overexpressing glioma cells shortened the survival time of tumor-bearing mice, whereas OSMR knockdown increased survival times. CONCLUSIONS: We analyzed genes whose expression was regulated by ANXA2 in glioma using invasive glioma models. Through this analysis, we identified that ANXA2 and OSMR regulate a phenotypic shift, suggesting that OSMR could be a promising target to treat and prevent glioma invasion.

ANGI-10. CHEMOTHERAPEUTIC STRESS INDUCES TRANSDIFFERENTIATION OF GLIOBLASTOMA CELLS TO PROMOTE VASCULAR MIMICRY

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Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumor affecting adults, with a median survival of approximately 21 months after diagnosis. A key factor underlying the limited efficacy of current treatment modalities is the remarkable plasticity exhibited by GBM cells, which allows them to effectively adapt to changes induced by our current treatment strategies. In addition to their plasticity, GBM tumors are also highly vascularized with aberrant vessels that further promote its aggressiveness. Recent research has demonstrated that GBM cells have the ability to transdifferentiate into endothelial cells (ECs), which suggests that GBM cells may use their properties of plasticity and vascularity in concert, leading to the creation of tumor-derived blood vessels. The mechanism behind this transdifferentiation remains unclear. Here, we show that treatment with temozolamide (TMZ)-based chemotherapy (the current standard of care) induces time-dependent increases in expression of markers for glioma stem cells (GSCs) and immature and mature ECs over 8 days of treatment *($p < .001$)* in multiple patient-derived xenograft (PDX) lines. In addition, GBM tumors growing as orthotopic xenografts in nude mice showed significantly increased expression of GSC markers (CD15 and CD133) and EC markers (CD105 and CD144) after 8 days of TMZ treatment *($p < .01$)*. *Ex-vivo* FACS analysis of these orthotopic xenografts showed the presence of immature and mature EC populations in addition to GSC populations. To assess the functionality of these increased EC populations, a tube forming assay was performed. Results showed that the tube forming capacity of PDX lines was significantly increased *($p < .01$)* after therapy. Furthermore, immunofluorescence analysis revealed increased tumor-derived vessels in TMZ-recurrent tumors. Overall, this study identifies chemotherapeutic stress as a new driver of transdifferentiation of tumor cells to endothelial cells and highlights cellular plasticity as a key player in therapeutic resistance and tumor recurrence.

ANGI-11. SEX DIFFERENCES IN IMAGING-BASED ASSESSMENT OF GLIOBLASTOMA INVASION

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INTRODUCTION: Neuroimaging dogma for glioblastoma asserts that hyperintensity on T1Gd MRI reveals the bulk of the tumor, while T2/FLAIR signal indicates edema. However, it is unclear whether this edema results from immune response or increased tumor cells. Further, one significant driver of the known sex differences in glioblastoma may be differences in immune response, due to the X-linkage of many immune genes. Based on this, we hypothesized that assumptions regarding tumor cellularity in T2/FLAIR images should be tailored to the biological sex of the patient. METHODS: Using a retrospective cohort of 18 primary glioblastoma patients receiving multiple image-localized biopsies (82 total) and standard MRI, we assessed: distance of biopsy from T1Gd and T2 areas; a pathologist's score of percent tumor cell density; and an imaging-based invasion metric, D/ρ. This metric is derived from the biomathematical Proliferation-Invasion model of glioma growth, which features two parameters, net growth rate (ρ) and net invasion rate (D). Their ratio D/ρ is related to degree of invasion, and is estimated from volumetric measurements of MRI abnormalities. Additionally, 25 patient-derived xenograft models implanted in females were grown until moribund, at which point brains were excised and stained for DAPI (to show all cells) and Lamin (to highlight tumor cells). Image processing of lamin-stained sections defines contours of intensity correlating with cell density. RESULTS: Outside both the T1Gd and T2 region, male patient biopsies had higher tumor cell densities than females. Males also tended to have higher invasion metrics. Although each set derived from different patients, preclinical metrics of invasion were positively correlated with clinical invasion in females but negatively correlated in males. CONCLUSION: Our preliminary finding that cell distribution patterns correlate with imaging metrics differently between the sexes supports the hypothesis that the degree of tumor cell density represented on certain MRI sequences may be sex-specific.

