torres-Trejo, Michael Newton, , Paige Willey, and Ramin Altaha; Mary Babb Randolph Cancer Center, WV School of Medicine

INTRODUCTION: Bevacizumab as a second-line agent for the treatment of glioblastoma multiforme (GBM) has a median duration of response of 4 months without any survival benefit in currently published studies. Case Report: PG is a 58-year-old Caucasian woman diagnosed in March 2005 with GBM in her right temporal lobe at total surgical resection. In April 2005, she was treated with concomitant temozolomide at 75 mg/m2 and brain irradiation. Four weeks following the completion of chemoradiation, she began monthly temozolomide that was titrated up to 360 mg/m2. In November 2005, magnetic resonance imaging (MRI) of the brain showed mass in the medial right temporal lobe and para hippocampal gyrus, and the temozolomide was stopped. The patient declined further surgical resection and was taken onto salvage therapy with bevacizumab at 10 mg/kg and irinotecan at 125 mg/m2 on days 1 and 15 of a 28-day cycle. A repeat MRI done in January 2006 revealed a definite interval decrease in size and enhancement of the contrast-enhancing mass in the right hippocampus by about 60%. After 4 cycles of chemotherapy, the treatment interval was then increased to every 3 weeks. MRI performed 1 year after initiation of chemotherapy showed no evidence of disease progression. After this time, treatment interval was progressively increased to 4, 8 and finally to 12 weeks in light of stable disease. The patient has completed 27 cycles of bevacizumab and irinotecan given every 12 weeks and remains stable at about 5 yrs from diagnosis.

CONCLUSION: This unusually prolonged survival in our patient could be a result of factors. In patients who initially respond to bevacizumab, there might be benefit in increasing the duration between bevacizumab treatments.

AI-22. A SYNTHETIC FRAGMENT OF BETA-AMYLOID PEPTIDE SUPPRESSES GLIOBLASTOMA PROLIFERATION, ANGIOGENESIS, AND INVASIVENESS IN VIVO AND IN VITRO
Susan E. Murphy,*, Michael Newton,*, Alejandro Torres-Trejo,*, Rachel Albright1, Michael Mullan2, Daniel Paris3, and Steven Brem;* Moffit Cancer Center;* The Roskamp Institute

BACKGROUND: The recent clinical success of bevacizumab in suppressing angiogenesis, prolonging progression-free survival, and decreasing tumor volume is proof of principle that inhibitors of angiogenesis are effective for in glioblastoma multiforme. An important consideration in drug discovery in angiogenesis research directed to glioblastoma is naturally occurring inhibitors that play a role in health and disease within the CNS. We reported that full length Abeta1-42 is a dose-dependent inhibitor of angiogenesis and suppressed human U87 subcutaneous xenografts. We developed a small peptide sequence of beta-amyloid, Abeta41-42, and determined it to be a potent, novel anti-angiogenic molecule. The purpose of this study was to evaluate the effect of this peptide in an invasive orthotopic glioma model. METHODS: C57BL/6 mice implanted with GL261 glioma orthotopically were treated with Abeta41-42 at 50 mg/kg/day or with control vehicle starting at day 9. Brains were harvested (day 27) and processed for H&E (invasion), Ki-67 (proliferation) and CD31 (microvascular density). Glioma cell invasion was determined by Marrigel-invasion assay. Proteins from cells treated with and without Abeta1-40 also showed reductions in glioma growth, invasion, and invasiveness. Furthermore, parallel experiments in transgenic mice overexpressing Abeta1-40 also showed reductions in glioma growth, invasion, and angiogenesis. The VEGF-p75NTR link could be important to drug discovery and translation to clinical control of the malignant phenotype of glioma.

AI-23. DUAL ROLE OF THE RHOF GEF LARG IN GliOBLASTOMA CELL INVASION
Yue Peng Yang, Matthew Emmis2, Nhan Tran2, and Marc Symons1;* The Feinstein Institute for Medical Research;* Translational Genomics Research Institute

The invasion of glioblastoma cells into the normal brain is a critical factor that limits current therapeutic approaches and leads to high rates of disease recurrence and patient mortality. The brain parenchyma has a unique environment composed of abundant tightly packed neuronal and glial processes that restrict the extracellular pore size of brain. Thus, in addition to relying on extracellular matrix (ECM) proteolysis, glioblastoma
cells also need contractile forces to squeeze their nuclei through narrow spaces. To assay these processes, we monitored glioblastoma cell invasion through 3-dimensional Matrigel, which is dependent on proteolytic activity, and the invasion of glioma cells across a 100 micrometer-dehiscence, pseudomeningocele, CSF leak, wound/bone infection). Categorical variables were compared using Fisher’s exact test, and continu-
ous variables were compared using the Wilcoxon rank sum test. 

