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Abstracts

ANGIOGENESIS AND INVASION

AI-01. CASE REPORT: OPTIC NEUROPATHY IN A PATIENT WITH GLIOBLASTOMA RECEIVING BEVACIZUMAB
Robert A. Fishman, Erik Hopp, Tracey Stevens, and Lara Kunshner; Alleggheny General Hospital

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 have recently been reported of visual neurotoxicity following bevacizu- mb treatment for GBM (Sherman J, Areagui A, Fathallah B, Bierman P. J., Linsky K, Larner S, Newman D, Schiff D, Neurology 2009;73:1924– 1926). We report here the case of a 24-year-old woman with neurofibroma- tosis type 1 and right thalamic GBM (WHO grade IV) who developed for a severe, subacute optic neuropathy during treatment with bevacizumab and irinotecan for recurrent GBM following standard initial chemoradiation therapy with temozolomide. After completing 4 cycles of salvage chemotherapy with bevacizumab and irinotecan, the patient developed diffuse blurring of vision, which were unresponsive over 3–4 weeks, and eventually total blindness. The following cessation of bevacizumab, the patient’s vision improved, with return of light perception and eventual return of baseline vision. Our review of the literature has revealed a few proposed mechanisms that may lead to bevacizumab-induced optic neuropathy; these mechanisms include arterial thrombosis, decreased optic nerve tolerance to radiation therapy, and upregulation of VEGF receptors with ensuing neovascularization and optic ischemia following radiation therapy. Our patient’s follow-up will include stringent eye exams by an ophthalmologist, to include confronta- tional visual field testing, external and anterior segment examination, and dilated fundus examination. Our completed poster will include a summary of our case, magnetic resonance images of the tumor, and results of ophthal- momological examination during and following bevacizumab treatment.

AI-02. DEGRADOME PROFILING FAILS TO IDENTIFY A UNIQUE PROTEASE SIGNATURE IN PRIMARY MALIGNANT BRAIN TUMORS
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Core proteolytic enzymes are gaining increasing attention as potential therapeutic targets in cancer. Using proteolytic activity profiling, we aimed to determine a core protease signature in malignant gliomas. Patient material from 23 glioblastoma (GBM) specimens were included with a median of 84% glioma in the specimen. Proteolytic activity was measured on tissue homogenates using four fluorogenic substrate arrays. The proteolytic activity was measured in glioma cell lines and patient material. Proteolytic activity of U251MF, U87MF and the parental U251 cell line were compared. We were unable to detect a unique proteolytic signature in this glioblastoma cohort.

AI-03. ROLE OF NEUROPILIN1 AND ITS CO-FACTORS VEGF AND SEMA3A IN REGULATING HETEROGENEOUS MICROENVIRONMENT OF GLIOMA MULTIFORME
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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 Neuropilin1 (Nrp1) with its co-factors to activate the vascular endothelial growth factor (VEGF165-VEGFR2 signaling axis or the Semaphorin3A (SEMA3A)-PlexinA1 signaling axis, thereby regulating neo-angiogenesis, migration and invasion. METHODS/RESULTS: Twenty-five paired flash-frozen and paraffin-embedded sections from the hypoxic and non-hypoxic areas of GBMs were obtained on the basis of intra-operative magnetic resonance imaging–based stereotactic and neuropathological verifi- cation. Five non-neoplastic human brain specimens were used as controls. Laser capture microdissection (LCM) on serial flash frozen sections was used to isolate tumor cells and endothelial cells, and mRNA was subjected to reverse transcription and quantitative PCR with α-hemoglobin (αHb) as a housekeeping gene. Real-time PCR data for Nrp1, VEGF165, VEGFR2, SEMA3A, PlexinA1, and αHb were analyzed using the comparative ΔΔCT method. In GBM, a ratio of Nrp1:αHb > 1 was found at both the RNA level and protein level. Mean Nrp1 protein expression in the peripheral tumor cells was 2 times higher than that in the central tumor cells. We analyzed the role of Nrp1 in regulating the angiogenesis and invasion of GBMs by evaluating the role of VEGF, SEMA3A or Nrp1 in regulating the growth of GBM cells under different conditions. Nrp1 expression was regulated in a cell-type specific manner. We found that Nrp1 expression was increased in the central tumor cells and endothelial cells, respectively. This suggested that spatial gradients of SEMA3A and VEGF may promote differential Nrp1 binding within the GBM center and periphery. Using genetic and pharmacological approaches to modulate Nrp1, VEGF165, SEMA3A, and PlexinA1, we found that Nrp1 facilitated activation of VEGF165-VEGFR2 or SEMA3A-PlexinA1 signaling results in the activation and deactivation of alphaVbeta3 integrin, respectively, with subsequent activation of focal adhesion kinase and metalloproteinases. CONCLUSIONS: Our studies demon- strate how molecular heterogeneity related to the Nrp1-activated mediation of the VEGF and SEMA3A signaling pathways differs between regions of GBMs to contribute to pathological heterogeneity.

