STEM-25. SINGLE-CELL SIGNATURES UNCOVER GLIAL PROGENITOR HETEROGENEITY AND MOLECULAR DETERMINANTS FOR GLIOMA GROWTH

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The major glial subtypes in the brain, oligodendrocytes and astrocytes, are heterogeneous populations, however, their progenitor diversity and contribution to malignant transformation remain elusive. Despite recent progress in the evaluation of oligodendrocyte and astrocyte heterogeneity in adult brain regions, the diversity and molecular profiles of their progenitors remains incompletely understood. In addition, a comprehensive understanding of transcriptional dynamics of glial progenitors and their lineage determinants underlying normal development and malignant transformation is currently lacking. To address the heterogeneity of glial progenitors, we performed targeted high-throughput single-cell RNA sequencing of prospective astrocyte lineage cells and oligodendrocyte precursor populations isolated by fluorescence activated cell sorting from the neonatal cortices and mapped single-cell expression profiles and molecular features of glial subtype progenitors. We found that astrocyte lineage cells are much more dynamic than previously appreciated and exhibit distinct lineage developmental trajectories in the developing neonatal cortex. In contrast to the astrocyte lineage, the progenitors of oligodendrocytes (OPC) exhibited a cellular continuum, which included a previously unrecognized primitive OPC subpopulation prior to the committed OPCs. Application of scRNA-seq to a murine model of malignant glioma revealed cycling OPC serving as a specific cellular niche contribute to glioma formation. We also established a new algorithm to identify transcription-factor-driven networks and discovered a set of regulators critical for controlling glial lineage specification and glioma growth. Thus, our single-cell analyses reveal distinct dynamics and heterogeneity of glial progenitors during brain development. Moreover, we identify analogous glial progenitors as the important source for glioma growth, and further define a molecular link between gliogenesis and gliomagenesis, thereby pointing to potential cellular and molecular targets for glioma treatment.

STEM-26. MICROENVIRONMENTAL FGF2 INDUCES GLIOBLASTOMA STEM CELLS THROUGH THE FGFR1-ZEB1 AXIS

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Glioblastoma is the most lethal and aggressive brain cancer in adults. Poor prognosis is due to resilience to therapy and tumour recurrence, which have been linked to glioblastoma stem-like cells (GSCs). FGF2 and its cognate receptors have been linked to malignancy and progression in glioma, and FGF2 is frequently employed in GSC culture paradigms. The specific mechanisms of how this growth factor promotes stemness and malignancy in glioblastoma remain incompletely understood. Therefore, we analysed expression of FGF receptors (FGFRs) and the effects of FGF2 on patient-derived glioblastoma cell lines. We found that FGF2 induces expression of the stemness-associated transcription factor ZEB1, increases sphere formation frequency and cell migration. Analysis of FGF receptor expression and function using knockdown approaches in patient-derived glioblastoma cell lines revealed that FGFR1 is relevant for stem cell maintenance. FGFR1 knockdown reduces sphere and colony formation, and increases survival in a xenograft mouse model. Analysis of large-scale gene-expression datasets revealed association of FGFR1 in mesenchymal glioblastoma, and increased FGFR1 expression in 30–40% of cases. We identify FGFR1 as a potential GSC marker and therapeutic target.

STEM-27. THE ALTERATION OF IMMUNOSUPPRESSIVE FUNCTION IN GLIOBLASTOMA WITH UNDIFFERENTIATED TRANSFORMATION

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Glioma stem-like cells (GSCs) are strongly related to the treatment resistance in glioblastoma (GBM). GSCs differentiate into differentiated glioma cells (non-GSCs), and lose the stem cell features such as self-renewal, pluripotency and tumorigenesis. However, several study have demonstrated that GSCs and non-GSCs could convert between each other. Recently, immunotherapy has been attracting attention as a new GBM therapy. Indoleamine 2,3-dioxygenase (IDO), the enzyme for Tryptophan metabolism, is involved in the ability of GBM to escape from immune surveillance, and show immunosuppressive function. In this study, we investigated the difference of IDO expression between GSCs and non-GSCs to search a new therapeutic target for GBM. We used the human malignant glioma cell lines U-251MG and Rev-U-251MG, which was established by culturing U-251MG cells in serum-free media. Rev-U-251MG formed spheres with increased level of Nestin and Nanog expression. We regarded Rev-U-251MG as a GSCs cell line model and investigated the expression of IDO. The expression levels of IDO mRNA and protein were increased in Rev-U-251MG compared to the expression in U-251MG. The expression levels of *IDO1* mRNA were analyzed by the quantitative reverse transcription PCR ($p<0.01$, n=6), and IDO1 protein were analyzed by Western blotting. These results suggest that the GSCs strongly escape from immune surveillance while producing more IDO compared to non-GSCs. It is important to know about the alteration of immunosuppressive function between GSCs and non-GSCs to establish a new treatment strategy for GBM. The possibility was suggested that GSCs immunosuppressive function via expression of IDO could be a new target for the GBM therapy.