ANGI-13. PLEXIN-B2 FACILITATES DIFFUSE GLIOMA INVASION BY REGULATING CELL ADHESION AND ACTO-MYOSIN DYNAMICS

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Diffuse invasion of glioblastoma (GBM) cells into brain tissue is a key factor for its high lethality. GBM cell migration is affected by functions of plexins, which are transmembrane receptors of semaphorins that regulate cell adhesion and cytoskeletal dynamics. Expression of Plexin-B2 is upregulated in GBM and correlates with malignancy. We show here that Plexin-B2 activity regulates biomechanical properties of GBM cells, promoting invasive growth. Plexin-B2 activity increased the capacity of GBM to invade as dispersed single cells by reducing the cell-cell adhesiveness between GBM cells, indicating that a major function of Plexin-B2 activity is to downregulate cell-cell adhesion systems. RNA-Seq analyses also revealed that GBM stem cells (GSC) with deletion of Plexin-B2 altered expression of genes related to cell adhesion and the matrisome, indicating compensatory mechanisms in cellular dynamics. Interestingly, in vivo intracranial transplant studies demonstrated that growth and invasion of Plexin-B2 mutant GSC was impaired, with mutant cells invading shorter distances and migrating mainly as groups of cells forming chains. Plexin-B2 mutant cells also were more likely to adhere to the vasculature, rather than to fiber tracts, suggesting altered biomechanical properties. This shift may be related to high stiffness of basal lamina of the vasculature, as Plexin-B2 KO cells have a preference for migration on stiff substrate in vitro. Intriguingly, the loss in Plexin-B2 expression also changed the distribution of the mechanosensor transction factor YAP, with high expression of Plexin-B2 correlating with increased nuclear YAP. Structure-function analyses revealed that the RasGAP domain as main signaling output of Plexin-B2. The Rap proteins are pleiotropic regulators of cell adhesion and actomysosin contractility. Our data also showed that overexpression of Plexin-B2 can lead to decreased levels of Rap1/Rap2. Thus, Plexin-B2 acts as a key regulator of the adhesion and contractility of GBM cells, thereby facilitating their diffuse invasion.

CELL BIOLOGY AND METABOLISM

CBMT-01. ALANINE FUELS ENERGY METABOLISM OF GLIOBLASTOMA CELLS

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Despite available aggressive treatment options, glioblastoma multiforme (GBM) has a median survival of 16 months indicating an urgent unmet need to develop new therapeutic strategies. Earlier, we have demonstrated that both glucose and acetate are the two major nutrients leading to synthesis of approximately ~60% acetyl-CoA in the brain tumors. Therefore, there must be other nutrients that may contribute to bioenergetic needs of highly proliferative GBM cells. Alanine is one of the non-essential amino acids (NEAA) that is constantly produced in cancer cells through Warburg glycolysis and its role in cancer metabolism is not well understood. Recently, it has been shown that alanine produced by pancreatic stellar cells contributed to the energy metabolism in pancreatic cancer cells. However, it is not known whether GBM can use alanine as an energy source. Here, we test whether GBM cells have the ability to metabolize alanine as a fuel to meet its increased energy requirements. Patient-derived GBM cells were cultured with 2.0 mM [3-^{13}C]alanine for the final 24 hours, harvested in 50% methanol, snap-frozen in liquid N_2, freeze-thaw cycle 3 times and lysates were stored at -80 °C. Derivatized material from the frozen lysates were used for GC-MS analysis to determine carbon mass isotopomer distribution (MID) of various glycolytic and TCA cycle intermediates. Our results indicated that [3-^{13}C] alanine entered the GBM cells and produced [3-^{13}C]lactate via pyruvate. Also, alanine-derived [3-^{13}C]pyruvate led to the generation of [2-^{13}C] acetyl-CoA, which entered TCA cycle and produced M+1 ^{13}C isotopomers of citrate, glutamate, malate and aspartate. MID showed the following ^{13}C enrichment (M+1) values: citrate, 6.9 % ± 0.3%; glutamate, 4.4% ± 0.3%; malate, 2.1% ± 0.6%; 2.1% ± 0.5%. This preliminary data shows that GBM cells are capable of utilizing alanine to generate energy and produce precursors for biomolecular synthesis.