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 sur- rounding 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 increased by EFEMP1 at the mRNA level, it appears that EFEMP1 activation

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AI-05. USE OF HUMAN PROGENITOR CELLS FOR CELL-BASED DELIVERY OF ANGIOSTATIC GENES TO BRAIN TUMORS
Valentina Marchetti, Faith Barnett, Matthew Wang, Lea Schepke, Javier Sanchez-Cespedes, Charles De Ross, Glen Nemerow, Bruce Torbett and Martin Friedlander; The Scripps Research Institute

Cell-based delivery of antitumor 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 these cells. These were transducible with lentivirus vectors (Barnett et al., CNS 2004). Previous studies have described the angiogenic molecule T2, a fragment of tryptophanyl-tRNA synthetase (Trrps) (Tezma et al., PNAS 2003). We have also demonstrated that T2 has a potent antiangiogenic effect in vivo (Dorrell et al., PNAS 2007). In these studies, we defined a subpopulation of the EPCs, CD44 high expressing cells, which migrate to the tumor mass as well as microspheres in vivo in a 9L tumor model. CD44+ cells were injected into glioma xenografts expressing antitumor genes to brain tumors. Studies to assess the efficacy of antitumor genes in this cell-based tumor model are underway. Supported by grants from the National Eye Institute to MF (EY11254 and EY 017540).

AI-06. METASTATIC GLIOBLASTOMA (GBM): IMPLICATIONS IN THE AGE OF BEVACIZUMAB
Samuel A. Goldlust, 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 sequela 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 metastases. Our objective was to determine whether the incidence of metastatic GBM has increased as bevacizumab has come into routine use. DESIGN/METHODS: We identified patients with GBM and extraparenchymal disease retrospectively from 2000–2009 using IRB-approved data bases. Histology, demographics, treatment, and survival were reviewed for survival analyses. RESULTS: The incidence of extraparenchymal disease was 22% in 2,366 patients with GBM. Of these, 36 were identified (including 17 women, median age 50 years) with clinically confirmed GBM, 240 had received bevacizumab at any time during treatment. As the antiangiogenesis drug bevacizumab has come into routine use, the incidence of metastatic GBM has increased (P < 0.01). CONCLUSION: 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 bevacizumab as an adjuvant to the conventional chemotherapeutic agent was more efficient than as a salvage therapy.

