orphan adhesion G-protein coupled receptor (GPCR), is enriched in CD133-expressing GSCs (n=3 biospecimens; P<0.04), at the mRNA and protein level. Immunohistochemical staining of 9 GBM biospecimens revealed that GPR133 is restricted to hypoxic areas of pseudopalisading necrosis, which also express hypoxic markers Hif1a and CA9. No GPR133 staining was observed in normal brain. Induction of hypoxia in patient-derived GBM cultures led to upregulation of GPR133 transcript (n=5 cultures; P<0.008). Consistent with the hypothesis that GPR133 expression is regulated by oxygen tension, we found multiple hypoxia response elements (HRE) in the genomic locus of GPR133 and Hif1a knockdown decreased the level of GPR133 transcript (P<0.05). GPR133 knockdown decreased the CD133+ cell population, tumor cell proliferation and tumorsphere formation in vitro under both normoxic and hypoxic conditions (P<0.05). GPR133 knockdown essentially abolished in vivo tumor xenograft initiation (P<0.002) and prevented the death of mouse hosts (n=4 mice/group; P<0.01). GPR133 knockdown decreased intracellular cAMP levels (P<0.05), and forskolin rescued the in vitro knockdown phenotype (P<0.03), suggesting that GPR133 signals through intracellular cAMP. Finally, analysis of TCGA data from 160 GBM patients reveals that higher GPR133 mRNA levels correlate with worse survival (P=0.0062). In summary, our findings offer compelling evidence that GPR133 plays an important pro-tumorigenic role in GBM, especially in the context of hypoxia, and represents a novel therapeutic target.

STMC-26. MULTIVALENT CATIONIC LIPOSOMES FOR siRNA TRANSFECTION OF PATIENT DERIVED GLIOMA INITIATING CELLS

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Glioma initiating cells (GICs) have been implicated as the root cause of treatment failure and tumor recurrence in glioblastoma multiforme (GBM). Therapeutic targeting of GICs is therefore essential to ensure effective treatment without relapse. A novel approach to modulate protein expression in cancer cells is the delivery of siRNAs using liposomes. However, past attempts to transfect cells using neutral liposomes have proven challenging and inefficient owing to the highly refractory nature of these cells. We therefore sought to develop a multivalent cationic liposome (MVCL) formulation for efficient and reproducible transfection of cancer initiating cells. The high valency and cationic charge on these liposomes allows binding to more siRNA molecules while increasing endocytosis into the cell due interactions with the negatively charged cell membrane. To test the ability of these liposomes to transfect GICs in vitro, we transfected CD133+ patient derived GICs with siRNA against the iron storage protein H-ferritin (Hft). Using this method of siRNA delivery, we were able to knockdown the expression of Hft in GICs compared to controls. The most effective Hft knockdown was observed at 48 and 72hours post transfection and was accompanied by a compensatory increase in the expression of light chain subunit of ferritin, L-ferritin. Past studies using Hft shRNA show reduced cell proliferation in GICs upon Hft downregulation. Consistent with this finding, we observed increased release of LDH in Hft siRNA treated GICs versus control and vehicle treated cells. These data suggest that Hft downregulation by itself might be lethal to GICs; a finding that is different from our previous report using astrocytoma cell lines. Thus, we show that MVCL liposomes possess the ability to efficiently transfect and deliver siRNA to patient derived cancer initiating cells providing a new means to modulate protein expression in GICs and a potential new target in H-ferritin.

STMC-27. NOVEL LYSINE DEMETHYLASE KDM1A INHIBITORS INDUCE DIFFERENTIATION AND APOPTOSIS OF GLIOMA STEM CELLS VIA UNFOLDED PROTEIN RESPONSE PATHWAY

