expression as well as ZIKV infectivity against GSCs, indicating that integrin is required for ZIKV infection. Of integrin b units, only silencing of integrin b_5 prevented the killing of GSCs by ZIKV infection, suggesting ZIKV infection required the a_vb_5 integrin. Supporting this hypothesis, blockade of the a_vb_5 integrin substantially reduced ZIKV infection of GSCs in glioblastoma organoid assays and in clinical glioblastoma specimens. Sox2 expression additionally suppress GSC expression of all members of the interferon-stimulated genes (ISG family), thereby suppressing innate anti-viral response to facilitate ZIKV infection. CONCLUSIONS: Collectively, our results reveal that ZIKV infection of GSCs is mediated by integrin $\alpha_v\beta_5$ leading to SOX2 expression which negatively regulates antiviral immunity thereby facilitating ZIKV infection.

STEM-13. FUNCTIONAL CHARACTERIZATION OF THE ZFHX4-CHD4 INTERACTION IN GLIOBLASTOMA CANCER STEM CELLS

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Glioblastoma stem cells (GSCs) they may be one reason for inevitable recurrence of GBM. We previously discovered that Zinc Finger Homeobox 4 (ZFHX4), a 450kD transcription factor, is required to maintain the GSC state. ZFHX4 interacts with CHD4, a core member of the nucleosome remodeling and deacetylase (NuRD) complex, which activates or represses gene expression via two distinct functions - histone deacetylation and ATP-dependent chromatin remodeling. CHD4 suppression phenocopies ZFHX4 suppression. The precise nature and function of the ZFHX4 interaction with CHD4 is not understood. Here we report that the ZFHX4-CHD4 interaction requires a single zinc-finger domain. An incremental truncation screen revealed that ZFHX4 amino acids 1838 to 2387, which contains zinc fingers 14 and 15, are required to bind CHD4. Disrupting the zinc coordination of zinc finger 14 impaired the ZFHX4-CHD4 interaction. Moreover, by overexpressing ZFHX4 amino acids 1838 to 2487, we decreased CHD4 recruitment to transcription regulatory regions of the stem cell genes SOX2 and SOX9, decreased transcription, and reduced clonogenic self-renewal. These results may provide the structural basis for new treatments to target GSCs and prevent recurrence in this devastating disease.

STEM-14. GLIOBLASTOMA STEM CELL TARGETING CHIMERIC ANTIGEN RECEPTOR T CELLS MODIFIED USING PHAGE DISPLAY ISOLATED PEPTIDES

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Glioblastoma (GBM) is the most aggressive primary brain tumor with high mortality rates and resistance to conventional therapy. Their resistance to conventional therapy has been attributed to the presence of cancer stem cells (CSCs), a sub-population of tumor cells capable of self-renewal and tumor initiation. Developing novel strategies to specifically target GSCs may allow more effective therapeutic strategies. Using in vivo phage display biopanning, we have identified several peptides with the potential to selectively target and bind GSCs. We wished to leverage the GSC targeting properties of the peptides to augment therapeutic delivery vehicles for the development of novel targeting strategies. We used a combination of GSC targeting peptides to modify the antigen-binding domain of chimeric antigen receptors, by arranging the peptides in tandem at the N-terminus of the CAR molecule. These tandem peptides were tested for binding to GSCs in vitro and in vivo. The functionality of the CAR-T cells was evaluated by measuring cytokine release in the supernatant after overnight co-culture through ELISA. Apoptosis was evaluated by flow cytometry with Annexin V staining. Two different GSC-targeting peptide CAR-T cells demonstrated specific targeting GSCs. Following co-culture with GSCs, GSC targeting CAR-T cells were activated with release of Interferon gamma and subsequently induced GSCs specific apoptosis. These results demonstrate the use of phage display biopanning to isolate GSC targeting peptides which may be used to develop novel GBM specific cytotoxic therapies.