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

A glioblastoma multiforme xenograft was established in athymic nude mice by injecting the mice with glioblastoma cells that stably express luciferase (U87MG-LucNeo). The mice were imaged 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 intraperitonally administered 4 weeks after nitrosourea treatment. For a salvage therapy, cetuximab (0.5 mg, 2 times) was intraperitonally administered when recovery of the bioluminescence signal was detected after nitrosourea monotherapy. The antitumor effects of cetuximab adjuvant therapy were superior to those of cetuximab salvage therapy. This study was consistent with the results from a survival comparison among nitrosourea 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 VASCULOCENIC MIMICRY IN THE DEVELOPMENT OF GLIOPLASMA
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 tumors (P < 0.01). Table formation in Matrigel was also significantly reduced at 6 hours, and completely abolished at 15 hours, with a sublethal 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 nanoparticles 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 < 0.001). Table 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 angiogenic 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 gliomas. This finding was associated with poor prognosis. In this study, we examined VM in tumors at different sizes of xenografts to analyze its possible functions during tumor development. METHODS: Tumor models in nude mice were established using human glioma cell lines as vehicle and delivering antitumor genes to brain tumors. Studies to assess the efficacy of antiangiogenic molecules in this cell-based tumor model are underway. Supported by grants from the National Eye Institute to MF (EY11254 and EY 017540).

AI-09. NOVEL ANTIANGIOGENIC AND HYPOXIC EFFECTS OF SACP-DOPS AGAINST GLIOMA: ALONE AND IN COMBINATION WITH ONCOTYLC VIRUSES
Jeffrey A. Wotton, Zhengtao Chu, Xiaoyang Qu, and Balveen Kaur; ¹Neuroscience Graduate Studies Program, The Ohio State University; 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 glycosphingolipid synthesis. 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 nanoparticles 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 < 0.001). Table 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 angiogenic 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 phosphatidylinerine (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, 1% CO2) was a significantly greater (P < 0.01) at several dosages (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 OVAs have shown additive effects. This study supports the further development of SapC-DOPS as a novel antitumor and antiangiogenic agent for GBM.
AI-10. EFEMP1 EXPRESSION IN GLIOMA: FRIEND OR FOE? 
Yi-Hong Zhou, 1 Yuanjie Hu, 1 Peter D. Poli 1, Eric Siegel 1, Daniel I. Ro 1, S. Marlon 1, Nelson Hsu 1, Shadi N. Milan 1, Siddharth Mohan 1, Liping Yu 1, Kenneth R. Hess 2, and Mark E. Linsey 1. 1University of California, Irvine; 2University of Arkansas for Medical Sciences; 2Ziren Research LLC; 4University of Texas MD Anderson Cancer Center

EGF-containing fibrin-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 in contrast 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 U251HF cells with suppressed tumorigenesis and angiogenic ability and found that EFEMP1 overexpression increased cell invasiveness through Matrigel substrate via modulation of CXCR7 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, 1 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 a 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, a small molecule antagonist of CXCR7, similarly reduced proliferation and invasion. Mechanistically, CXCR7 expression in glioma cells was induced by hypoxia. Depleting HIF-2alpha in U251MG cells by siRNA suppressed CXCR7 expression, while overexpression of HIF-2alpha resulted in CXCR7 expression. In vivo, 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-2alpha. Targeting CXCR7 and HIF-2alpha might provide novel opportunities for improving brain tumor therapy.