**RESULTS:** Two hundred eleven patients underwent second surgery (n = 162) or third surgery (n = 49) craniotomy for recurrent glioblastoma, with 26 (12.3%) cell patients) wound healing complications. Seventy percent of patients received bevacizumab, 23 received preoperative bevacizumab, and 18 received postoperative bevacizumab. Significantly more patients receiving preoperative bevacizumab developed healing complications (34.8%) than patients treated without bevacizumab (10.0%, P = 0.01). Postoperative bevacizumab caused 5.6% impaired healing, similar to patients treated without bevacizumab (P = 0.9). Preoperative bevacizumab duration did not influence healing (OR = 0.98, P = 0.55). More healing complications occurred in patients receiving bevacizumab for the invasive behavior of glioblastoma cells, we identified LARG, a GEF that specifically activates members of the Rho subfamily of small GTPases. In addition, RNA- mediated depletion of LARG by siRNA resulted in decreased glioma invasion through Matrigel and the formation of invadopodia, a specialized domain of the plasma membrane that concentrates the extracellular proteolytic activity of the cell. Depletion of LARG also inhibits nuclear squeezing. In addition, depletion of LARG inhibits glioblastoma cell invasion into ex vivo brain tissue. In vivo, glioma micro-focuses also need contractile forces to squeeze their nuclei through narrow spaces. To assay these processes, we monitored glioblastoma cell invasion through 3-dimensional Matrigel, which is dependent on proteolytic activity, and the invasion of glioma cells across a 100 micrometer-dehiscence, pseudomeningocele, CSF leak, wound/bone infection). Categorical variables were compared using Fisher’s exact test, and continuous variables were compared using the Wilcoxon rank sum test. **RESULTS:** Two hundred eleven patients underwent second surgery (n = 162) or third surgery (n = 49) craniotomy for recurrent glioblastoma, with 26 (12.3%) cell patients) wound healing complications. Seventy percent of patients received bevacizumab, 23 received preoperative bevacizumab, and 18 received postoperative bevacizumab. Significantly more patients receiving preoperative bevacizumab developed healing complications (34.8%) than patients treated without bevacizumab (10.0%, P = 0.01). Postoperative bevacizumab caused 5.6% impaired healing, similar to patients treated without bevacizumab (P = 0.9). Preoperative bevacizumab duration did not influence healing (OR = 0.98, P = 0.55). More healing complications occurred in patients receiving bevacizumab for more than 4 weeks after second surgery (28.6% vs. 10.2%, P = 0.1) and third surgery (44.4% vs. 9.1%, P = 0.03) craniotomy, with second surgery more than 4 weeks after the last bevacizumab dose. These complications should be acknowledged as increasing treatment of glioblastoma with bevacizumab creates more glioblastomas needing surgery for recurrence during antangiogenic treatment.

**AI-1-25. THE SRC ACTIVATING PROTEIN, AFAP1, IS POSITIONED TO PROMOTE INVASION IN GLIOMASTOMA MULTIFORME**

David A. Clump1, Johnathan A. Engil1, Arian H. Mintz1, Jess Cunnick2, and Daniel G. Flynn2, UPMC, The Children’s Hospital of Pennsylvania Medical College

Glioblastoma multiforme (GBM) is the most frequent as well as the most lethal of the primary brain tumors. Despite multiple modality treatment, long-term survival is limited by the failure to control invasive cell subpopulations that lead to local glioma recurrence as well as by the topographically diffuse nature of the disease. Src family kinase (SKF) inhibitors are among the most effective agents at decreasing invasion of glioma cells in culture, and therefore have been considered for translation to the clinic. We are currently assessing the role of the Src family kinase SH2 domain binding partner, AFAP1, in glioma invasion. Using immunohistochemistry, we found that AFAP1 is overexpressed in GBM tissue specimens. Additionally, using Western blot analysis and immunofluorescence of glioblastoma cell lines, we characterized the inter- actions of AFAP1 with the actin cytoskeleton, highlighting its role in promotion of actin-rich motility structures. As cSrc activity contributes to the invasive phenotype of GBM, further understanding of the modulators of this pathway, including AFAP1, may facilitate progression of the field toward individualized therapy.

