*Neuro-Oncology* 14:vi1–vi6, 2012.
doi:10.1093/neuonc/nos219

NEURO-ONCOLOGY

# Abstracts

## LAB-ANGIOGENESIS AND INVASION

### AI-01. CAIX REGULATION OF EXTRACELLULAR PH AND INVASION IN GLIOBLASTOMA

Martin A. Proescholdt[1], Marsha J. Merrill[2], Eva-Maria Stoerr[1], Annette Lohmeier[1], and Alexander Brawanski[1]; [1]University Regensburg Medical Center, Regensburg, Germany; [2]Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

OBJECTIVE: Glioblastoma multiforme is the most frequent primary brain tumor in adults. The major obstacle for successful treatment is the invasive growth pattern. Metabolically, glioblastomas are highly glycolytic, leading to increased levels of lactic acid production. The carbonic anhydrase IX (CAIX) moderates the extrusion of hydrogen ions into the extracellular space, which may enhance tumor invasion by activating proteolytic enzymes. We therefore induced glycolysis in glioblastoma cells and investigated the extracellular pH, cathepsin B expression, and its subcellular distribution and secretion parallel to the invasive behavior of the cells upon CAIX knockdown. METHODS: U251 glioblastoma cells were transfected with a CAIX siRNA construct and cultured in a Biocoat Matrigel invasion chambers with an 8-μm pore size membrane. The chambers were incubated in a humified 5% $CO_2$ modular with either 21% oxygen and 25 mM glucose (control) or 0% oxygen plus 125 mM glucose (glycolysis). Invasion was quantified by counting the cells on the lower membrane surface. Extracellular pH was measured using a pH-meter. Cathepsin B expression and localization was investigated by RT-PCR, Western blot, and immunofluorescence staining, respectively. The cathepsin B secretion into the supernatant was measured using a cathepsin B activity assay. RESULTS: In vitro glycolysis caused a significant increase of cathepsin B expression and secretion combined with massive invasion of glioblastoma cells. In addition, the subcellular distribution of the enzyme was shifted to the cell periphery. The extracellular pH dropped significantly under glycolytic conditions, antagonized by CAIX knockdown. In addition, CAIX knockdown did not influence cathepsin B expression but attenuated the intracellular change of cathepsin B distribution and reduced both the cathepsin B secretion and glioma cell invasion. CONCLUSION: Our data demonstrate that CAIX moderates invasion in glycolytic glioma cells via acidification of the extracellular milieu and enhanced secretion of cathepsin B.

### AI-02. BREVICAN TRANSACTIVATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR VIA SRC KINASE AND CELL-SURFACE SULFATIDES, PROMOTING GLIOMA CELL MOTILITY

Hosung Sim, Bin Hu, Colleen A. Pineda, Sung-Ok Yoon, and Mariano S. Viapiano; The Ohio State University, Columbus, OH, USA

Malignant gliomas are the most common type of primary brain tumors and have a highly invasive behavior that makes them essentially incurable. Glioma invasion is triggered by multiple molecules that favor adhesion and motility through neural tissue. These include molecules secreted by the tumor cells and those originally from the neural extracellular matrix, which are modified by the infiltrating cells. Brevican, a predominant proteoglycan of the brain extracellular matrix, is also secreted and cleaved by glioma cells, generating fragments that promote tumor invasion. The molecular signaling triggered by brevican to promote cell motility is largely unknown. Here, we report that the N-terminal domain of brevican, but not the full-length protein, activates Src-kinase, thereby transactivating epidermal growth factor receptor signaling. Src activation involves a novel interaction of N-terminal brevican with cell-surface sulfatides, suggesting a signaling pathway from membrane lipids to MAPK activation that causes an Src-dependent increase of cell motility. These results disclose a potential signaling mechanism activated by lecticans in glial cells and suggest novel anti-invasive strategies based on targeting brevican cleavage and its interaction with cell-surface glycolipids.

### AI-03. JAK-1/2 INHIBITION IMPAIRS BONE MARROW-DERIVED CELL RECRUITMENT, PREVENTING LOW-GRADE TO HIGH-GRADE GLIOMA TRANSFORMATION

Prajwal Rajappa[1], William S. Cobb[1], Yujie Huang[1], David C. Lyden[1], Jacqueline Bromberg[2], and Jeffrey P. Greenfield[1]; [1]Weill Cornell Medical College, New York, NY, USA; [2]Memorial Sloan-Kettering Cancer Center, New York, NY, USA

INTRODUCTION: Low-grade gliomas are well differentiated, slow-growing, and lack neovascularization. High-grade gliomas are poorly differentiated, highly proliferative (Ki67+), and vascularized. Malignant transformation, the process by which low-grade gliomas become higher-grade tumors, is defined, in part, by the angiogenic switch, a period of rapid growth sustained by an evolving microenvironment supportive of neovascularization. We previously determined that bone marrow-derived cells (BMDCs) were necessary for this transformation. In order to examine the functional role of BMDC mobilization and recruitment in this process, we explored the utility of JAK inhibitors (potent cytokine signaling suppressors) within this context. Here we demonstrate that JAK inhibition blocks the recruitment of BMDCs to primary low-grade glial tumors, resulting in reduced neovascularization (CD31) and preventing malignant transformation. METHODS: A spontaneous, transgenic, PDGF-driven murine glioma model was utilized to recapitulate low-grade to high-grade progression over 6 weeks. Tumor-bearing 3-week-old animals were given JAK1/2 inhibitors via oral gavage for 3 weeks. Immunofluorescence was used to characterize the role of BDMCs within the tumor microenvironment. Flow cytometry was used to quantify BMDC recruitment. Explanted high-grade murine glioma cell lines were utilized to analyze the effects of JAK1/2 treatment upon cell proliferation. RESULTS: The number of mobilized BMDCs in the peripheral blood, bone marrow, and within tumors directly correlated with tumor grade in control animals. Animals treated with JAK inhibitors had delayed transformation radiographically and histologically and had a median survival duration more than twice as long as the controls. Flow cytometry demonstrated impaired Cd11b + /GR1 mobilization in the peripheral circulation and recruitment to tumors of treated animals. Proliferation of explanted high-grade cell lines treated with JAK inhibitors was not affected, highlighting their tumor-extrinsic effects on tumorigenesis. CONCLUSION: Our results suggest that treatment with JAK 1/2 inhibitors impaired recruitment of a myeloid-derived subset of BMDCs, delaying glioma progression in vivo and prolonging survival.

