P08.14 IN SITU DETECTION OF HYPOXIA INDUCIBLE FACTOR 2 ALPHA IN MALIGNANT GLIOMAS
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INTRODUCTION: Direct intratumoral measurements confirm that necrosis and hypoxia are fundamental features of the pathobiology of glioblastoma (GBM). Tumor cells adapt to hypoxia through up regulation of hypoxia inducible factors (HIFs), a conserved family of transcription factors. Overexpression of HIF-2 alpha (HIF-2α) appears to be expressed in glioblastoma tumor cells (not neural progenitors) and drives adaptations prolonged (>24 hour) hypoxia. Targeting HIFs is an attractive cancer treatment strategy given that tumor hypoxia is a constant environmental cue driving genetic instability, cell migration, angiogenesis, and chemoradiation resistance. GBM, a tumor uniformly characterized by an intratumoral hypoxic environment, is a prime candidate for HIF therapeutic targeting but specific drugs were not available. PT2385 is a novel, first-in-class, HIF-2α inhibitor which recently entered clinical trials for renal carcinoma. PT2385 is orally administered and is small, lipophilic with a plasma/brain ratio of 0.9 in rats. To establish HIF-2α as a therapeutic target in GBM, we investigated in situ expression of HIF-2α protein in tissue samples from the Wake Forest Brain Tumor Center of Excellence Brain Tumor Bank. METHODS: 22 formalin-fixed, paraffin-embedded glioma samples (grade IV) were analyzed for HIF-2α expression using immunohistochemistry. After rehydration, endogenous peroxidase was blocked, epitopes were retrieved using a microwave Tris EDTA pH 9.0 solution, non-specific epitopes were blocked, and HIF-2α antibody (Santa Cruz, clone 10B2, 1:500 dilution) was added. A rabbit anti-mouse secondary antibody and anti-mouse antibody was then added followed by detection chromagen. An inverted bright field microscope captured images and representative samples were digitally scanned. Localization and quantification of HIF-2α was independently verified by a neuropathologist. RESULTS: There was no detectable HIF-2α expression in the four Grade I and two Grade III gliomas studied. Of the 16 GBMs (Grade IV), HIF-2α was expressed in 13 (81%). HIF-2α was highly expressed in seven specimens (>10% cells positive), intermediate in six specimens (5-10% of cells positive), and minimal to none in three specimens. Staining was specific to both tumor cells and occasionally mononuclear cells (based on morphology). It was noted that HIF-2α was frequently present in perivascular and perinecrotic regions. CONCLUSIONS: HIF-2α expression for the majority of GBMs, but is absent in grade gliomas. Immunohistochemistry demonstrated a range of HIF-2α abundance along with regional staining patterns, clustered in perivascular and perinecrotic niches. HIF-2α appears to correlate with increasing malignancy grade. This is the first in situ description of HIF-2α in gliomas and further studies are in progress for preclinical in vitro and in vivo testing of PT2385, a first-in-class HIF-2α targeted agent, in GBM.

P08.15 NEW SYNTHETIC PEPTIDES DERIVED FROM HONEY IN GLIOMA CONTROL (PRECLINICAL STUDY)
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In spite of advancements in all types of malignancy control like surgery, chemotherapy, radiotherapy, immunotherapy as well as presence of many peptides in the market for cancer control, prognosis in many types of cancers still very poor. It is enough for anybody to have a look at the main causes of death at any country to know that cancer is one of them and to discover how the challenge and defect in its treatment. Many researches aim was just how the challenge and defect in its treatment. Many researches aimed to overcome this challenge through direct apoptotic action on its own ability, cell migration, angiogenesis, and chemoradiation resistance. GBM, a tumor uniformly characterized by an intratumoral hypoxic environment, is a prime candidate for HIF therapeutic targeting but specific drugs were not available. PT2385 is a novel, first-in-class, HIF-2α inhibitor which recently entered clinical trials for renal carcinoma. PT2385 is orally administered and is small, lipophilic with a plasma/brain ratio of 0.9 in rats. To establish HIF-2α as a therapeutic target in GBM, we investigated in situ expression of HIF-2α protein in tissue samples from the Wake Forest Brain Tumor Center of Excellence Brain Tumor Bank. METHODS: 22 formalin-fixed, paraffin-embedded glioma samples (grade IV) were analyzed for HIF-2α expression using immunohistochemistry. After rehydration, endogenous peroxidase was blocked, epitopes were retrieved using a microwave Tris EDTA pH 9.0 solution, non-specific epitopes were blocked, and HIF-2α antibody (Santa Cruz, clone 10B2, 1:500 dilution) was added. A rabbit anti-mouse secondary antibody and anti-mouse antibody was then added followed by detection chromagen. An inverted bright field microscope captured images and representative samples were digitally scanned. Localization and quantification of HIF-2α was independently verified by a neuropathologist. RESULTS: There was no detectable HIF-2α expression in the four Grade I and two Grade III gliomas studied. Of the 16 GBMs (Grade IV), HIF-2α was expressed in 13 (81%). HIF-2α was highly expressed in seven specimens (>10% cells positive), intermediate in six specimens (5-10% of cells positive), and minimal to none in three specimens. Staining was specific to both tumor cells and occasionally mononuclear cells (based on morphology). It was noted that HIF-2α was frequently present in perivascular and perinecrotic regions. CONCLUSIONS: HIF-2α expression for the majority of GBMs, but is absent in grade gliomas. Immunohistochemistry demonstrated a range of HIF-2α abundance along with regional staining patterns, clustered in perivascular and perinecrotic niches. HIF-2α appears to correlate with increasing malignancy grade. This is the first in situ description of HIF-2α in gliomas and further studies are in progress for preclinical in vitro and in vivo testing of PT2385, a first-in-class HIF-2α targeted agent, in GBM.