**AI-1-26. IMPACT OF BEVACIZUMAB CHEMOTHERAPY ON CRANIOTOMY WOUND HEALING**

Aaron J. Clark, Nicholas A. Butowski, Susan M. Chang, Michael D. Prados, Jennifer Clarke, Mei-Yin C. Polley, Michael E. Suhbree, Michael W. McDermott, Andrew T. Parsa, Mitchel S. Berger, and Manish K. Aghi; University of California, San Francisco

INTRODUCTION: Recent FDA approval has increased bevacizumab use for the treatment of recurrent glioblastoma. Phase II trials reported 6% impaired wound healing for patients receiving bevacizumab initiated post-operatively. The impact of bevacizumab on subsequent craniotomy healing has not been addressed. METHODS: We retrospectively reviewed patients who had undergone craniotomy for recurrent glioblastoma from 2005 to 2009. We evaluated bevacizumab therapy/duration and healing problems (dehiscence, pseudomeningocele, CSF leak, wound/bone infection). Categorical variables were compared using Fisher’s exact test, and continuous variables were compared using the Wilcoxon rank sum test. **RESULTS:** Two hundred eleven patients underwent second surgery (n = 162) or third surgery (n = 49) craniotomy for recurrent glioblastoma, with 26 (12.3%) cell patients) wound healing complications. Seventy percent of patients received bevacizumab, 23 received preoperative bevacizumab, and 18 received postoperative bevacizumab. Significantly more patients receiving preoperative bevacizumab developed healing complications (34.8%) than patients treated without bevacizumab (10.0%, P = 0.01). Postoperative bevacizumab caused 5.6% impaired healing, similar to patients treated without bevacizumab (P = 0.9). Preoperative bevacizumab duration did not influence healing (OR = 0.98, P = 0.55). More healing complications occurred in patients receiving bevacizumab for more than 4 weeks after second surgery (28.6% vs. 10.2%, P = 0.1) and third surgery (44.4% vs. 9.1%, P = 0.03) craniotomy, with second surgery more than 4 weeks after the last bevacizumab dose. These complications should be acknowledged as increasing treatment of glioblastoma with bevacizumab creates more glioblastomas needing surgery for recurrence during antangiogenic treatment.

**AI-27. SCREENING GLIOMA TUMOR SPECIMENS FOR THERAPY SENSITIVITY**

Joseph P. Megyesi1, Penny Costello1, Warren MacDonald1, Erin Dyer1, David Macdonald1, Robert Hammond1, Yaliya Kalache1, Jay Eassaw2, and JMcIntyre2; University of Western Ontario; University of California

BACKGROUND: Surgical brain tumor specimens can be used to obtain valuable information regarding sensitivity to drug therapies. Data collected on surgical specimens using an in vitro growth and invasion assay were correlated with patient response to chemotherapy. METHODS: Surgically obtained glioma tumor specimens were cultured in a 3-dimensional collagen gel matrix and assessed for growth and invasion. Chemotherapy treatment curves were generated for each phase (I/II clinical trial) study drug that had produced a positive response and those predicted to have a negative response. RESULTS: Tumors from individual patients differed in terms of response profiles, especially when response was defined as at least an 80% reduction in tumor invasion distance. Similarly, different drugs varied in their ability to inhibit tumor growth and invasion, with only 9 of 31
AI-28. ISOCITRATE DEHYDROGENASE-1 (IDH-1) EXPRESSION DOES NOT CO-LOCALIZE WITH HYPOXIA INDUCIBLE FACTOR-1ALPHA (HIF-1ALPHA) EXPRESSION IN GLIOMAS
Susan C. Williams, Matthias A. Karajannis, Luis Chiriboga, Andreas von Deimling, and David Zagzag; 1New York University Langone Medical Center; 2Ruprecht-Karls-Universität Heidelberg

INTRODUCTION: Prior studies have demonstrated that in the enzyme cytosolic isocitrate dehydrogenase-1 (IDH-1) over-expression occurs more commonly in certain types of brain tumors, with the majority of secondary glioblastomas having progressed from lower grade lesions with an IDH-1 mutation. The role of IDH-1 in this progression is unclear but has been proposed to be linked to hypoxia inducible factor-1alpha (HIF-1alpha). Using immunohistochemistry, we analyzed glioma samples that were positive for the R132H IDH-1 mutation for HIF-1alpha expression to determine whether tumors harboring this IDH-1 mutation had increased HIF-1alpha expression and co-localization. METHODS: The New York University Langone Medical Center Pathology database was queried for all archival surgical specimens of glial neoplasms. Using immunohistochemistry on formalin-fixed paraffin-embedded sections, 135 glial neoplasms were analyzed for the R132H IDH-1 mutation. The tumors that were positive for this IDH-1 mutation were then analyzed for HIF-1alpha expression by immunohistochemistry. RESULTS: Evidence of IDH-1 R132H mutated tumor cells was present in 19 of 155 patients. Some of the tumors expressing this IDH-1 mutation also exhibited increased HIF-1alpha expression. However, we did not observe IDH-1 and HIF-1alpha co-localization in these tumors. CONCLUSIONS: Activation of HIF-1alpha has been implicated as a mechanism for tumor progression in gliomas harboring the IDH-1 mutation. Our results do not support an in situ link between HIF-1alpha expression and the R132H IDH-1 mutation.