AI-12. SPECIFIC KNOCODOWN OF UPA/UPAR ATTENUATES INVASION IN GLOBLASTOMA CELLS AND XENOGRAFTS BY INHIBITION OF CLEAVAGE AND TRAFFICKING OF NOTCH 1 RECEPTOR 
HarirRaghu, 1 Christopher S. Gondi, 2 Meena Gujrati, 2 Dzung H. Dinh, 2 and Jasti S. Rao; 1University 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, either singly or simultaneously, inhibited the anchorage independent growth of U251 and GBM xenograft cell lines by solid colony formation assay. The four Notch receptors and the Notch ligands, Delta and Jagged, were expressed in U251 cells and in the GBM xenograft cell lines respectively. 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 1 receptor between Gly 1743 and Val 1744 position, suggesting inhibition of activated cytosolic fragment-related 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 RBPjk-dependent manner. uPA/UPAR siRNA downregulated Notch-related nuclear activation of NFκB subunits and the P3F-K/ACT/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 puPA-, puPAR-, and pu2-treated pre-established intracranial tumors in 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 cells and xenografts, 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 Narayani, 1 Saroj D. Kunnamkatt, 2 Praveen Medaballi, 1 John Golfinos, 1 Erik Parker, 1 Edward Knopp, 1 Deborah Gruber, 1 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 improvement 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 conventional chemotherapy or chemoradiation. RESULTS: With a median follow-up of 7 months (range 1–58) or 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. 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 HGG, 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 Batrall, 1 Salomeh Jelveh, 1 Thomas Lindsey, 1 Richard Hill, 1 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 reconstituted by transplanting BM harvested from GFP transgenic mice. In these mice, brain tumor xenografts in an intracranial window chamber model (ICW) were generated using U87 glioma cells engineered to stably express mCherry fluorochrome. In-vivo imaging: We have established a novel imaging strategy in which two-photon laser capture microscopy (2PLM) on ICW was used to obtain high-resolution real-time imaging of tissue section. RESULTS: 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 improvement 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 conventional chemotherapy or chemoradiation. RESULTS: With a median follow-up of 7 months (range 1–58) or 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. 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 HGG, 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.
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 animal care protocol. Tissue specimens were analyzed by immune histochemistry. RESULTS: One day following tumor cell implantation, BMDCs were circulating intravascularly and lining the abluminal vessel wall. At 7 days, there was a significant migration of BMDCs with or without tumor environment differentiating into vascular endothelial cells plus three other distinct cell populations: macrophages, GFAP+ glial cells, and monocytes. In comparison, control needle injection alone demonstrated a temporary recruitment of BMDCs without any migration or differentiation of BMDC. In later stages of tumor growth, vessels in the tumor periphery demonstrated clear incorporation of BMDC into tumor 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-17. INTEGRIN ALPHA3 IS INVOLVED IN THE INVASIVE BEHAVIOR OF GLIOMA STEM-CELL LIKE CELLS

Mirutoshi Nakada, Emi Namba, Natsuki Furuyama, Yuja Yoshida, Dansuke 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 invasiveness 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 reverse transcription-PCR, and the role in guiding invasion pathway was further evaluated in glioma cell lines. RESULTS: Neurrophysaries 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+ and CD133- cells was determined. Integrin alpha3 was overexpressed in CD133+ cells compared with CD133- cells (4.4 glioma cell lines: 2.4-240-fold). The production of integrin alpha3 was confirmed in CD133+ cells by immunocytochemistry. Immunohistochemistry demonstrated the localization of integrin alpha3 to the invading cells, especially in the tumor cells around the vessels, which we supposed to be a stem cell niche. Glioma cell lines transfected with integrin alpha3 showed more pronounced invasive growth in migration and invasion assays. In glioma cell lines U87 and SNB19 with high expression of high expression of integrin alpha3, the depletion of integrin alpha3 by small interfering RNA (siRNA) decreased invasion. The migration activity of glioma cell lines by manipulating the integrin alpha3 gene was linked to the blockade of cell-to-cell adhesion molecules (e.g. EGF- and PDGF-mediated stimulation of glioblastoma invasion using: 1) PDGFR and EGFR; 2) migration of glial progenitors that overexpress EGFR through brain slice, using a rodent model of gliomatosis cerebri; 3) migration of EGFR-expressing tumor cells in a rodent model of glioblastoma that expresses PDGFR and EGFR. RESULTS: EGF and PDGF each stimulate 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 NMMII. 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 blocked EGF-stimulated migration in a rodent model of glioblastoma that expresses PDGFR and EGFR. In glioblastoma cells, even with activation of two promigratory signal transduction cascades. Taken together, our results highlight the potential value of targeting NMMII to block glioblastoma dispersion.