### AI-04. GUANYLATE-BINDING PROTEIN-1 AS A NOVEL EFFECTOR OF EPIDERMAL GROWTH FACTOR RECEPTOR-DRIVEN INVASION IN GLIOBLASTOMA

Ming Li[1], Akitake Mukasa[1], Maria del-Mar Inda[1], Jianhua Zhang[2], Lynda Chin[2], Webster Cavenee[1], and Frank Furnari[1]; [1]Ludwig Institute, University of California San Diego, La Jolla, CA, USA; [2]Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

Epidermal growth factor receptor (EGFR) activation by amplification or mutation is one of the most frequent genetic lesions in a variety of human tumors, including glioblastoma (GBM), which is characterized by independent but interrelated features of extensive invasion into normal brain parenchyma, rapid growth, necrosis, and angiogenesis. Although guanylate binding protein-1 (GBP1) was among the first interferon-inducible proteins identified, its function is still largely unknown. Here we show that activating EGFR promoted GBP1 expression in GBM cell lines through a signaling pathway involving Src and p38 MAPK. Moreover, we identified Yin Yang 1 (YY1) as the downstream transcriptional regulator of EGFR-driven GBP1 expression. GBP1 was required for EGFR-mediated matrix metalloproteinase-1 (MMP1) expression and glioma cell invasion in vitro. Deregulation of GBP1 expression did not affect glioma cell proliferation, whereas overexpression of GBP1 enhanced glioma cell invasion through MMP1 induction, which required its C-terminal helical domain and was independent of its GTPase activity. Reducing GBP1 levels by RNA interference in invasive GBM cells also markedly inhibited their ability to infiltrate the brain parenchyma of mice. GBP1 expression was high and positively correlated with EGFR expression in human GBM tumors and cell lines, particularly those of the neural subtype. Together, these findings establish GPB1 as a previously unknown link between EGFR activity and MMP1 expression and nominate it as a novel potential therapeutic target for inhibiting GBM invasion.

### AI-05. GENOTYPICAL CHARACTERISTICS OF GLIAL TUMOR CELLS PRESENT IN INTRATUMORAL ENDOTHELIAL PROGENITOR CELLS BUT NOT IN THEIR CIRCULATING COUNTERPARTS

Ping-Pin Zheng, Marcel van der Weiden, Peter J. van der Spek, Arnaud J. Vincent, and Johan M. Kros; Erasmus Medical Center, Rotterdam, Netherlands

INTRODUCTION: Malignant glioma patients usually have increased levels of circulating endothelial progenitor cells (EPCs). Circulating EPCs contribute to glioma neovasculature, although whether these cells can acquire tumor genetics while in the bloodstream or after being recruited into the tumors is unknown. In this study we performed a genotypical characterization of intratumoral and circulating EPCs of glioma patients. METHODS: We sought a set of common glioma genotypical aberrations (amplification of EGFR, deletion of PTEN, and aneusomy of chromosomes 7 and 10) in the EPCs inboth tumor and blood samples of 36 glioma patients. RESULTS: The EPCs present in the tumor tissues shared genetic aberrations with the tumor cells. EPCs with EGFR amplification were found in 46% of the tumors and with PTEN deletion in 36% of the tumors. EPCs with polysomy 7 and monosomy 10 were detected in 56% and 38% of the tumors, respectively, while centrosomal abnormalities in EPCs were found in 68% of the tumors. The genetic aberrations were absent in the circulating EPCs. CONCLUSION: The presence of genetic aberrations of glioma cells in intratumoral EPCs may point to transdifferentiation of glioma stem cells into EPCs. However, the possibility that circulating EPCs have acquired the genetic aberrations of the tumor cells after being recruited into the gliomas cannot be ruled out. These findings highlight the complexity of the cellular constituents of glioma neovascularization, which should be taken into account when developing anti-angiogenic strategies for gliomas.

### AI-06. A NEW GROW-OR-GO MODEL FOR STUDYING GLIOMA GROWTH AND INVASION

Hassan M. Fathallah-Shaykh[1], Olivier Saut[2], Jean-Baptiste Lagaert[2], and Thierry Colin[2]; [1]University of Albama at Birmingham, Birmingham, AL, USA; [2]University of Bordeaux, Bordeaux, France

Malignant gliomas remain associated with poor prognosis despite recent advances in therapeutic modalities, including anti-angiogenesis. The invasion of normal brain tissue by glioma cells increases neurological morbidity, restricts surgical options, and plays a role in mediating tumor recurrence. We derive a new mathematical model that describes glioma cell growth and brain invasion in the context of local hypoxia. The equations are designed to model the "grow-or-go" phenotype: that is, when cells switch from the replicative to the invasive phenotype when their local environment becomes deficient in nutrients and oxygen. Simulations, which replicate the necrotic, proliferative, and invasive components of malignant gliomas, reveal novel growth and invasion patterns and predict the dynamiceffects of anti-angiogenic drugs. We discuss the clinical applications of the model.

### AI-07. C-SRC AND NEURAL WISKOTT-ALDRICH SYNDROME PROTEIN (N-WASP) PROMOTE HYPOXIA-INDUCED ACCELERATED BRAIN INVASION BY GLIOMAS

Hassan M. Fathallah-Shaykh, Lita Araysi, and Zhuo Tang; University of Albama at Birmingham, Birmingham, AL, USA

Malignant gliomas remain associated with poor prognosis and high morbidity because of their ability to invade the brain. Human gliomas exhibit a phenotype of accelerated brain invasion in response to anti-angiogeneic drugs. Here we study eight human glioblastoma cell lines; four show accelerated motility in hypoxic conditions. Src inhibition by dasatinib abrogates this phenotype. Molecular discovery and validation studies link c-Src, neural Wiskott-Aldrich syndrome protein (N-WASP), focal adhesion kinase (FAK), beta-catenin, and protein kinase B (Akt) to the hypoxia-enhanced motility phenotype. Downregulating c-Src or N-WASP by RNA interference abrogates the hypoxia-induced enhancement in motility as shown by in vitro assays and in live brain sections. These findings support the idea that c-Src and N-WASP play key roles in mediating the molecular pathogenesis of hypoxia-induced accelerated brain invasion by gliomas.