STEM-28. TISSUE FACTOR PROMOTES THE GLIOMA STEM CELL PHENOTYPE, AND IS SUPPRESSED BY MUTANT IDH1

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Isocitrate dehydrogenase 1 mutant ($IDH1^{mut}$) gliomas have global genomic hypermethylation, are less aggressive than IDH1 wild-type ($IDH1^{wt}$) gliomas, and generally grow poorly *in vitro* and *in vivo*. Yet little data exist that connect specific hypermethylation targets to this unique phenotype. We previously reported that the gene encoding Tissue Factor (TF), *F3*, which promotes both thrombosis and malignant behavior, is among the most hypermethylated and downregulated genes in $IDH1^{mut}$ gliomas. In multiple $IDH1^{wt}$ and $IDH1^{mut}$ patient-derived glioma cell lines, *F3* was hypermethylated in $IDH1^{mut}$ cells compared to $IDH1^{wt}$ cells, with reduced TF protein expression. A demethylating agent, decitabine, increased *F3* transcription in $IDH1^{mut}$ glioma cells, but not in $IDH1^{wt}$ cells. TF knockdown greatly reduced proliferation, colony formation, glioma stem cell (GSC) marker expression, and xenograft growth of $IDH1^{wt}$/$EGFRvIII^{amp}$ GBM6 cells and $IDH1^{wt}$/$EGFR^{amp}$ GBM12 cells, but not of *NF1*-mutant GBM43 cells. Conversely, TF induction enhanced the proliferation and colony formation of $IDH1^{mut}$ GBM164 and TB09 cells, especially GBM164. TF also increased the *in vivo* "take rate" of intracranial GBM164 xenografts from 0% to 100%, but did not enable TB09 xenograft growth. TF activated receptor tyrosine kinases (RTKs) in GBM6, GBM12, and GBM164, but RTK expression was very low in GBM43 and TB09. Transcriptomic profiling showed that only two genes were downregulated after TF knockdown in GBM6 and GBM12, and also upregulated after TF induction in GBM164: *PROM1*, encoding CD133, and *CTNND2*, encoding δ-catenin. Neither gene was affected by TF manipulation in GBM43 or TB09. High *F3* mRNA correlated with enrichment of GSC markers, and worse outcome, in TCGA gliomas. These data suggest that: (i) TF promotes a GSC phenotype through RTKs; (ii) CD133 and δ-catenin may be critical effectors of TF-induced GSC behavior; (iii) TF methylation reduces $IDH1^{mut}$ glioma malignancy; (iv) TF is an attractive, novel therapeutic target in $IDH1^{wt}$ gliomas.

STEM-29. UNSATURATED FATTY ACID (UFA) METABOLISM REGULATES MEMBRANE-ENDOLYSOSOME-NUCLEAR INTER-ORGANELLE COMMUNICATION IN GLIOMA STEM CELLS

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Stem cells receive the signals from their niches that instruct them to self-renew and prevent them from differentiating through a cascade of inter-organelle communication processes. However, how the inter-organelle communication mechanism functions in the maintenance of stemness in glioma stem cells (GSCs) remains unclear. To identify potential glioma suppressors that affect the interaction of GSCs with their niches, we discovered that the RNA-binding protein Quaking (QKI) is a key regulator of cellular endocytosis. *QKI* is mutated or deleted in ~34% of human glioblastomas. Consistently, 92% of the *Nestin-CreERT2;*$Qki^{L/L}$*;*$Pten^{L/L}$*;*$p53^{L/L}$ (QPP) mice developed glioblastoma with a median survival of 105 days, yet the *Nestin-CreERT2;PtenL/L;p53L/L* mice did not develop any gliomas. Mechanistically, QKI regulates the RNA stability and alternative splicing of numerous protein and lipid components of endolysosomes, particularly the unsaturated fatty acids (UFAs). Notably, lower levels of QKI, endolysosomes, and stearoyl-CoA desaturase (SCD, the key enzyme for UFA biosynthesis) all correlate with poorer