The Role of Disseminated Tumor Cells in Recurrent Glioblastoma Multiforme

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Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor in humans. Invasion by GBM cells results in high recurrence (>90%) and a poor prognosis, with median survival rates of 12-14 months. Tumor recurrence is attributed to the survival and local regrowth of treatment-resistant cells. We have isolated glioma stem cells (GSCs) from a patient’s primary tumor prior to treatment (0203) and from various locations in the patients brain after recurrence at time of death (T2-T7). Through whole exome sequencing (WES) we identified the greatest concordance between the recurrent tumor (T3-T4) and a distant site of microscopic tumor burden (T6-T7), with ablation of 0203 gene signatures following treatment. These data suggest a common precursor between the recurrent tumor (T3-T4) and distant, disseminated tumor cells (DTCs) (T6-T7). We hypothesize that following treatment, DTCs migrate to the original tumor location and result in tumor recurrence.

Materials and Methods

1) Differentially labeled human glioma cells (0203) were orthotopically injected into NOD-SCID cell lines using single cell RNA sequencing to assess expression between primary and metastatic tumor populations. We used intracranial injections followed by analysis with long-term survival and treatment monitoring. To observe migration and interaction between 0203 and T7 tumor populations in vitro, we used transwell assays to analyze migration and interaction between 0203 and T7 tumor populations. We evaluated gene expression and tumor heterogeneity within the isolated patient GSC populations using single cell RNA-sequencing.

Results

Preliminary WES data suggest that the recurrent tumor may be derived from an ancestral population of DTCs. To test this, we investigated migratory potential between tumor subpopulations. The transwell assay demonstrates that induction of apoptosis in 0203-GFP-TK following treatment with GCV increases migration of T7 towards dying 0203. Interestingly, there was comparable movement in the control group where T7 were seeded alone. Additionally, immunoblot analysis of T7 reveals decreased expression of markers associated with migration or proliferation in the presence of 0203 and a subsequent recovery of normal expression levels once 0203 were killed off with GCV. Taken together, these data suggest that physiologically, 0203 may play a role in inhibiting migration or proliferation of T7 and other DTC populations in the brain.

Single cell RNA-sequencing provides a powerful tool to study transcriptome at a single cell resolution. Through multidimensional scaling, we analyzed global transcriptional interrelationships between the different cell populations. The t-SNE plot shows that 0203 have a relatively unique expression profile from the rest of the tumor populations, and that the recurrent tumor (T3-T4) and distant tumor (T6-T7) cluster with some overlapping regions that may represent transitional cells between populations. These transitional cells are of particular interest to us in the future as they may provide clues about the evolution of DTCs in becoming the recurrent tumor post-treatment. Moreover, we identified distinct subpopulations within the cell lines that overexpress AURKA/B, which would be interesting to see whether it plays a role in allowing DTC survival as they acquire otherwise lethal genetic abnormalities.

Further analysis of the scRNA-seq data will evaluate cellular heterogeneity within the original and recurrent tumors, and may identify a hierarchical characterization of the tumor recurrence, driven by proliferation of DTC subpopulations. Identifying a role for DTCs in tumor recurrence may lead to more effective therapies aimed at prolonging disease-free intervals following initial treatment of GBM.

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References