### AI-08. H-FERRITIN IS RADIOPROTECTIVE IN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

Kari A. Duck, Padmavathi Ponnuru, Elizabeth B. Neely, and James R. Connor; Pennsylvania State University College of Medicine, Hershey, PA, USA

Ferritin is traditionally considered an intracellular iron storage protein that provides protection to cells via its capacity to sequester iron. The role of ferritin is expanding to include iron delivery to cells and angiogenesis. The protective role of ferritin combined with its angiogenic functions make this protein potentially useful in therapeutic interventions. For example, decreasing ferritin expression may limit angiogenesis in tumor formation. The purpose of the present study is to investigate the effect of ferritin on brain microvascular endothelial cell sensitivity to radiation. Endothelial cells from rabbit brain microvasculature were cultured and exposed to gamma radiation (0-10 Gy) with or without treatment with recombinant H-ferritin (rHFrt, 0, 5 nM, and 50 nM). Proliferation assays were performed at 48 hours after exposure to radiation. The effect of rHFrt exposure on the expression of vascular endothelial growth factor (VEGF) and its receptors (VEGFR1 and VEGFR2) was also determined. Exposure to gamma radiation is associated with a decrease in endothelial cell proliferation and expression of VEGF and VEGFR. Pre-treating endothelial cells with rHFrt for 24 hours protected the endothelial cells from radiation. Moreover, extracellular ferritin increased the expression of VEGF and VEGFR1. Radiation decreased the phosphorylated form of VEGFR2, but the degree of this decrease was limited by the presence of H-ferritin. Ferritin enhances cell proliferation in the presence of gamma radiation. The upregulation of VEGF and its receptors after ferritin exposure is consistent with the increase in proliferation and suggests a mechanism by which ferritin supports endothelial cell survival. These data suggest that limiting ferritin in endothelial cells may enhance radiosensitivity to prevent tumor proliferation.

### AI-09. HYPOXIA UPREGULATES MIR-451 EXPRESSION IN GLIOMAS

Mine Esencay[1], Pilar Gonzalez[2], Avital Gaziel[1], Yasmeen Safraz[1], Helena Mira[2], Eva Hernando[1], and David Zagzag[1]; [1]New York University Langone Medical Center, New York, NY, USA; [2]Unidad de Neuro-Oncología Instituto de Salud Carlos III, Madrid, Spain

BACKGROUND: MicroRNAs (miRNAs) are short, non-coding RNAs with wide gene regulatory activity at the posttranscriptional level. We hypothesized that, given their ability to impact multiple genes and pathways, miRNAs could play a key role in the complex biological behavior of glioblastoma (GBM). Furthermore, specific miRNA manipulation could simultaneously block the angiogenic and invasive properties of these tumors. Glucose modulates miR-451 levels: abundant glucose allows relatively high miR-451 expression, promoting cell growth, whereas miR-451 levels decrease in low-glucose conditions, slowing proliferation but enhancing migration (Godlewski et al., 2010). METHODS AND RESULTS: Using RT-PCR, we analyzed the level of miR-451 in 25 gliomas (oligodendroglioma [n = 3], astrocytoma grade II [n = 7], anaplastic astrocytoma [n = 8], and GBMs [n = 17]). We observed that astrocytic tumors had a higher level of expression than oligodendroglioma, and the expression within astrocytic tumors correlated with tumor grade. We then performed in situ hybridization and observed that hypoxic pseudopalisading cells, at the edge of necrotic zones within GBM tumors, express high levels of miR-451. This finding prompted us to investigate whether miR-451 levels are upregulated in response to hypoxia. MiR-451 levels were indeed upregulated in LN308 and GL261 glioma cells exposed to hypoxia compared with normoxia in vitro. Next, we tested the effect of miR-451 overexpression on the pro-angiogenic response of glioma cells in vivo. We found that injecting miR-451-transduced GL261 glioma cells intracranially resulted in tumors that were more vascularized than the scrambled-transduced controls. CONCLUSION: Our data show that miR-451 is upregulated by hypoxia in vivo and in vitro and is involved in glioma angiogenesis. We are thus interested in further understanding the role of miR-451 in the GBM pro-angiogenic response to hypoxia, and we plan to test the therapeutic potential of suppressing miR-451 in GBM in vivo.

### AI-10. THE DIFFERENTIAL EXPRESSION OF MICRORNA-200 SUPERFAMILY IN PRIMARY BREAST CANCER AND BRAIN METASTASES

Ryan A. McDermott, Ilya Ulasov, Natalya Kaverina, Patrik Gabikian, and Maciej Lesniak; University of Chicago, Chicago, IL, USA

Metastatic brain tumors are among the most common and lethal brain lesions, with the incidence of diagnosis annually outnumbering all other intracranial tumors combined. In order for tumor cells to metastasize and travel to the brain, they first undergo epithelial to mesenchymal transition (EMT). During migration, tumor cells gain or lose cellular proteins that regulate proliferation, angiogenesis, and invasion. While these changes are mainly governed by cellular factors, the role of epigenetic contribution is

still under investigation. MicroRNAs (miRNAs) have been shown to regulate the behavior of breast cancer cells, but their involvement in brain metastasis development remains unknown. The objective of this study was to evaluate expression of miRNA involvement in the formation of brain metastases from primary breast tumors. To determine a molecular profile of brain metastases, we analyzed two pairs of matched metastatic brain lesions and their primary specimens by using the Affymetrix miRNA platform. By using a cluster analysis, we identified 10 miRNAs that mainly belong to the miRNA-200 superfamily and are expressed differentially between primary tumors and their corresponding brain metastases. A further QRT-PCR analysis validated the array data. Next, we defined the targets of these miRNAs using a bioinformatic approach. We selected the CXCL12, EphA2, MMP16, RUNX1, and KiSS1 genes, which not only have binding sites for the miRNA-200 superfamily but also play a role in metastasis. Regulation of target gene expression by the miRNA-200 superfamily was determined via a luciferase reporter gene assay. In conclusion, the results of this study contribute to the current knowledge about the involvement of the miRNA-200 superfamily in the regulation of proteins that contribute to metastasis. Further understanding the complex role of miRNAs in the formation of brain metastases may add a new insight into the pathogenesis of brain metastasis and facilitate development of new therapeutics against metastatic brain tumors.