AI-18. SLIT2 INHIBITS BRAIN TUMOR INVASION BY DOWNREGULATING MMP14

Mohamad Seyed Sadri1, Deborah Maret1, Emad Seyed Sadri1, Vincent Su1, Jad Alhami1, Jean-Sebastien Denault2, Damien Faury3, Nabi Jado3, Andre Nantel4, and Rolando Del Maestro1; 1MNI - McGill University; 2BRID - NRCC; 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 (Werbowetskie-Ogilvie 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 cell lines treated by SLIT2, while the glioma cells tested had negligible transcriptional response. We selected ten candidates (MMP14, CathD, ColIVa1, Iga3, Arhgdia, Vim, App, PPI33, AAMP) for qPCR validation. Our results demonstrated that MMP14 expression 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, PPI33, AAMP) for qPCR validation. Our results demonstrated that medulloblastoma cells downregulated MMP14 expression in medulloblastoma cells decreases invasion and proliferation, rendering them more susceptible to temozolomide. Our results suggest that targeting the signaling pathways downstream of Slit-Robo may be useful in modifying the invasive paradigm of brain tumor cells.

AI-16. A KEY TRANSCRIPTIONAL REGULATOR OF BRAIN TUMOR CELL INVASION AS A NOVEL THERAPEUTIC TARGET

Sean McAllister, Liliana Soroceanu, Arash Pakdel, Chandani Limbad, Peter Canoll1, and Steven S. Rosenfeld1; 1Columbia University; 2University of Washington

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 invasive and metastatic responsiveness of high grade gliomas (glioblastoma multiforme, or GBM). Id-1 expression correlates with human glioma cell invasiveness and proliferation. 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-19. INVASION IS AN IMPORTANT PROGNOSTIC FACTOR IN NEWLY DIAGNOSED HIGH-GRADE GLIOMAS
Saroj D. Kunnakkat, Donato Perretta, Praveen Medabalmi, Michael L. Gruber, Debnath Gruber, John Giovannetti, Jask Parkar, and Ashwatha Narayana; New York University Langone Medical Center

PURPOSE: While the importance of mitosis and angiogenesis is well understood in the progression 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 for 1 year. Progression-free survival (PFS) and overall survival (OS) in these patients were compared with 17 patients with localized HGG who went on to have disease progression in a diffuse manner following treatment that included bevacizumab given at 10 mg/kg every 2 weeks. Sixteen patients with localized HGG who had local recurrence after similar therapy, acted as a control group. RESULTS: The patient characteristics were similar in each group. With a median follow-up of 14 months (range, 1–41 months), the median PFS and OS for newly diagnosed multifocal HGG patients were 5.8 months (95% CI, 0.0–4.2 months) and 12.1 months (95% CI, 0.0–26.2 months), respectively. For patients with localized HGG who had recurrence as multifocal disease, the median PFS and OS were 9.3 months (95% CI, 4.9–13.6 months) and 24.5 months (95% CI, 19.9–28.0 months), respectively. CONCLUSIONS: Invasion is an important prognostic factor in patients with newly diagnosed HGG and confers poor prognosis. Efforts to block invasion should be a priority in clinical trials employing antiangiogenic therapy.

