Single-Cell Microfluidic Genetic Profiling Reveals Two Distinct Subsets of Brain Tumor-Initiating Cancer Stem Cells Co-Existing in Human Glioblastoma Before and After Therapeutic Intervention

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Introduction

Glioblastoma is the most common and aggressive primary human brain cancer. The existence of distinct molecular subtypes of glioblastoma and possibility for intra-tumoral heterogeneity present significant therapeutic challenges. Brain tumor-initiating cells (BTICs), self-renewing multi-potent cells critical for tumor maintenance and growth, are attractive targets of glioblastoma therapy. In this study, single-cell microfluidic genetic profiling of primary human glioblastoma was performed to characterize intra-tumoral BTIC heterogeneity, identify unique surface markers of BTIC subsets, and associate BTIC subsets with clinically-relevant bulk tissue molecular subtypes.

Methods

Fresh human glioblastoma tumors obtained directly from neurosurgical resections were immediately dissociated and passaged in neural stem cell media to enrich the subpopulation of tumor cells with stem-like properties. Microfluidic genetic profiling of BTICs at the single-cell level was performed to characterize intra-tumoral BTIC heterogeneity. Surface markers of distinct BTIC subpopulations were validated in fluorescence-activated cell sorting (FACS) and colony forming assays. In vitro and in vivo testing of purified BTIC subsets were performed to verify multipotency, self-renewal, and capacity to generate glioblastoma tumors in murine cranial xenotransplantation models.

Results

Two distinct subsets of BTICs were identified co-existing in human glioblastoma, both in de novo tumors and in recurrent tumors after initial complete surgical resection and chemoradiation. The finding that both BTIC subsets were present in reproducible proportions across patients, and from both de novo and recurrent tumors, suggest these subpopulations are not differentially affected by current clinical interventions. Each BTIC subtype was characterized by distinct surface markers, and single-cell molecular profiles relating to distinct bulk tissue molecular subtypes. Both BTIC subtypes were validated in vitro and in vivo as demonstrating multi-potency, self-renewal, and capacity to generate glioblastoma tumors in murine xenotransplants.

Conclusions

These data support BTIC subpopulation heterogeneity as an underlying source of intra-tumoral bulk tissue molecular heterogeneity. Identification of cell surface markers of distinct BTIC subpopulations will support future study of glioblastoma cancer stem cells, and the potential development of BTIC subpopulation-specific therapeutic strategies.

Learning Objectives

1) Cancer stem cells and glioblastoma cancer biology

2) Single cell microfluidic analyses

3) Genomic profiling and molecular subtypes

4) FACS surface marker validation and prospective cell subpopulation isolation

5) In vitro and in vivo validation of stem cell-like properties in cancer stem cell studies

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