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Glioma Stem Cells (GSCs) play a central role in glioblastoma (GBM) development and chemo/radiation resistance, and their elimination is critical for the development of efficient therapeutic strategies. Recently, we demonstrated that lysine-specific histone demethylase 1A (KDM1A/LSD1) is overexpressed in GBM. In the present study, we determined whether KDM1A modulates GSCs stemness and differentiation and tested the utility of two novel KDM1A specific inhibitors (NCL-1 and NCD-38) to promote differentiation and apoptosis of GSCs. The efficacy of KDM1A targeting drugs was tested on purified GSCs isolated from established and patient-derived GBMs using both in vitro assays and in vivo orthotopic preclinical models. Our results suggested that KDM1A is highly expressed in GSCs and knock down of KDM1A using shRNA reduced GSCs stemness and induced the differentiation. Pharmacological inhibition of KDM1A using NCL-1 and NCD-38 significantly reduced the proliferation, neurosphere formation and induced apoptosis of GSCs with little effect on differentiated cells and KDM1A-knockout cells. In preclinical studies using orthotopic models, NCL-1 and NCD-38 significantly reduced GSC-driven tumor progression and improved mice survival. RNA sequencing analysis showed that KDM1A inhibitors modulate several pathways related to stemness, differentiation and apoptosis. Mechanistic studies showed that KDM1A inhibition induced activation of the unfolded protein response (UPR) pathway. Western blot analysis demonstrated increased expression of BiP, cleaved ATF-6, p-PERK and CHOP. Further immunohistochemical analysis demonstrated increase in expression of UPR components in tumor sections. Chromatin immunoprecipitation assays revealed significant enrichment of H3K4-me2 at the promoters of the UPR genes ATF3 and DDIT3 in GSC following treatment with KDM1A inhibitors. More importantly, in vivo studies demonstrated that KDM1A inhibitors NCL-1 and NCD-38 are safe without any significant toxicity. These results strongly suggest that selective targeting of KDM1A using NCL-1 and NCD-38 is a promising therapeutic strategy for elimination of GSCs.

STMC-28. INTACT EGFR DEFINES HUMAN GERMINAL MATRIX AND GLIOBLASTOMA POPULATIONS WITH SHARED AND EPIGENETICALLY IMPRINTED STEM CELL PROPERTIES

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Epidermal growth factor receptor (EGFR) signaling is important for neural development and is frequently dysregulated in glioblastoma (GBM), but its developmental relationship to human gliomagenesis remains poorly understood. Using an EGF-ligand-binding strategy, we isolated EGFR+/– populations from fresh human germinal matrix (GM) and GBM tissues, enriching for cells with intact ligand-binding domain (EGFR+^{INTACT}), and directly compared their downstream functional and molecular phenotypes. In both GM and GBM, only EGFR+ ^{INTACT} populations displayed stem cell properties *in vitro*, and in GBM, tumor initiation *in vivo*. Chromatin accessibility mapping revealed remarkable overlap between neoplastic and developing EGFR+ populations. Shared open chromatin regions annotated cell cycle and stemness gene sets and distinct regulatory motifs genome-wide, with footprints for ASCL1, SOX4/10, E2F1 and GABPA specifically at *EGFR*. Our study defines a novel population of EGFR+^{INTACT} stem-like cells derived from fresh GM and GBM samples and implicates developmentally imprinted transcriptional regulators for their proliferation.

STMC-29. GENETIC ENGINEERING OF GLIOMA CELLS WITH HISTONE-2B-GFP TAG TO TRACK AND CHARACTERIZE QUIESCENT GLIOMA STEM CELLS

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Glioblastoma (GBM) is the most common malignant brain tumor with a dismal prognosis and limited therapy options. Advances in culture methods have led to the establishment of patient-derived glioma stem cell (GSC) lines that preserve genomic and physiologic characteristics of GBM. Quiescent populations of GSCs have been proposed as the main source of GBM recurrence after therapy. We are applying CRISPR to introduce a doxycycline inducible histone-2B-GFP (H2B-GFP) labeling system at the AAVS1 locus, a "safe harbor" for transgene insertions. The H2B-GFP label allows to track and isolate quiescent glioma cells that retain high GFP label (dividing cells dilute H2B-GFP), and to characterize their cancer stem cell properties. We have transfected GSC lines with an AAVS1 Cas9 plasmid and a targeting plasmid carrying Tet-ON transactivator M2rtTA and tetO-H2B-GFP. Cells with integrated targeting vector were selected with G418, and insertions were confirmed by PCR over 5' and 3' arms. H2B-GFP GSC lines were transplanted intracranially into the striatum of SCID mice, and after an initial pulse period of 2 weeks (doxycycline-ON), tumors are allowed to expand during chase periods of 2, 4, or 8 weeks. Subcohorts of mice are subjected to radiation therapy to elucidate the contribution of quiescent GSC to radiation resistance. We are using histology to reveal the quiescent cell populations (GFP-high) in tumors, their spatial distribution and proximity to vascular niches, and their molecular features. GFP-high (quiescent) and GFP-low (proliferating) populations of tumor cells are separated by FACS, and populations are assessed by in vitro assays to elucidate whether the proliferative history of GSCs leaves a lasting imprint on future proliferation, differentiation, or migration behaviors. Finally, we are also using FACS sorted populations to isolate RNA and generate expression profiles by RNA-Seq, which will allow us to identify new molecular markers of quiescent glioma stem cells.