AI-20. EXTRACELLULAR EFEMP1 BLOCKS AKT PHOSPHORYLATION AND DECREASES EGFR IN GLIOMA CELLS
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Previous studies in our lab have shown that EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) demonstrates an antiproliferative 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 EGFR receptor has also been reported. The EGFR signaling pathway plays a major role in the malignant behavior of glioma. EGFR alterations, inactivation and/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 EFEMP1. We treated LN229 and U251 cells with 50 ng/mL of a human recombinant EFEMP1 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 EFEMP1 on Akt is a consequence of downregulation of EGFR at either the mRNA or protein level. Given our previous findings connecting EFEMP1 to the downregulation of VEGFA gene expression, it appears that EFEMP1 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
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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 a new contrast-enhancing mass in the medial right temporal lobe and para hippocampal gyrus, and the temozolomide was stopped. The patient declined further surgical resection and was started on 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 reflection of variations in tumor biology. The staggered dosing of bevacizumab used in this patient could be attenuating the development of resistance. 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 SUPPRESS GLIOMA PROLIFERATION, ANGIOGENESIS, AND INVASIVENESS IN VIVO AND IN VITRO
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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 the treatment of human glioblastoma. An important 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 Abeta1-40 is a dose-dependent inhibitor of angiogenesis and suppressed human U87 subcutaneous xenografts. We developed a small peptide sequence of beta-amyloid, Abeta1-20, 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 cells orthotopically were treated with Abeta1-20 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 a Matrigel-invasion assay. Cells from mice treated with and without Abeta1-20 were analyzed for expression levels of p75NTR and VEGF. RESULTS: In mice treated with Abeta1-20, tumor volumes were decreased (P < 0.05) with a reduction in microvascular density and inhibition of glioma invasiveness, the tumor cell proliferation rate was reduced in the treated group (P < 0.03). Toxicity related to the drug was not observed. In vitro, Abeta1-20 inhibited invasion in GL261 cells (P < 0.05). Western blot analysis showed VEGF decreased and p75NTR increased in a dose-dependent manner in cells treated with Abeta1-20. CONCLUSION: Systemic delivery of a potent antiangiogenic peptide, Abeta1-20, leads to reductions in glioma proliferation, angiogenesis, 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 RHO GEF LARG IN GLIOBLASTOMA CELL INVASION
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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 space of the brain. Thus, in addition to reliance 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 angiogenesis formation across a basement membrane, which involves nuclear squeezing. This squeezing process was mediated by the kinase ROCK, a Rho effector that stimulates actomyosin contractility (Beadle et al., Mol. Biol. Cell (2008) 19: 3357). Rho family GTPases play a central role in tumor invasion and are regulated by guanine nucleotide exchange factors (GEFs). In an effort to screen for GEFs that are necessary for the invasive behavior of glioblastoma cells, we identified LARG, a GEF that specifically activates members of the Rho subfamily of small GTPases. We further showed that LARG-mediated depletion of LARG by siRNA or by RNAi-mediated knockdown 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. Intracranial implants of primary human glioma in rats displayed distinct tumor phenotypes with increasing in vivo passage number. Invasive, nonanaplastic tumors were established in the first passages, while angiogenic phe-notypes were associated with the first passage, while angiogenic phe-notypes were associated with glioblastoma with bevacizumab creates more glioblastomas needing surgery for recurrent glioblastoma than patients treated without bevacizumab (28.6% vs. 10.0%, P = 0.01). Categorical variables were compared using Fisher's exact test, and continu-ous variables were compared with Wilcoxon rank-sum test. RESULTS: Two hundred eleven patients underwent second (n = 162) or third (n = 49) craniotomy for recurrent glioblastoma, with 26 (12.3%) cell culture and 273 (35.0%) tissue culture. One hundred twelve patients received no 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 (12.3%, 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, receiving complications before the second (28.6% vs. 10.2%, P = 0.1) and third (44.4% vs. 9.1%, P = 0.03) craniotomy, with the latter statistically significant. We found a trend toward increased healing complications in patients stopping bevacizumab less than 28 days preoperatively compared with those stopping bevacizumab 28 or more days preoperatively (OR = 6.5, P = 0.07), but even patients stopping bevaci-zumab 28 or more days before surgery trended toward more healing compli-cations than patients treated without bevacizumab (28.6% vs. 10.0%, P = 0.1). CONCLUSIONS: While confirming the minimal effect of postoperative bevacizumab on healing, we found that preoperative bevacizumab dramati-cally impaired healing after second/third craniotomy. This effect is more striking for patients undergoing a third craniotomy and for patients with a shorter delay between bevacizumab and surgery, but healing was impaired even with 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-24. NESTIN-EXPRESSING CELLS IN TUMOR ANGIOGENESIS
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Nestin is a type VI intermediate filament protein originally described in neural stem cells. Nestin is also expressed in other cell types in both normal and diseased tissues, including cancer. The role of nestin-expressing cells in tumors is not well understood. We studied orthotopic implants of human glioma in mice and rats. Glioma implanted large numbers of host mouse or rat nestin-positive cells. Nestin was revealed a network of tumor and host cells in the glioma and was present in many types of solid tumors and that nestin-positive cells are attracted by such tumors. These host nestin-positive cells, together with the tumor micro-foci that invadopodia formation and ROCK-dependent nuclear squeezing. In addition, our observations suggest that targeting LARG-mediated signaling events presents novel avenues to limit glioblastoma invasion.