### AI-11. ERG AS A RELIABLE MARKER OF VASCULAR CELLS IN BRAIN TUMORS

Amir Iranmahboob, Matthew Haber, Mine Esencay, Girish Fatterpekar, Eytan Raz, Dimitris Placantonakis, and David Zagzag; New York University Langone Medical Center, New York, NY, USA

INTRODUCTION: The ETS related gene (Erg), a transcription factor expressed abundantly in endothelial cells, has been linked to angiogenesis. To date, the literature has evaluated Erg expression mostly in prostate and kidney neoplasms. Very little research has been done to assess Erg expression in tumors of the central nervous system (CNS). METHODS: Thirty-six CNS neoplasms were analyzed for Erg immuno-staining. A representative sample of slides for each case containing Erg staining was evaluated using a threshold method with custom Matlab 2008b software. Pixels were defined as positively Erg-stained if the value was greater than 2 times the standard deviation from the mean of the total image value. The sum of pixels with Erg-positive staining was divided by the total number of pixels in the field of view. This corresponds to the percent surface area of Erg staining in the field of view. This automated methodology for calculating percent staining was applied to all of the cases in a blinded manner. Percent staining was averaged for different types of tumors. RESULTS: Endothelial cells were strongly and diffusely immunoreactive for Erg. Percent staining for Erg in endothelial cells was greatest for hemangioblastoma ($n = 8$; $3.74\% \pm 0.64\%$), and it was statistically significantly greater than meningioma ($n = 12$; $2.65\% \pm 0.85\%$, $p < 0.002$), glioblastoma ($n = 7$; $2.19\% \pm 0.87\%$, $p < 0.006$), metastatic carcinoma ($n = 5$; $1.85\% \pm 0.58\%$, $p < 0.001$), and schwannoma ($n = 4$; $1.75\% \pm 0.81\%$, $p < 0.001$). Staining was also detected in normal endothelial cells in tissues adjacent to the tumors. We are currently analyzing the pattern of immunoreactivity for each of these types of tumors. CONCLUSION: Erg is a novel marker for endothelial cells within brain tumors. Future studies are required to understand the biological function of Erg in these tumors.

### AI-12. HIGH LEVELS OF IL-8 AND IL-13 AT BASELINE AND ABSENCE OF EARLY DECREASE OF VEGF IN PLASMA OF PATIENTS WITH RECURRENT GLIOBLASTOMA NOT RESPONDING TO BEVACIZUMAB

Marica Eoli[1], Cristin Rabascio[2], Lucia Cuppini[1], Elena Anghileri[1], Serena Pellegatta[1], Angelica Calleri[2], Patrizia Mancuso[2], Paola Porrati[1], Francesco Bertolini[2], and Gaetano Finocchiaro[1]; [1]Neurological Institutte C. Besta, Milan, Italy; [2]European Institute of Oncology, Milan, Italy

Bevacizumab, an anti-VEGF antibody, has shown significant activity in patients with glioblastoma (GBM), a highly vascularized tumor. In this study, we investigated the mechanisms of GBM resistant to bevacizumab. We treated 49 recurrent GBM patients with poor prognostic factors with bevacizumab (10 mg/kg) and irinotecan (125 or 340 mg/m$^2$) every 2 weeks. After a median follow-up of 27 weeks, patients had a median overall survival (OS) and progression-free survival (PFS) of 33 and 18 weeks, respectively. PFS at 6 and 12 months were 32% and 12%. OS at 6 months was 60%. Toxicity and side effects were modest. Using the Bio-Plex$^{TM}$ cytokine assay, we measured the plasma levels of IL-1b, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-4, IL-17, FGF basic, G-CSF, IFN-gamma, MCP-1, MIP-1b, PDGF-beta, and TNF-alpha in these patients before treatment and every 8 weeks. In 12 patients with disease progression 8 weeks after treatment (nonresponders), plasma levels of IL-8 and IL-13 were significantly higher than in responders (ie, patients free from progression 8 weeks after treatment): $17.1 \pm 11.7$ pg/mL vs $11.06 \pm 9.31$ ($P = 0.03$) for IL-8 and $147.4 \pm 378.2$ pg/mL vs $21.9 \pm 17.4$ ($P = 0.03$) for IL-13. No significant reduction of VEGF or other cytokines was present in nonresponders. On the contrary, plasma VEGF decreased significantly in responders 8 weeks after treatment ($25 \pm 37.2$ pg/mL vs $13 \pm 30.8$, $P = 0.002$). The data encourage further investigation on the potential role of IL-8 and IL-13, two pro-angiogenic cytokines, in generating resistance to anti-VEGF therapy. They also support larger studies on the predictive role of VEGF decrease during early phases of anti-angiogenic therapy with bevacizumab.

### AI-13. THE ROLE OF PROTO-ONCOGENES IN TUMOR-ASSOCIATED MACROPHAGE POLARIZATION AND PROMOTION OF GBM TUMOR PROGRESSION

Darren F. Seals, Karen L. Burger, Denise M. Gibo, and Waldemar Debinski; Wake Forest School of Medicine, Winston-Salem, NC, USA

Glioblastoma (GBM) commonly recurs at sites distant from tumor resection cavities. GBM tumor microenvironments are also infiltrated by immune cells. We have preliminarily found that CD68 +, CD163 +, and M2-polarized, CD204+ tumor-associated macrophages (TAMs) are highly present in GBM specimens. These TAMs may support tumor cell invasive behavior. The differentiation of monocytes into M2-polarized macrophages is facilitated by an AP-1 factor, the proto-oncogene fos-related antigen 1 (Fra-1). Fra-1 also participates in the control of GBM cell migration and matrix metalloproteinase (MMP) activity. Macrophage and cancer cell invasive behavior can also be regulated by Tks5, a substrate of the proto-oncogene Src. Tks5 protein levels positively correlate with invadopodia-associated cancer cell invasion, and Tks5 directly controls podosome-associated matrix degradation and invasion in macrophages through collective changes in adhesion, chemotaxis, and the expression/activity of MMP9. New preliminary data now suggest the involvement of Fra-1 and its gene target JunB in these Tks5-regulated processes and in the cross-talk between tumor cells and macrophages. First, along with podosome-associated matrix degradation, Fra-1, JunB, Src, and Tks5 are all upregulated in model M2-polarized THP-1 macrophages relative to pro-inflammatory M1 macrophages. This upregulation in proto-oncogenic signaling also occurs in response to conditioned media from GBM cell cultures. When Tks5 expression is silenced in THP-1 macrophages, there are concomitant decreases in Fra-1 and JunB protein levels. Secondly, the co-culture of THP-1 and GBM cell lines increases the functional levels of secreted MMP2 and MMP9 beyond that of monocultured cells. These same co-culture conditions also enhance the migratory capacity of the GBM cell line in a scratch wound assay. Thus, proto-oncogenes such as Fra-1, JunB, and Src/Tks5 may modulate the development of M2-polarized TAMs within GBM tumor microenvironments may facilitate GBM tumor cell invasion, and therefore may be novel potential prognostic factors and desirable therapeutic targets for this disease.

