Abstracts

by ligands recognized by the toll-like family of receptors (TLRs). TLR activation limits proliferation in developing neural systems, yet these pathways are generally drivers of proliferation in many tumors including gliomas. N-cadherin in radioresistant glioblastoma and subsets of developmental programs activated in self-renewing cancer stem cells (CSCs). While mechanisms responsible for TLR-mediated tumor progression are largely unknown, TLR ligands are currently under investigation as antigens for immunotherapy approaches. Using ex vivo patient-derived xenografts, we find that proliferation of non-CSCs is attenuated by TLR ligands, whereas CSC proliferation is unaffected by ligand exposure. Profiling revealed that CSCs have reduced TLR4 compared to non-CSCs and that down-regulation of TLR4 expression correlates with aggressive disease progression. Overexpression of TLR4 in CSCs reduced stem cell signaling and attenuated proliferation, self-renewal, and tumor growth. Mechanistically, we found that TLR4 signaling is directly connected to the stem cell transcriptional program via way of binding kinase 1 (TBK1) and PDX1. Glioma and breast CSCs also express TLR4 and TBK1, and knockdown of TLR4 is necessary and sufficient for CSC self-renewal via epigenetic regulation of core self-renewal transcription factors. The activation of TBK1 downstream of TLR4 is responsible for RBBP5 suppression. Our findings reveal a unique pathway through which TLR4 can potentiate the stem cell state, linking innate immune signaling responses to stem cell maintenance. These data suggest that strategies to activate the immune system may also negatively impact stem cell programs in tumor cells, indicating that approaches that alter the immune system also target the treatment-refractory CSC population.

STEMC-16. EZH2-MEDIATED ARL13B REGULATE CILOGENESIS IN GBM
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Glioblastoma stem cells (GSCs) are known to be responsible for GBM therapeutic resistance. Previously we have reported exceptional plasticity within GBM during chemotherapy, which is associated with increases in CSC sub-populations. The initial investigation indicated that Polycomb group protein EZH2 is critical for therapeutic stress-induced cellular plasticity. Chemical inhibition, as well as shRNA-mediated knockdown of EZH2, suppresses cellular plasticity-mediated increases in GSCs. To elucidate molecular mechanisms of EZH2-mediated therapeutic resistance, gene expression analysis was performed in the presence of the EZH2 inhibitor DZNep. The results indicate that the expression of ARL13B, a member of the ADP-ribosylation factor-like family protein critical for cilia formation and maintenance, was most affected by DZNep (more than 13-fold downregulation). Knockdown of EZH2 by shRNA completely abolished the expression of ARL13B. TCGA data analysis revealed that EZH2 expression was inversely correlated with survival of patients with grade III (p=0.03) and IV GBM (p=0.007). Immunofluorescence staining showed that ARL13B colocalized with cilia in primary GBM cell lines as well as in a patient-derived xenograft (PDX) model. Chemotherapeutic stress on PDX models significantly enhanced formation of cilia (2 fold, p-value <0.0002) and length of primary cilium (1.5 fold, p-value =0.0001), as well as in post-therapy recurrent GBM PDXs (1.5 fold, p-value <0.0003). Moreover, Sh activated in Glioblastoma (Gli) those are required for Shh signaling were co-localized with the primary cilium. These observations suggest that therapeutic stress induced ARL13B expression and formation of primary cilium, which can regulate Shh signaling during chemotherapy, may contribute to promote therapeutic resistance in GBM.

STEMC-17. N-CADHERIN UPREGULATION MEDIATES SLOW PROLIFERATION AND THERAPEUTIC RESISTANCE IN GLIOMA STEM CELLS
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Glioblastoma is the most frequent and malignant brain tumor with an overall survival of only 14.6 months. Although these tumors are treated with surgery, radiation and chemotherapy recurrence is inevitable. A critical population of tumor cells in terms of therapy, the so-called cancer stem cells (CSCs), has been identified in gliomas and many other cancers. These tumor cells have a stem cell-like phenotype and are suggested to be responsible for tumor growth, chemoresistance and recurrence. However, functional evidence for migrating glioma cells having a stem cell-like phenotype is currently lacking. In the present study, the aim was to characterize the phenotype of migrating tumor cells using a novel migration assay of N-cadherin using tumor cell lines. By demonstrating that N-cadherin expression had low expression of cell cycle genes and high expression of stemness-related genes. In conclusion, our data indicate that increased N-cadherin expression induces a state of slow growth in GSCs through a reduction of Wnt/β-catenin signaling, which underlies their therapeutic resistance. Therefore, N-cadherin represents a new important target to antagonize tumor recurrence in glioblastoma.

STMC-18. ANOMALOUS COMPARTMENTALIZATION OF CANCER STEM CELL SIGNATURES IN GLOBLASTOMA
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Cancer stem cells (CSCs) can be defined by functional attributes that include tumorigenesis, self-propagation, and maintained self-renewal, but their gene signatures and specific anatomic contexts remain obscure. To characterize candidate signatures and explore specific loci in glioblastoma enriched for putative CSCs, we analyzed 270 transcriptional profiles of anatomic GBM (40), choroid plexus features and CSCs, including GBM by TCGA data analysis revealed that expression of the N-cadherin was significantly upregulated in glioblastoma (Gli) those are required for Shh signaling across tumors despite intratumor and intertumor heterogeneity. These features provided a framework for defining three anatomic compartments enriched for putative CSC profiles on the basis of published CSC and cell type signatures. The distribution of these features across tumors revealed the presence of intratumor heterogeneity. These features are consistent with subtype-selective and anatomic feature-enriched gene expression.

STMC-20. MIGRATING GLIOMA CELLS EXPRESS STEM CELL MARKERS AND GIVE RISE TO NEW TUMORS UPON Xenografting
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Glioblastoma (GBM) is the most frequent and malignant brain tumor with an overall survival of only 14.6 months. Although these tumors are treated with surgery, radiation and chemotherapy recurrence is inevitable. A critical population of tumor cells in terms of therapy, the so-called cancer stem cells (CSCs), has been identified in gliomas and many other cancers. These tumor cells have a stem cell-like phenotype and are suggested to be responsible for tumor growth, chemoresistance and recurrence. However, functional evidence for migrating glioma cells having a stem cell-like phenotype is currently lacking. In the present study, the aim was to characterize the phenotype of migrating tumor cells using a novel migration assay of N-cadherin using tumor cell lines. By demonstrating that N-cadherin expression had low expression of cell cycle genes and high expression of stemness-related genes. The results showed pronounced migration of five different GBM spheroid cultures. The results showed pronounced migration of five different GBM spheroid cultures, but not of the commercial cell line U87MG. An in vitro limiting dilution assay showed preserved but reduced spheroid formation capacity of migrating cells. The results showed preserved but reduced tumorigenic capacity. Profiling of mRNAs revealed no substantial deregulation of 16 predefined CSC-related genes and the HOX-gene list in migrating cells compared to spheroids. Deter- Minating mRNA expression of N-cadherin revealed that mRNA expression was identical. In conclusion, migrating tumor cells preserve expression of stem cell markers and functional CSC characteristics. Since CSCs are reported to be highly resistant to therapy, these results emphasize that the CSC phenotype should be taken into consideration in future treatment of GBM.