AI-29. FOCAL ADHESSIONS DYNAMICS IN MALIGNANT GLIAL CELLS WITH VARIABLE DRR EXPRESSION
Abdulrazag Ajan, S. Husaine, and K. Petretta; Montreal Neurological Institute

Gliomas are the most common primary brain tumors. Regardless of the tumor grade, except for grade 1, tumor invasion of surrounding brain tissue is a common finding. The invasive behavior of these tumors is a major challenge for reaching a cure from these neoplasms. Understanding how focal adhesion cytoskeleton interaction is less examined in gliomas than in other cell types. We will present data of focal adhesion dynamics with variable DRR expression in a human glioma cell line. Our work includes quantification of focal adhesions in glioma cell lines with different DRR expression and evaluation of the change in dynamics of focal adhesions using live-tissue imaging obtained from the same cell lines. Our work adds to the current knowledge of focal adhesion cytoskeleton interaction in glioma.

AI-30. ONGENOCIC EGFRvIII SENSITIZES GBM CELLS TO PROANGIOGENIC EFFECTS OF THE COAGULATION SYSTEM
Nathalie Magnus, Delphine Garnier, Brian Meehan, and Janusz Rak; McGill University Montreal Children's Hospital Research Institute

INTRODUCTION: Tissue factor (TF) is a procoagulant receptor frequently overexpressed in human glioblastoma multiforme (GBM), in which thrombotic events are particularly frequent. Analysis of GBM cell lines suggests that TF is a regulatory target of several major genetic alterations associated with this disease, including activation of the epidermal growth factor receptor (EGFR) kinase activity of its mutant (EGFRvIII).

CB-01. REGULATION OF AMINOACYLASE EXPRESSION IN NEUROBLASTOMA
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Neuroblastoma, a cancer of the sympathetic nervous system, is the most common extracranial solid tumor in children. MYCN amplification and increased BDNF/Tkr6 signaling are features of high-grade tumors, yet only 25% of malignant tumors display those features. Thus, the identification of additional biomarkers and therapeutic targets is essential. Since aminocyclase 3 (ACY3) could serve a similar function in neuroblastoma, ACY3 expression was examined in TrkB-positive, MYCN-amplified SM-KCNR and TrkB-negative, non-MYCN amplified SK-N-AS and SK-N-SH neuroblastoma cell lines. ACY1 and ASAP exhibited distinct spatial localization in SM-KCNR and SK-N-Sh cells, while ACY3 displayed nuclear expression in all three lines. ACY1 was the only aminocyclase whose expression was up-regulated upon neuronal differentiation of SK-N-Sh cells in media containing 10% serum. ACY3 expression was greater in the least aggressive SK-N-AS line and significantly reduced in the most aggressive SMS-KCNR line. Conversely, ACY3 expression was highly expressed in the most aggressive SMS-KCNR cells. In vivo, aminocyclases are expressed in common sites of neuroblastoma origin. Bioinformatics data mining of Kaplan-Meier survival data highs-risk neuroblastoma. Aminocyclase 3 (ACY3) could serve a similar function in neuroblastoma, ACY3 expression was examined in TrkB-positive, MYCN-amplified SM-KCNR and TrkB-negative, non-MYCN amplified SK-N-AS and SK-N-SH neuroblastoma cell lines. ACY1 and ASAP exhibited distinct spatial localization in SM-KCNR and SK-N-Sh cells, while ACY3 displayed nuclear expression in all three lines. ACY1 was the only aminocyclase whose expression was up-regulated upon neuronal differentiation of SK-N-Sh cells in media containing 10% serum. ACY3 expression was greater in the least aggressive SK-N-AS line and significantly reduced in the most aggressive SMS-KCNR line. Conversely, ACY3 expression was highly expressed in the most aggressive SMS-KCNR cells. In vivo, aminocyclases are expressed in common sites of neuroblastoma origin. Bioinformatics data mining of Kaplan-Meier survival data highs-risk neuroblastoma. Aminocyclase 3 (ACY3) could serve a similar function in neuroblastoma.

CB-02. NPAS3 IS A LATE-STAGE-ACTING PROGRESSION FACTOR IN GLIOMAS WITH TUMOR SUPPRESSIVE FUNCTIONS
Manjit Rana, 1Tim-Rasmus Kiehl, 1Kelvin So, 1Peter Gould, 1Norbert Njewing, 1Deepak Kannasarat, 1Centre de recherche du CHUL, 2University Health Network; 3Centre de recherche du CHUL; 4University Health Network; 3University of Vermont; 4Laval University

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