P08.16 ATP3 REDUCES MIGRATION CAPACITY BY REGULATION OF MATRIX METalloPROTEINASES IN GliOBLASTOMA IN VITRO
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OBJECTIVE: Glioblastoma is associated with poor survival and a high recurrence rate in patients due to the inevitable uncontrollable infiltrative tumor growth. The elucidation of the molecular mechanisms may offer opportunities to prevent relapses. In this study we investigated the role of the activating transcription factor 3 (ATF3) in the context of experimental nitric oxide donor (NO) treatment with JS-K (O2-(2,4-dinitrophenyl)-1-[4-ethoxy carbonyl]pyrazole-1-yl)diazonium-1,2-dilolate) on migration of GBM cells in vitro. METHODS: RNA microarray was performed to identify ATF3 as one of the genes upregulated by experimental NO therapy in U87 glioma cells. To elucidate its role in tumor growth and treatment response of malignant gliomas, ATF3 was downregulated by siRNA and evaluation of expression of target genes STAT3 and NFkB was investigated by qRT-PCR, Western Blot and immunocytochemistry. The underlying molecular mechanisms of migration capacity and proliferation were studied by Western Blot, zymography, immunostaining and PCR while migration was assessed by wound closure and invasion assay. RESULTS: RNA microarray revealed that gene expression of ATF3 is 15-fold upregulated after exposure to 15 µM JS-K for 48h. We demonstrate that ATF3 is directly involved in the regulation of matrix metalloproteinase expression and activation. MMP2, 7 and 9 were downregulated and MMP3 activity was reduced compared to controls. Inhibition with specific inhibitors like aminoglutethimide and chloroquine of the transporter for NO entry into the gap (p=0.02) and reduced invasive capacity to 30% (p=0.00004). The proliferation rate was 2-fold reduced by ATF3 (p=0.003) after 72h whereas the influence of NO on viability did not change. CONCLUSIONS: Overexpression of ATF3 leads to a significantly reduced migration and invasion capacity by induction of tissue inhibitors of matrix metalloproteinases. ATF3 is directly involved in expression and activation of MMPs as well as the oncogenic regulators STAT3 and NFkB. Our study highlights for the first time the role of ATF3 as a potential novel therapeutic target and can therefore be important for specific anticancer therapy to overcome the high treatment resistance of glioblastoma.

P08.17 MITOTIC CHECKPOINT INHIBITION AUGMENTS TUMOR-TREATING FIELD (TTTFIELD) EFFECTS ON GliOBLASTOMA CELLS
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INTRODUCTION: Treatment of glioblastoma (GBM) patients with Tumor Treating Fields (TTFields) in addition to standard therapy showed significant increase in progression free and overall survival (EP-14 trial). TTFields are alternating electric fields with low intensity (1-3 V/cm) and intermediate frequency (100-400kHz) that disrupt cell division through the inhibition of spindle fiber formation. The chemotherapeutic agent Vincristine is also reported to inhibit spindle fibers and in combination with TTFields is known to slow down cell division. Our study highlights for the first time the role of Vincristine as a potential novel therapeutic target and can therefore be important for specific anticancer therapy to overcome the high treatment resistance of glioblastoma.

RESULTS: In vitro studies showed inhibitory effects for those peptides by themselves upon 17 tumor cell lines out of 18 cell lines; U87MG, MDA-MB-468, K562, A375, MGC63, SH-4, RD, KPI, 5637, 2774, ML-1, Cal-27, Colo-205, 769P, E0-1, HLE, MDA-MB-436 and Calu-3 as well as the case with in vivo study in U87MG tumor mouse model. Pharmacokinetic of peptide F was also studied using 3 rats that showed very short half life. At Debnik1, we try to modify and improve peptide F and other peptides to get more potent effects plus safety, who knows, we may do it so, it is clear how those results could move the field of malignancy in general and especially gliomas management forward.