### AI-14. CXCR4/CXCR7 HETERODIMER MEDIATES GLIOMA CELL MIGRATION TOWARD SDF-1α

Mine Esencay and David Zagzag; New York University Langone Medical Center, New York, NY, USA

Gliomas, the most common and malignant brain tumors of the central nervous system, exhibit a high invasive capacity, preventing effective therapy. Therefore, intense efforts are ongoing to delineate the molecular mechanisms governing glioma cell migration and invasion. In this report, we show that U87MG, LN229, and LN308 glioma cells express CXCR7 protein, and exposure to hypoxia upregulates CXCR7 protein expression in these cell lines. CXCR7-positive U87MG, LN229, and LN308 glioma cells migrated toward stromal-derived factor (SDF)-1α. shRNA-mediated knockdown of CXCR7 expression reduced the migration of LN229 and LN308, but not U87MG, glioma cells toward SDF-1α in hypoxic conditions. Inhibiting CXCR4 in LN299 and LN308 glioma cells that were knocked down for CXCR7 expression did not further reduce migration toward SDF-1α. Knockdown of CXCR7 expression in LN229 and LN308 glioma cells also decreased levels of SDF-1α-induced phosphorylation of ERK1/2 and Akt, suggesting a role for CXCR7 in glioma cell migration. Inhibiting CXCR4 in these cells that were also knocked down for CXCR7 did not further reduce levels of phosphorylated ERK1/2 and Akt. Analysis of immunoprecipitated CXCR4 from LN229 and LN308 glioma cells revealed co-precipitated CXCR7. Taken together, our findings indicate the presence of a functional heterodimer of CXCR4/CXCR7 that mediates glioma cell migration toward SDF-1α, and our results support the development of therapeutic agents targeting these receptors.

### AI-15. A NOVEL TNFRSF MEMBER, TROY (TNFRSF19), IS UPREGULATED IN GLIOBLASTOMA AND MEDIATES GLIOBLASTOMA CELL INVASION AND SURVIVAL

Nhan L. Tran[1], Serdar Tuncali[1], Jean Kloss[2], Zhongbo Yang[2], Cassie A. Schumacher[3], Cassandra Diegel[3], Julianna T. Ross[1], Bart O. Williams[3], Jennifer M. Eschbacher[4], and Joseph C. Loftus[2]; [1]Translational Genomics Research Institute, Phoenix, AZ, USA; [2]Mayo Clinic Arizona, Scottsdale, AZ, USA; [3]Van Andel Research Institute, Grand Rapids, MI, USA; [4]Barrow Neurological Insitute, Phoenix, AZ, USA

Glioblastoma (GB) is the most malignant form of all primary adult brain tumors. Of the features that characterize GB, arguably none is more clinically significant than the propensity of glioma cells to aggressively invade the surrounding normal brain tissue. These invasive cells render complete resection impossible and confer resistance to chemotherapy and radiation. To improve treatment of malignant glioma, we must find a way to stop the dispersing tumor cells. Members of the tumor necrosis factor (TNF) ligand superfamily and their cognate receptors regulate various cellular responses including proliferation, migration, differentiation, and apoptosis. The TROY gene (TNFRSF19) encodes an orphan member of the TNFR superfamily: a type I cell-surface receptor that lacks a cytoplasmic death domain but contains a single TNF receptor-associated factor (TRAF)-binding site. We demonstrate that TROY expression correlates inversely with patient survival and drives GB cell migration and invasion. Our results showed that TROY overexpression in glioma cells activated Rac1 signaling in a Pyk2-dependent manner to drive invasion and migration. Knockdown of TROY in a primary GB xenograft significantly prolonged survival in vivo. In addition, when we activated expression of TROY in mouse astrocytes in vivo using an RCAS vector system for glial-specific gene transfer in G-tva transgenic mice, astrocyte migration was induced within the brain, corroborating the in vitro importance of the TROY signaling cascade in GB invasion. TROY expression significantly increased resistance of GB cells to both IR- and TMZ-induced apoptosis in vitro via the Akt and NF-kappaB activation. Inhibition of either Akt or NF-kappaB activity suppressed the survival benefits of TROY signaling in response to TMZ treatment. These findings suggest that the aberrant expression and/or signaling by TROY contributes to, and possibly drives, the malignant dispersion of glioma cells.

### AI-16. CXCR7 MEDIATES CIRCULATING BONE MARROW-DERIVED CELL TRACKING TO THE GLIOBLASTOMA VASCULATURE

Melissa Whiteman[1], Marissa Dombovy-Johnson[2], Alexis Vangellow[2], Yang Liu[2], Eleanor Carson-Walter[2], and Kevin A. Walter[2]; [1]University of Texas Medical Branch, Galveston, TX, USA; [2]University of Rochester, Rochester, NY, USA

Glioblastoma multiforme (GBM) is a genetically heterogeneous, highly vascular brain tumor, making anti-angiogenic therapies promising therapeutic options. Although most antivascular therapies aim to inhibit classic angiogenesis, alternate evidence suggests that circulating bone marrow-derived cells (BMDCs), including endothelial progenitor cells (EPC), can be recruited to the tumor vasculature through the process of vasculogenesis. These cells home in on the tumor site and are recruited by growth factors secreted by the tumor cells. We describe the recruitment of human BMDCs to brain tumor vessels via carotid injection of fluorescently labeled cells into mice bearing intracranial GBM xenografts. Our approach exploits circulating cells that are easily collected from blood, avoids long-term propagation in cell culture, and can utilize the entire BMDC population as well as isolated specific progenitor cell types. Dual labeling with endothelial, hematopoetic, and mesenchymal progenitor markers revealed that BMDCs recruited to tumor endothelium express known angiogenic and endothelial cell markers as well as glioma endothelial markers, including TEM1/endosialin and CXCR7, a recently described marker for CXCL12. Injection of flow-sorted CXCR7 +/CD45- cells resulted in similar recruitment to the tumor vasculature. Labeled cells were not retained in the adjacent non-neoplastic brain tissue. Mechanistically, incubation of BMDCs with an anti-CXCR7 monoclonal antibody prior to carotid injection decreased the percent of vessels that contained stained cells by approximately 54% 7 days after injection as compared with brain tissue injected with control BMDCs. In vivo delivery of a small molecule CXCR7 antagonist blunted BMDC tropism for the xenograft vasculature by more than 90%, further supporting a role for CXCL12:CXCR7 signaling in this migratory response. Exploiting the homing capacity of circulating BMDCs for brain tumor vessels may facilitate the development of novel targeted therapies.