AI-25. THE SRC ACTIVATING PROTEIN, AFAP1, IS POSITIONED TOWARD THE INVASIVE PHENOTYPE OF GBM, PREDICTING A POSITIVE RESPONSE TO CHEMOTHERAPY
Joseph P. Megyesi1, Penny Costello1, Warren Macdonald1, Erin Dyer1, David Macdonald1,2, Robert Hammond1, Yahiya Kalache1, Jay Easaw2, and J McIntyre2; 1University of Western Ontario; 2University of Calgary

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 subpopu-lations 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 their activity is currently being evaluated in early clinical trials for GBM in con-current with radiotherapy and temozolomide. SKFs are thought to play an important role in modulating signals that affect changes in actin-based motility structures. The Src SH3/SH2 binding partner, AFAP1-10, is distinctive in that it relays signals from PKCalpha to acto-myosin nuclear squeezing, resulting in the reorganization of actin-myosin structures. Here, we demonstrated using immunohistochemistry that AFAP-1 is overexpressed in GBM tissue specimens. Additionally, using Western blot analysis and immunofluorescence of glioblastoma cell lines, we characterized the inter-action between AFAP-1 and the Src family kinase-AFAP-1 complexes and the promotion of actin-rich motility structures. As Src activity contributes to the invasive phenotype of GBM, further understanding of the modulators of this pathway, including AFAP-1, may facilitate progression of the field toward individualized therapy.

AI-26. IMPACT OF BEVACIZUMAB CHEMOTHERAPY ON CRANIOTOMY WOUND HEALING
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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 recurrent 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 continu-ous variables were compared with Wilcoxon rank-sum test. RESULTS: Two hundred eleven patients underwent second (n = 162) or third (n = 49) craniotomy for recurrent glioblastoma, with 26 (12.3%) cell culture and 273 (35.0%) tissue culture. One hundred twelve patients received no 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 (12.3%, 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 before the second (28.6% vs. 10.2%, P = 0.1) and third (44.4% vs. 9.1%, P = 0.03) craniotomy, with the latter statistically significant. We found a trend toward increased healing complications in patients stopping bevacizumab less than 28 days preoperatively compared with those stopping bevacizumab 28 or more days preoperatively (OR = 6.5, P = 0.07), but even patients stopping bevacizumab 28 or more days before surgery trended toward more healing compli-cations than patients treated without bevacizumab (28.6% vs. 10.0%, P = 0.1). CONCLUSIONS: While confirming the minimal effect of postoperative bevacizumab on healing, we found that preoperative bevacizumab dramati-cally impaired healing after second/third craniotomy. This effect is more striking for patients undergoing a third craniotomy and for patients with a shorter delay between bevacizumab and surgery, but healing was impaired even with 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
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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 corre-lated with patient response to chemotherapy. METHODS: Surgically obtained glioma tumor specimens were cultured in a 3-dimensional collagen gel matrix and observed for growth and invasion. Chemotherapy drug effects on mean invasion and growth were expressed as a ratio relative to control conditions. Temozolomide (TMZ) sensitivity was correlated with methyl-guanine-methyl transferase methylation status. Length of patient sur-vival was compared between TMZ-treated patients whose screening results had predicted 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 invasion, with invasion, on 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
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INTRODUCTION: Prior studies have demonstrated that in the enzyme cytosolic isocitrate dehydrogenase-1 (IDH-1) occur more commonly in certain types of brain tumors, with the majority of secondary glioblastomas having progressed from lower grade lesions with an IDH-1 mutation. The role of IDH-1 in this progression is unclear but has been proposed to be linked to hypoxia inducible factor-1alpha (HIF-1alpha). Using immunohistochemistry, we analyzed glioma samples that were positive for the R132H IDH-1 mutation for HIF-1alpha expression to determine whether tumors harboring this IDH-1 mutation had increased HIF-1alpha expression and co-localization. METHODS: The New York University Langone Medical Center Pathology database was queried for all archival surgical specimens of glial neoplasms. Using immunohistochemistry on formalin-fixed paraffin-embedded sections, 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 ADHESIONS DYNAMICS IN MALIGNANT GLIAL CELLS WITH VARIABLE DRR EXPRESSION
Abdulrazzag Alian, S Husaine, and K. Petrecca; 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. Understanding how focal adhesion dynamics change in relation to DRR expression will improve the overall understanding of the mechanisms involved in glioma migration.

