Spatial Context of Tumor Immune Microenvironment of Matched Primary and Recurrent Glioblastomas

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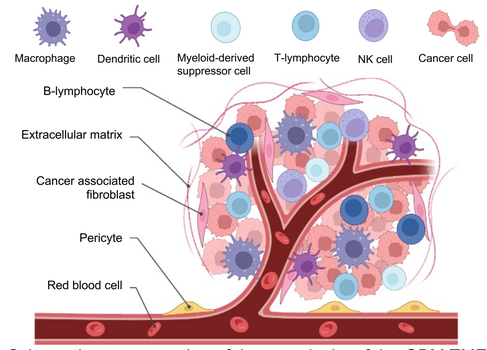
Introduction

Glioblastoma multiforme (GBM) is the most malignant and prevalent form of primary astrocytomas. It is the most lethal form of primary brain tumors in adult patients with an average life expectancy of approximately 14 to 15 months from the diagnosis[1]. The disease progression and response to chemotherapy and radiation are deeply associated with the tumor microenvironment (TME)[2].

Tumor-infiltrating lymphocytes (TILs), such as T-cells and NK T-cells, and macrophages are two populations that play a central role in fighting the disease in the TME of GBM[3,4]. The spatial context of these cells in GBM microenvironment as well as differences in the immune environment between newly diagnosed and recurrent GBM are not well studied.

An unbiased multiplex imaging approach using the co-detection by indexing (CODEX) technology was applied to investigate the distribution of T-cells and macrophages in primary and recurrent GBM tumors.



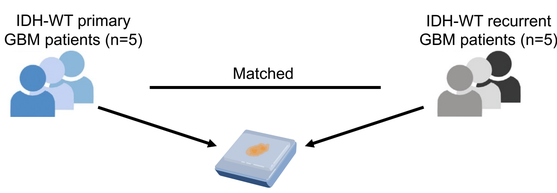
Schematic representation of the complexity of the GBM TME.

Research Objectives

1. Define different cell-types in the GBM TME and investigate the spatial immune context and interactions between different cell populations.
2. Identify differences in the distribution and spatial context of immune cells between primary and recurrent GBM tumors.
3. Identify differences in the distribution and spatial context of immune cells between the necrotic core of the tumor, infiltrating edge, and the healthy tissue in the brain.

Methodology

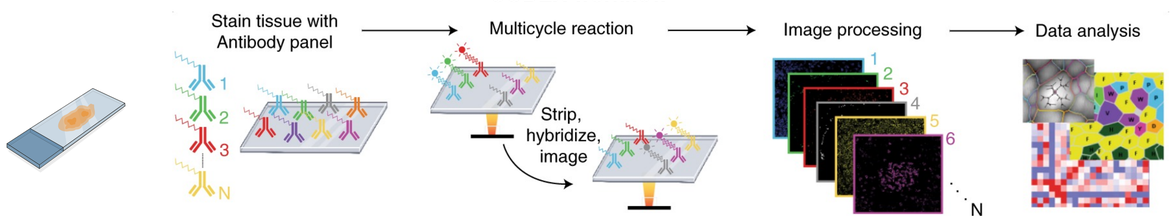
Study cohort and antibody panel for CODEX



Antibody Panel			
CD45 (pan immune marker)			
T-cells	Macrophages	Monocytes	Functional
CD3	CD68	CD14	GranzymeB (Gr.B)
CD4	CD163	CD16	Galectin-9 (Gal9)
CD8			

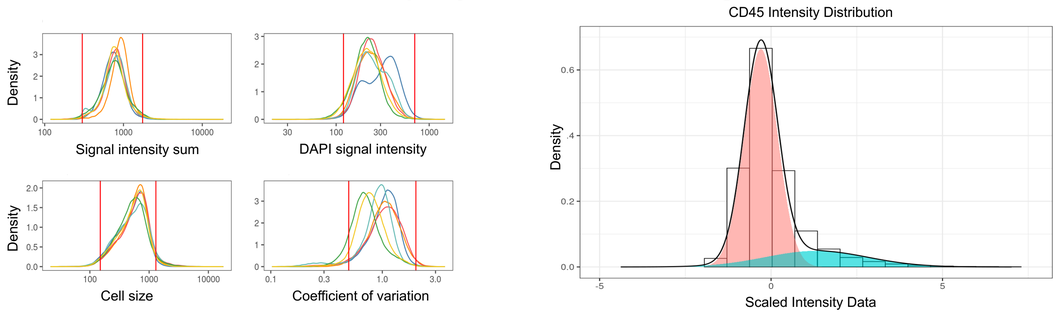
Tumor resected from 5 matched IDH-WT GBM patients were preserved using the standard protocol for formalin-fixed paraffin embedding. Sections of the embedded tissue were processed using the CODEX method to obtain the multiplex images with 42 markers (only a subset of 10 were used for this project).