STMC-30. TRAFFICKING AND EFFECT ON SURVIVAL OF BEVACIZUMAB IN GLIOBLASTOMA

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Most patients with recurrent glioblastoma (GBM) are treated with bevacizumab, a humanized monoclonal antibody (mAb) that binds VEGF-A and inhibits its binding to VEGFR. Approximately 30% of GBM patients are non-responsive to bevacizumab and the underlying mechanism for the lack of response is unknown. It has been assumed that bevacizumab solely targets circulating VEGF-A. We hypothesized that bevacizumab and human IgGs in general gain access to the perivascular niche that contains cancer stem cells (CSCs) in GBM. We found that bevacizumab gains access to the perivascular tumor area through leaky blood vessels and was internalized by tumor cells in an orthotopic established xenograft mouse model of GBM. In vitro, CSCs (CD133+) from GBM rapidly internalized bevacizumab into membrane protrusions that contained actin and internalization was significantly inhibited by a macropinocytosis inhibitor (EIPA), suggesting CSCs internalize bevacizumab via macropinocytosis. Furthermore, bevacizumab or human IgG were largely detected in the Rab4+ "fast" recycling compartment at 5 min, and in the LAMP1+ compartment (late endosome/lysosome) at 3 hr in the CSCs. We observed similar trafficking patterns for bevacizumab in our in vivo model. CSCs from GBM do not express the neonatal Fc receptor, the canonical pathway for recycling of IgG. Bevacizumab induces autophagy in CSCs due to VEGF deprivation and this mechanism is blocked with the addition of growth factors. Taken together, our data show that in GBM, bevacizumab gains access to the perivascular tumor space, is macropinocytosed by CSCs where it is trafficked to a recycling compartment or to the late endosome/lysosome, and that it induces autophagy promoting survival of these tumor cells. These data suggest that alterations in endocytosis or recycling in the CSCs could impact the fate of therapeutic IgGs like bevacizumab and ultimately influence a patients' response to GBM therapy.

STMC-31. STIMULATION OF MICROGLIA AND MACROPHAGES AND GROWTH ATTENUATION OF BRAIN TUMOR-INITIATING CELLS WITH TUMOR NECROSIS FACTOR-ALPHA

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Microglia and macrophages (M/Ms) are functionally plastic entities that are compelled by glioblastoma (GBM) to adopt anti-inflammatory phenotypes and become major players in GBM progression. Understanding how to reverse this compulsion and maintain a pro-inflammatory tumor microenvironment is critical to developing effective therapeutics for GBM. Our studies have uncovered that GBM-associated M/Ms (GAM/Ms) can be pharmacologically compelled to shed the influence of GBM and secrete inhibitory factors that decrease the proliferation of GBM stem cell (GSC) lines and xenografts (Nature Neurosci 17:46-55, 2014). GSCs are a cellular reservoir that support GBM treatment resistance so it is important to development therapeutics that target this population. Our recent studies show that the most potent M/M-secreted factor behind GSC inhibition is tumor necrosis factor-alpha (TNF). We found that TNF decreases GSC proliferation and self-renewal through cytotoxic effects as well as G1 cell cycle arrest. TNF also induces differentiation in molecularly diverse GSCs. Additionally, we found that TNF can compel freshly-isolated human GAM/Ms to adopt a pro-inflammatory phenotype and inhibit GSCs in co-culture. The TNF receptors, TNFR1/2, are differentially expressed on GSCs and M/Ms. TNFR1, associated with apoptosis, is expressed by GSCs, while TNFR2, associated with survival mechanisms, is expressed on GAM/Ms. Moreover, TNFR1 on GSCs co-labels with OLIG2, one of the most specific markers of stemness in GBM, supporting the notion that TNF can target GSCs. Normal brain expresses low to non-existent levels of TNFR1, lending further support to this notion. Given the lack of studies investigating the effect of TNF on GSCs and the immunomodulatory effects TNF can exert on GAM/Ms, we feel it is a promising strategy to harness the effects of this powerful pro-inflammatory cytokine against GBM.