### AI-17. CXCR7 CONTRIBUTES TO BRAIN TUMOR ANGIOGENESIS THROUGH THE EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK1/2) PATHWAY

Yang Liu, Eleanor Carson-Walter, and Kevin Walter; University of Rochester, Rochester, NY, USA

The critical roles of the chemokine CXCL12/SDF-1α and its receptor, CXCR4, have been well documented in primary tumor growth and metastasis. Recently, it has been reported that CXCR7 serves as a second receptor for CXCL12, exerting an important role in promoting the growth of many types of tumors. However, the functional contribution of CXCR7 to malignant brain tumors, especially its role in tumor angiogenesis, remains largely unknown. We found that elevated CXCR7 mRNA levels are associated with poor survival for patients with glioma and, specifically, glioblastoma (GBM). We used immunohistochemical staining to localize CXCR7 protein expression to GBM tumor cells and vessels in clinical specimens and immunofluorescent staining to confirm its expression in cultured human brain microvascular endothelial cells (HBMECs). We inhibited expression of CXCR7 in HBMECs using small interfering RNA (siRNA). Targeted suppression of CXCR7 RNA and protein was confirmed by qRT-PCR and flow cytometry. Phenotypically, CXCR7 knockdown significantly impeded CXCL12-stimulated tube formation and migration by HBMECs. CXCL12 promoted tube formation and migration on HBMECs through activation of extracellular signal-regulated kinases (Erk1/2). Mechanistically, siRNA-mediated depletion of CXCR7 reduced CXCL12-induced phosphorylation of Erk1/2. In addition, treatment of HBMECs with a small molecule antagonist of CXCR7, CCX771, blocked CXCL12-induced tube formation in matrigel assays. Therefore, CXCR7 mediates CXCL12 signaling in HBMECs in part by activating the Erk1/2 pathway. Targeting CXCR7 may provide novel opportunities for improving brain tumor therapy, and our data suggest the possibility of using a small molecule CXCR7 antagonist as an anti-angiogenic agent for malignant brain tumors.

### AI-18. INSIGHTS ON THE ROLE OF MICROENVIRONMENT IN THE RECURRENCE OF MALIGNANT GLIOMAS TO ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR THERAPY: OPPORTUNITIES FOR NEW COMBINED THERAPIES

Nahir Cortes-Santiago, Konrad Gabrusiewicz, Dan Liu, Mohammad B. Hossain, Joy Gumin, Xuejun Fan, Charles Conrad, Kenneth Aldape, Mark Gilbert, Aditya Raghunathan, W.K. Alfred Yung, Juan Fueyo, and Candelaria Gomez-Manzano; The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Supported by a strong pathobiological rationale (malignant gliomas are highly vascularized tumors) and by clinical results (improved progression-free survival), anti-vascular endothelial growth factor (VEGF) therapies are offered to patients with malignant gliomas. Specifically, bevacizumab, a humanized monoclonal antibody inhibiting VEGF, is an FDA-approved agent for second line treatment of these patients. However, preclinical data from our group and others, as well as some clinical data, show a heightened invasive phenotype in the recurrence of gliomas after treatment with bevacizumab and other VEGF signaling pathway interfering agents. We undertook this study to explore the microenvironment mechanisms responsible for this elusive resistance. In our studies, we collected robust evidence of the overrepresentation of a subpopulation of myeloid cells, Tie2-expressing monocytes (TEMs), co-existing with a tumoral invasive pattern in animals treated with anti-VEGF agents. These cells accumulate in the tumor/normal interphase regions, and their presence was significantly higher than in brain tissue of glioma-bearing mice treated with other therapies, such as temozolomide. Of clinical interest, we detected this myeloid subpopulation in patients for whom anti-VEGF therapies failed. Using both M2-polarized monocyte cultures and monocytes isolated from the blood of healthy donors, we observed that the molecular profile expression of TEMs is related to tumor remodeling, as assessed by expression arrays and validated by flow cytometry and ELISA. Data from co-culture studies support the role of TEMs in enhancing the migration properties of glioma cells. Furthermore, our results suggest that increased levels of Angiopoietin 2 (Ang2) attract TEMs to the tumor/normal interphase, a fact further demonstrated in animal models. Together, our results suggest a microenvironment-based mechanism for the escape of malignant gliomas from anti-VEGF therapies and encourage regimens based on antiangiogenic therapies to be modified by targeting either TEMs and/or the recruiting cytokine Ang2 in order to improve the clinical outcome for malignant glioma patients.

### AI-19. ANGIOGENESIS IN GLIOBLASTOMA TUMORS IS PROMOTED BY INTEGRIN α3β1

Eunnyung Bae, Ping Huang, Monica Burgett, Gaelle Muller-Greven, Niladri Kar, and Candece L. Gladson; Cleveland Clinic, Cleveland, OH, USA. *co-first authors

Angiogenesis is a prominent and characteristic feature of glioblastoma (GBM) tumors. As migration is necessary for angiogenesis, we hypothesized that the tumor-associated endothelial cells (ECs) in GBM are more migratory. To test this, we isolated ECs from GBM and compared their chemotactic migration and tubule formation with that of normal brain ECs. Using Boyden chambers with collagen-coated, 3-micron pore filters, we found that GBM-isolated ECs were approximately 5 times more migratory toward bFGF than were normal brain ECs. As integrins mediate cell-migration, we analyzed the integrin repertoire and found higher expression of integrin α3β1 in GBM-isolated ECs than in normal brain ECs, consistent with a prior report of the increased level of integrin α3 expression in ECs in GBM biopsies. In tubulomorphogenesis assays on matrigel, GBM-isolated ECs formed an increased number of tubules that budded abundant sprouts, which were absent in the normal brain ECs in the same condition. The sprouts stained positive for integrin α3, and a neutralizing antibody to integrin α3 significantly reduced sprouting, suggesting that integrin α3β1 is necessary for sprouting in GBM-isolated ECs. Furthermore, the localization of integrin α3β1 in the GBM-isolated ECs was significantly different than that in normal brain Ecs; in GBM-isolated Ecs, integrin α3β1 localized largely to cell-cell contacts. As VE-cadherin is important in EC-EC junction formation, we analyzed its expression. We found a dramatic reduction in VE-cadherin expression in GBM-isolated ECs as compared with normal brain ECs. Taken together, these data suggest that in GBM-isolated ECs, the increased expression of integrin α3β1 and its altered localization to cell-cell contacts along with decreased VE-cadherin expression likely contribute to the increased sprouting and migration that promotes angiogenesis in GBM.