CODEX workflow



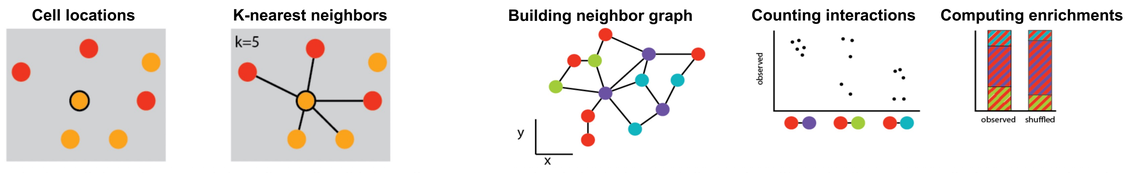
Each antibody was partially reduced and conjugated with a DNA oligonucleotide to recover the oligonucleotide-antibody conjugate. Subsequently, the tissue was prepared for antigen retrieval, stained with the oligonucleotide-antibody conjugate, hybridized with the corresponding fluorescent oligonucleotide and imaged[5]. After treating the tissue with all the 42 markers in a multicycle reaction, images were processed for data analysis.

Data quality control and thresholding



After cell segmentation, quality control and filtering was performed on each cell using basic QC metrics such as total sum of the signal, DAPI intensity, cell size, and coefficient of variation. Then, the intensity data was normalized (arcsin transformation) and scaled to account for batch effects. For thresholding, the scaled intensity data was fitted to a two component gaussian distribution. The threshold was calculated by finding the mean of the lower curve and the threshold was set to two standard deviations from that mean.

Spatial Analyses



To conduct spatial analyses and describe cell-cell interactions, unsupervised clustering and dimensionality reduction was applied across regions in each patient. A graph of cell-cell interactions using XY coordinates was generated using k = 5 nearest neighbors' parameter. Pairwise adjacency was computed and evaluated statistically using the hypergeometric test to determine if certain clusters or cell-type interactions were enriched or depleted between the two groups.