STMC-32. THE MESENCHYMAL SUBTYPE OF GLIOMA STEM-LIKE CELLS EXHIBITS REDUCED ENDOPLASMIC RETICULUM STRESS

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GBM is a lethal brain tumor that contains two major cancer stem cell subtypes (Proneural, PN and Mesenchymal, MES) bestowed with tumor initiating properties. Of these two subtypes, the MES glioma stem-like cells (GSCs) are associated with increased radioresistance, and thus it is important to identify essential molecules that regulate their survival and proliferation. Here, we performed 2D gel proteomic comparison of PN versus MES tumors and discovered higher expression of the endoplasmic chaperone protein GRP78 in the MES subtype of GBMs. In addition, TCGA analyses showed significantly higher expression of GRP78 and SOD2 in the MES GBM subtype compared to all other subtypes of GBM (Proneural, Classical, Neural). MES GSCs contained lower baseline levels of the unfolded protein response (UPR) and reactive oxygen species (ROS), verifying our hypotheses that MES GSCs exhibit reduced ER stress. Consistently, MES GSCs showed more resistance to low-glucose and tunicamycin-stimulation both of which induce the ER stress and oxidative stress. Intriguingly, lesser ER stress translates to increased protein synthesis and as consequence we found MES GSCs show increase protein synthesis and larger cell size. Currently we are exploring strategies to knockout GRP78 and study its impact on MES GSC self-renewal, proliferation and tumorigenesis.

STMC-33. DIFFERENTIAL MIGRATION PATHWAYS OF THERAPEUTIC STEM CELL CARRIERS TO GLIOMA AFTER INTRANASAL DELIVERY

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Neural stem cells (NSCs) and mesenchymal stem cells (MSCs) possess natural tropism to brain tumors. The unique stem cell intranasal delivery strategy bypasses the blood brain barrier and was recently demonstrated to convey therapeutic benefits in xenograft mouse models in conjunction with radiation and oncolytic virotherapy. It is imperative to understand the tumor tropism of therapeutic stem cells delivered intranasally to formulate optimal therapeutic strategies. To overcome the limitations of in vivo tracing of small number of stem cells delivered intranasally, we leveraged our mesoporous nanoparticle (MSN)-based radiolabeling technology to analyze NSCs and MSCs with MSN-Zr89 enabled Positron Emission Tomography (PET) with high sensitivity. PET imaging and immunocytochemistry demonstrated striking differences in the primary migration routes of MSCs and NSCs, in that MSCs predominantly migrated toward glioma xenografts through the caudal regions of the mouse brain, such as trigeminal ganglia, whereas NSCs predominantly migrated through the rostral regions of the brain, especially the olfactory bulbs. We hypothesize that the chemotaxic cytokine receptor profiles of NSCs and MSCs are major determinants in the migratory path in response to diverse brain micro-environmental cues, and genetic editing can augment tumor tropism. Mouse cytokine array analysis of brain tissue lysates collected from the olfactory bulbs and trigeminal ganglia showed EGF as a top candidate of responsible environmental cues. Based on the expression differences in EGFR in MSCs and NSCs, we utilized the clustered regularly interspaced palindromic repeats (CRISPR)-based endogenous gene activation to significantly upregulate the EGFR expression levels in NSCs, with improved chemotaxic migration capacity toward EGF in vitro, potentially helpful for enhancements in tumor tropism and therapeutic benefits in survival. Further optimization of tumor tropism via additional cytokine receptor expression profile gene editing offers possibility to enhance the tumor coverage of therapeutic stem cell carriers for brain malignancies of diverse intracranial locations.

STMC-34. ROLE OF INTERLEUKIN-8/CXCR2 REGULATED EPIGENETIC PLASTICITY IN GBM RECURRENCE

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Emerging evidence has revealed the enrichment of cancer stem cells (CSCs) in glioblastoma (GBM) and other cancers post-therapy. This can occur by dedifferentiation of non-CSCs to CSCs within the tumor population, which may be responsible for promoting the therapeutic resistance. To elucidate the molecular mechanisms of post-therapy cellular plasticity, a gene expression analysis, comparing the pre- and the post-therapy GBM CSCs (GSCs), revealed that Interleukin-8 (IL-8) transcripts are significantly elevated in post-therapy CD133+ GSCs. IL-8 is a pro-inflammatory chemokine that is upregulated both at mRNA and protein level upon thera-