### AI-20. MODELING DISTINCT INVASIVE PHENOTYPES OF GLIOBLASTOMA

Jane R. Engler, Aaron E. Robinson, Annette Molinaro, and Joanna J. Phillips; University of California San Francisco, San Francisco, CA, USA

Invasion of neoplastic cells deep within non-neoplastic brain parenchyma, a fundamental feature of glioblastoma (GBM), significantly contributes to the poor prognosis of the disease. Tumors can have diverse patterns of intraparenchymal invasion, including perivascular invasion and diffuse, single-cell invasion. While the therapeutic implications of this intratumoral heterogeneity are unclear, recent studies suggest that, in a subset of patients, altered patterns of invasion may be a mechanism of resistance to antiangiogenic therapy. To identify the molecular mechanisms that drive different invasive patterns of glioma, we have established two murine gliomas with distinct invasive phenotypes. Derived from the same parental Ink4a/Arf-/- neural progenitor cells and transduced with hEGFRvIII, the tumors were selected based upon their in vivo pattern of tumor cell invasion: perivascular (PV) vs diffuse single-cell invasion (DI). When propagated as neurospheres, the tumors retain these invasive phenotypic patterns upon subsequent orthotopic transplantation ($P = 0.043$, $n = 19$). Thus they provide a useful tool to study tumor cell invasion in an immunocompetent host. ZsGreen tagged tumor cells were sorted from tumors and transcriptionally profiled. Tumors with a DI pattern had a significantly higher expression of genes associated with interactions with the extracellular environment, including cell-adhesion molecules, chondroitin sulfate biosynthesis, and heparan sulfate biosynthesis, than did tumors with a PV pattern of invasion (> 2-fold increase, FDR < 1%, $n = 12$). Consistent with the potential differences in interactions with the extracellular environment, DI tumor cells displayed greater adherence to fibronectin than did PV tumor cells ($P = 0.03$). We are currently identifying determinants of differential invasion at the protein level, with an emphasis on extracellular proteins, using knockdown and antibody-mediated inhibition in both murine and human tumor cells. As response to therapy may be influenced by tumor cell invasion and as extracellular determinants of invasion are potential therapeutic targets, it is critical that we better understand this fundamental process.

### AI-21. SPATIOTEMPORAL REGULATION OF GBM NEOVASCULARIZATION AND RESPONSE TO THERAPY

Gelareh Zadeh, Kelly Burrell, and Richard Hill; University of Toronto, Toronto, ON, Canada

Although antiangiogenic (AA) therapy has held promise in treatment of glioblastoma multiforme (GBM), tumor recurrence and revascularization following therapy persists. Our study aims to establish the spatiotemporal contribution of bone marrow-derived progenitor cells (BMDCs) to tumor neovascularization, specifically to understand the role of BMDCs in GBM revascularization following AA therapy, radiation treatment, and chemotherapy. We used two GBM models in this study: intracranial glioma xenografts created in intracranial windows, generated in chimeric mice with GFP + bone marrow, and a transgenic model of GBM, RasB8, with reconstituted GFP + BM. Mice were treated with chemotherapy (TMZ), radiation, and AA therapy using VEGFTRAP. Using 2-photon high-resolution in vivo images, we studied the pattern of integration of GFP + BMDCs into tumor vasculature. Vasculature in different GBM regions (central/peripheral) and at different stages of tumor growth and in response to therapy were isolated using laser capture microdissection and RNA extracted for quantitative real-time PCR analysis to establish a differential angiogenic profile. We found a distinct pattern of integration of BMDCs into tumor vasculature based on tumor-growth stage and region. At early stages of tumor formation (less than 1 week), 90% of BMDCs integrated into the vasculature of both the center and the periphery, whereas at later stages of tumor growth (more than 3 weeks), less than 10% of BMDCs integrated in the central GBM vasculature while 90% persisted in the periphery. Vessels demonstrated a distinct angiogenic profile in the center vs the periphery of GBMs. Radiation and VEGFTRAP treatment altered the expression profile, significantly increasing expression of Angiopoietin2/Tie2 pathway. Our results suggest that the tumor microenvironmental factors that vary with tumor region and growth-stage closely influence mechanisms of GBM neovascularization. We show that BMDCs promote tumor revascularization following therapy. Moreover, our findings demonstrate a switch from VEGF-dependent mechanisms of neovascularization to an Angiopoietin/Tie2 pathway mechanism in response to either radiation or AA therapy.

### AI-22. GLIOBLASTOMA RESISTANCE TO ANTIANGIOGENIC THERAPY ASSOCIATED WITH AN INFLAMMATORY AND MESENCHYMAL PHENOTYPE

Yuji Piao, Ji Liang, Verlene Henry, Lindsay Holmes, Erik Sulman, and John F. deGroot; The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Antiangiogenic therapy reduces vascular permeability and delays progression but may ultimately promote an aggressive treatment-resistant phenotype. The aim of the present study was to identify mechanisms responsible for glioblastoma resistance to antiangiogenic therapy. Glioma stem cell (GSC) NSC11 and U87 cell lines with acquired resistance to bevacizumab were developed from orthotopic xenografts in nude mice treated with bevacizumab. Genome-wide analyses were used to identify changes in tumor subtype and specific factors associated with resistance. Mice with established parental NSC11 and U87 cells responded to bevacizumab, whereas glioma cell lines derived at the time of acquired resistance to anti-VEGF therapy were resistant to bevacizumab and prolonged survival compared with untreated controls. Gene-expression profiling comparing anti-VEGF therapy-resistant cell lines with untreated controls demonstrated an increase in genes associated with a mesenchymal origin, cellular migration/invasion, and inflammation. Consistent with these data, mice bearing NSC11 and U87-resistant tumors showed significantly greater infiltration of CD74 and $F4/80^{+}$ myeloid cells, respectively, compared with mice carrying wild-type tumors. Gene set enrichment analysis (GSEA) demonstrated that bevacizumab-treated tumors showed a significant correlation to published mesenchymal gene signatures. Invasion-related genes were also upregulated in both NSC11 and U87-resistant cells, which had higher invasion rates in vitro than did their respective parental cell lines. Our studies identify multiple pro-inflammatory factors associated with resistance and identify a proneural to mesenchymal transition (PMT) in tumors resistant to antiangiogenic therapy. A better understanding of resistance mechanisms may provide new strategies to prolong the efficacy of this therapy and accelerate the integration of combination therapies into clinical trials.