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/Tnfkb 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 aminocytase 1 (ACY1), an amino acid decacetylase, is a putative tumor suppressor in small cell lung and renal cell carcinomas, we investigated whether ACY1 or family members aminocytase 2 (ACY2) or aminocytase 3 (ACY3) could serve as similar tumor suppressors in neuroblastoma. Aminocytase expression was examined in Trkb-positive, MYCN-amplified SMS-KCN and Trkb-negative, non-MYCN amplified SK-N-AS and SK-N-SH neuroblastoma cell lines. ACY1 and ACY3 exhibited distinct spatial localization in SMS-KCN and SK-N-SH cells, while ACY3 displayed nuclear expression in all three lines examined. ACY1 was the only aminocytase whose expression was up-regulated upon neuronal differentiation of SK-N-SH cells in media containing 10% serum. ACY2 expression was greater in the least aggressive SK-N-BE line and significantly reduced in the most aggressive SMS-KCN line. Conversely, ACY3 expression was highly expressed in the most aggressive SMS-KCN cells. In vivo, aminocytases are expressed in common sites of neuroblastoma origin. Bioinformatics data mining of Kaplan-Meier survival data (Yates’ test) of high-risk neuroblastomas revealed that aminocytase expression was associated with poor prognosis and that low expression of ACY1 or ACY2 is also correlated with poor prognosis, suggesting that the loss of these aminocytases may contribute to neuroblastoma tumorigenesis.

AI-30. ONCOGENIC EGF-RIII 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) by types of its mutant (EGFRvIII).

METHODS: U373 and U87 glioma cell lines and their EGFRvIII expressing or TF transfected counterparts were compared for growth in vivo and FVIIa-dependent procoagulant activities (Xa generation assay) of expression of TF and PAR1/2 (RT-PCR, ELISA), and production of angiogenic factors (VEGF and IL-8). RESULTS: We found that EGFRvIII upregulates TF, PAR-1, PAR-2, and FVII in GBM cell lines, which also become highly procoagulant and hypersensitive to stimulation with FVIIa, PAR-1, and PAR-2 activating peptides. This stimulation evokes production of VEGF and IL-8 in EGFR/EGFRvIII-expressing GBM cells, but not in their indolent counterparts transfected with TF alone (TF-U873). However, TF transfection renders indolent GBM cells capable of forming tumors, but only after a long latency. CONCLUSIONS: TF signaling interacts with and amplifies the proangiogenic effects of EGFRvIII-driven oncogenic pathways, and TF also may have an independent but modest role in GBM progression.