Results

Increased CD4⁺ CD68⁺ macrophages in the recurrent than primary TME

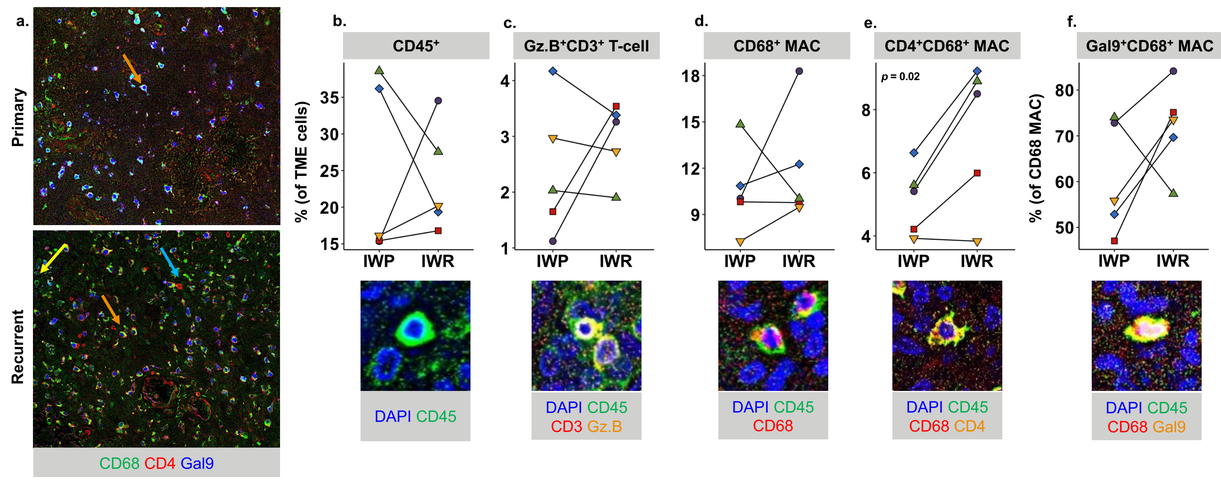


Fig. 1 Percentage of different cell populations in the primary and recurrent TME of matched IDH-WT GBM patients.
(a) Visual representation of the distribution of CD4 presenting CD68 macrophages expressing Gal9 in the primary and recurrent TME. The orange arrow depicts a triple positive cell with expression of CD4, CD68 and Gal9. The blue arrow illustrates a CD4 T-cell. The yellow arrow points to a CD68 macrophage that is not expressing Gal9. (b) Distribution of CD45⁺ immune cells (p = 0.92, paired t-test). (c) Distribution of CD3e⁺ T-cells expressing cytotoxic marker granzyme B (p = 0.39, paired t-test). (d) Distribution of CD68⁺ macrophages (p = 0.54, paired t-test). (e) Distribution of CD4 presenting CD68⁺ macrophages (p = 0.02, paired t-test). (f) Percentage of CD68⁺ macrophages expressing Gal9 in the primary and recurrent TME of matched IDH-WT GBM patients (p = 0.20).

Training model to quantify enriched and depleted cell-cell interactions

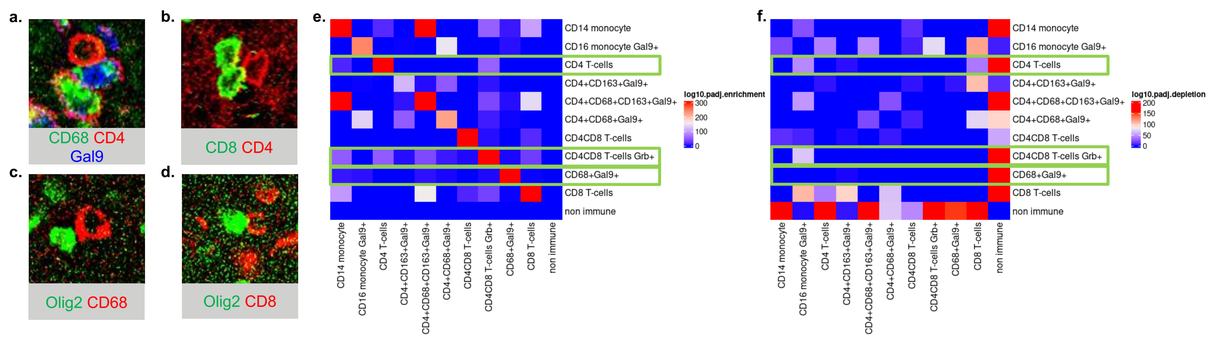


Fig. 2 Spatial analysis data from a primary tumor to illustrate the results of the training model to quantify enriched and depleted cell interactions.
2a-d Visual representation of cell-cell interactions. (a) Two CD4 presenting CD68 macrophages expressing Gal9 are interact with CD4 T-cell. (b) CD8 T-cell interacts with a CD4 T-cell. (c) CD68 macrophage interacts with Olig2 (nonimmune marker used to identify proliferating tumor cells). (d) CD8 T-cell interacts with Olig2. (e) Pairwise adjacency plot from region 1 of P5 for enriched interactions between clusters identified from the unsupervised analysis displaying log10 (adjusted p-value). Red represents interactions that are highly enriched in this region. (f) Pairwise adjacency plot from region 1 of P10 for depleted interactions identified from the unsupervised analysis displayed log10 (adjusted p-value). Red represents interactions that are greatly depleted in this region.

Conclusions

- There is an enormous amount of heterogeneity between the distribution of T-cells and macrophages across patients and the tumor subtype.
- CD4 expression of myeloid lineage cells was detected in both primary and recurrent tumors. There was a significant increase in the number of the CD68 macrophages expressing CD4 in the recurrent TME compared to their matched primary counterparts.
- Gal9 was expressed in over 60% of all macrophages in the TME and the percentage of CD68 macrophages expressing Gal9 increased in the recurrent setting for a large majority of patients.
- PD1 and PDL1 were mainly undetectable in all patients in this cohort. Gal9 and its binding partner TIM3 appear to be the dominant checkpoint marker in the GBM TME.

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