STEM-15. SMALL BUT FIERCE: THE ROLE OF EXTRACELLULAR VESICLES IN MESENCHYMAL TRANSITION AND THERAPEUTIC RESISTANCE IN GLIOBLASTOMA

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Glioblastoma (GBM) is a dismal disease and despite optimal treatment, long-term survival remains uncommon. Molecular classification revealed three distinct GBM subgroups and has helped to shine light on the tumor's inter/intratumoral heterogeneity. Interestingly, recent evidence shows plasticity between these subtypes in which the proneural (PN) glioma stem-like cells undergo transition into the more aggressive mesenchymal (MES) subtype leading to therapeutic resistance. Extracellular vesicles (EVs) are considered a heterogeneous group of membrane-limited vesicles secreted by nearly every cell. In the context of GBM, these biological nanoparticles act as multifunctional signaling complexes and play an important role in intercellular communication allowing cancer cells to exchange information with each other, the tumor microenvironment, and distant cells. We show that MES cells derived EVs modulate PN cells to increase migratory potential, stemness, invasiveness, aggressiveness, and therapeutic resistance by inducing mesenchymal transition through NF-KB/STAT3 signaling. Furthermore, we shine light on the role of EVs derived from irradiated GBM cells and their potential impact on microglia and resulting treatment resistance.

STEM-16. IGF1/N-CADHERIN/B-CATENIN/CLUSTERIN SIGNALING AXIS MEDIATES ADAPTIVE RADIORESISTANCE IN GLIOBLASTOMA

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Glioblastoma (GBM) is composed of heterogeneous tumor cell populations including those with stem cell properties, termed glioma stem cells (GSCs). GSCs are innately less radiation sensitive than the tumor bulk and are believed to drive GBM formation and recurrence after repeated irradiation. However, it is unclear how GSCs adapt to escape the toxicity of repeated irradiation used in clinical practice. To identify important mediators of adaptive radioresistance, we generated radioresistant human and mouse GSCs by exposing them to repeat cycles of irradiation. Surviving subpopulations acquired strong radioresistance *in vivo*, which was accompanied by increased cell-cell adhesion, slower proliferation, an elevation of stemness properties and N-cadherin expression. Increasing N-cadherin expression rendered parental GSCs radioresistant, reduced their proliferation, and increased their stemness and intercellular adhesive properties. Conversely, radioresistant GSCs lost their acquired phenotypes upon CRISPR/Cas9-mediated knockout of N-cadherin. Mechanistically, elevated N-cadherin expression resulted in the accumulation of b-catenin at the cell surface, which suppressed Wnt/b-catenin proliferative signaling, reduced neural differentiation, and protected against apoptosis through Clusterin secretion. Restoration of wild type N-cadherin, but not mutant N-cad lacking b-catenin binding region, led to induce radioresistance in N-cadherin knockout GSCs, indicating the importance of the binding between N-cadherin and b-catenin. We also demonstrated that N-cadherin upregulation was induced by radiation-induced IGF1 secretion, and the radiation resistance phenotype could be reverted with picropodophyllin (PPP), a clinically applicable blood-brain-barrier permeable IGF1 receptor inhibitor, supporting clinical translation. Moreover, the elevation of N-cad and Clusterin are related to prognosis of GBM in the TCGA dataset. In conclusion, our data indicate that IGF1R inhibitor can block the N-cadherin-mediated resistance pathway. Our study deepens our understanding of adaptive radioresistance during repeated irradiation in GBM, and validates the IGF1/N-cadherin/b-catenin/Clusterin signaling axis as a novel target for radio-sensitization, which has direct therapeutic applicability.

STEM-17. NOT ALL GBM STEM CELLS ARE EQUAL: IMPLICATIONS FOR RESEARCH AND THERAPY

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Glioblastoma (GBM) kills almost all patients within 2 years. A subpopulation of cells, GBM stem cells (GSCs), contributes to treatment resistance and recurrence. A major therapeutic goal is to kill GSCs, but no targeted therapy yet exists. Since their discovery, GSCs have been isolated using single surface markers, such as CD15, CD44, CD133, and a-6 integrin. It remains unknown how these single surface marker-defined GSC populations compare to each other in terms of signal transduction and function and whether expression of different combinations of these markers is associated with distinct phenotypes. Using mass cytometry and fresh operating room specimens, we found that 15 distinct GSC subpopulations exist *in vivo* and they differ in their MEK/ERK, WNT, and AKT pathway activation status. In culture, some subpopulations were lost and previously undetectable ones materialized. GSCs highly expressing all four surface markers had the greatest self-renewal capacity and *in vivo* tumorigenicity as well as the strongest WNT pathway activation. This work highlights the signaling and