### AI-23. TARGETING INTERCELLULAR CELL-ADHESION MOLECULE-1 (ICAM-1) PROLONGS SURVIVAL IN AN ORTHOTOPIC GLIOMA STEM CELL MODEL

Yuji Piao, Ji Liang, Verlene Henry, Lindsey Holmes, and John F. de Groot; The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Glioblastoma, characterized by cellular heterogeneity, vascular proliferation and extensive tissue infiltration, is the most common malignant brain tumor. Gene-expression profiling comparing invasive anti-VEGF therapy-resistant cell lines with untreated controls demonstrated an increase in genes associated with a mesenchymal origin, inflammation, and cellular migration/invasion. One highly overexpressed gene identified in tumors with acquired resistance to bevacizumab was intercellular cell-adhesion molecule 1 (ICAM1, CD54). ICAM1 is a cell-adhesion glycoprotein of the

immunoglobulin supergene family that interacts with β2 integrins, mediates leukocyte transendothelial migration, and activates T cells during inflammatory processes. ICAM1 overexpression in tumors resistant to antiangiogenic therapy was validated by real time PCR and Western blot. In a panel of glioma stem cell lines, ICAM1 expression was higher in mesenchymal than in proneural cell lines. GFP-tagged ICAM1 shRNA lentivirus was used to knock down ICAM1 in multiple cell lines. An in vitro matrigel transwell migration assay showed a significant reduction in cell migration in shICAM1 cell lines compared with scramble controls. However, cell proliferation was unaffected by ICAM1 knockdown in vitro. Next, we injected shICAM1 and scramble glioma stem cells into the brains of nude mice. Animals bearing tumors formed from shICAM1 cells survived significantly longer than mice injected with scramble control cells. The impact of ICAM1 on glioma resistance to antiangiogenic therapy in vivo will be presented. Our studies identify ICAM1 as a potentially important mediator of tumor migration/invasion in glioblastoma. Targeting ICAM1 may provide a new strategy to prolong the efficacy of antiangiogenic therapy and prevent this invasive phenotype.

## AI-24. VASCULAR ENDOTHELIAL GROWTH FACTOR BLOCKADE RESULTS IN EPITHELIAL MESENCHYMAL TRANSITION IN GLIOBLASTOMA

Wang Rong, Kosuke Funato, Petrina Georgala, Fumiko Shimizu, Leif Droms, and Viviane Tabar; Memorial Sloan-Kettering Cancer Center, New York, NY, USA

The use of vascular endothelial growth factor (VEGF) blockade has been associated with a paradoxical increase in invasion and metastasis of various solid cancers, including glioblastoma (GBM). The mechanisms responsible for this often terminal event remain unclear. Here we investigated whether VEGF blockade via bevacizumab (Bev) affects GBM cells in a cell-autonomous fashion. We observed freshly dissociated GBM cells obtained from the surgical suite under time-lapse photography and demonstrated a higher mean velocity, as well as a longer recorded distance in cells treated with Bev for 48 hours (about 40%, $P < 0.005$). Similarly, a matrigel invasion assay demonstrated a 5-fold increase in invasion capacity among cells treated with Bev vs controls. Similar data were obtained in a transwell migration assay. Phenotypic analysis showed an upregulation in the expression of PDGFR, Vimentin, SMA, and YKL40. GBM explants treated ex vivo with Bev demonstrated a similar upregulation but tended to have a perivascular distribution. We also obtained paired samples from patient tumors before and after Bev therapy and identified a statistically significant upregulation of similar markers. Finally, RTqPCR arrays demonstrated a dramatic increase (5-1000 times) in the expression of Slug, Twist, CTGF, Col5A, and Notch signaling activity among the migratory cells treated with Bev. This was associated with a decrease in E cadherin and cMet, suggesting a transition from an epithelial to a mesenchymal state. Ongoing work will establish the pathways governing this transition in GBM and will define the cell subpopulations capable of this phenotypic plasticity.

## AI-25. EGFR AMPLIFICATION CORRELATES WITH INCREASED MIGRATION EFFICIENCY IN HUMAN GLIOBLASTOMA

Jonathon J. Parker[1], Kalen R. Dionne[1], Rada Massarwa[1], Marci Klaassen[1], Nicholas K. Foreman[1], Lee Niswander[1], Peter Canoll[2], B.K. Kleinschmidt-DeMasters[1], and Allen Waziri[1]; [1]University of Colorado School of Medicine, Aurora, CO, USA; [2]Columbia University, New York, NY, USA

PURPOSE: The extent of brain invasion varies among glioblastoma (GBM) patients and remains a major source of treatment failure. Epidermal growth factor receptor (EGFR) amplification is common in GBM, and this pathway has been previously associated with invasive behavior through in vitro modeling and analysis of clinical imaging. However, there has been no direct association between EGFR amplification and characteristics of tumor-cell migration in human GBM. To further explore this issue, we used an organotypic slice culture system to compare migration speed and efficiency of tumor cells within human GBM and correlated these findings with EGFR amplification. METHODS: Tissue slices were generated from freshly resected primary GBM and cultured under standard levels of oxygen and glucose. Tumor cells were labeled with a ZsGreen-expressing retrovirus and imaged using time-lapse confocal microscopy. Individual cell-migration paths were generated and used to calculate mean migration speed (ratio of total distance to time of migration) and efficiency (ratio of displacement distance to total distance traveled) for each tumor. EGFR amplification was assessed in donor tumors using fluorescence in situ hybridization. RESULTS: Mean migration speeds were distributed over a nearly 3-fold range across our cohort, while mean migration efficiency varied from 0.43 to 0.77. Faster migration speeds were significantly correlated with higher migration efficiency in tested tumors. Critically, EGFR-amplified tumors exhibited significantly greater migration efficiency than did non-amplified tumors (0.70 vs 0.54, $P < 0.05$). A similar strong trend was noted between efficiency and higher mean migration speed in tumors ($R2 = 0.47$, $P = 0.06$). CONCLUSIONS: Our study confirms the presence of baseline differences of tumor-cell migration in human GBM. These data provide support for prior studies suggesting that EGFR amplification is associated with a more invasive tumor phenotype. We predict that the slice model may provide a personalized testing platform for EGFR-specific therapies in patients with GBM.