CHARACTERIZING INTRA-TUMORAL HETEROGENEITY IN POST-TREATMENT RECURRENT GLIOBLASTOMA VIA CLUSTERING ANALYSIS OF RNA SEQUENCING AND QUANTITATIVE HISTOPATHOLOGY

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Introduction

• Distinguishing between tumor and treatment effect in post-treatment glioma, although essential for clinical management, is difficult because contrast-enhancing regions are a mixture of both recurrent tumor and reactive tissue, and definitive histopathological criteria do not exist.

• This study disentangles the marked intra-tumoral heterogeneity in the treatment-recurrent setting by algorithmically categorizing intraoperative MRI-localized biopsies into three clinically-relevant tissue clusters based on quantitative histopathology and RNA sequencing analysis.

• This cluster analysis has also led to the development of novel tissue-specific gene sets that can be used to phenotypically characterize future post-treatment recurrent MRI-localized biopsies.
Methods

• A retrospective cohort of 84 MRI-localized biopsies from 37 adult patients with post-treatment recurrent glioblastoma underwent mRNA extraction and subsequent sequencing using a highly multiplexed PLATE-seq protocol. Sequencing reads were aligned, counted, and normalized for downstream analysis.

• For 48 of these 84 biopsies, a neighboring piece of tissue underwent quantitative histopathology based on labeling index (LI) for SOX2, CD68, NeuN, Ki67, and H&E.

• Correlation analysis between histopathological stains and gene expression for these 48 samples was performed, and the top 1000 genes sorted by correlation and variance were used to perform hierarchical clustering for determination of three gene sets that each characterize a specific clinically-relevant tissue cluster.

• These gene sets were then used to perform single-sample gene set enrichment analysis (ssGSEA) to categorize the 48 “training” biopsies with IHC as well as the 36 “testing” biopsies without IHC into clinically-relevant tissue clusters.
Figure 1

Correlation heatmap between normalized gene expression and immunohistochemical labeling indices with subsequent hierarchical clustering revealed three orthogonal tissue-specific gene sets:

- **Gene Set 1**: 358 genes strongly correlated with SOX2, H&E, and Ki67
- **Gene Set 2**: 295 genes strongly correlated with NeuN
- **Gene Set 3**: 347 genes strongly correlated with CD68

![Correlation heatmap](image)
Panels A and B are heatmaps depicting ssGSEA enrichment scores from the three tissue-specific gene sets for the training and testing samples, respectively. Hierarchical clustering using gene enrichment scores from these three gene sets allows categorization of each MRI-localized biopsy into one of three clinically-relevant tissue clusters, demonstrated in panels C (training samples) and D (testing samples).
Discussion

• Using 48 MRI-localized biopsies with both RNA sequencing and histopathological data, correlation between normalized gene expression and immunohistochemical labeling indices allowed development of three orthogonal tissue-specific gene sets that characterize the heterogeneity existing in post-treatment recurrent glioma:
  • Gene Set 1: genes positively correlated with SOX2 (tumor burden), H&E (cellularity), and Ki67 (proliferation), which represent genes associated with recurrent tumor.
  • Gene Set 2: genes positively correlated with NeuN (neurons), which represent genes associated with infiltrated brain tissue.
  • Gene Set 3: genes positively correlated with CD68 (microglia), which represent genes associated with reactive tissue consistent with treatment effect.
• Single-sample gene set enrichment analysis utilizes these phenotypically-derived gene sets to effectively categorize MRI-localized biopsies of post-treatment recurrent glioma into three tissue types: Cluster 1 = recurrent tumor; Cluster 2 = infiltrated cortex; and Cluster 3 = reactive tissue consistent with treatment effect.
• These gene sets can be applied to categorize novel post-treatment recurrent tissue, as demonstrated using the PLATEseq data of the testing set, in absence of quantitative histopathology.
Summary and Future Directions

• Three novel tissue-specific gene sets that characterize the intra-tumoral heterogeneity of post-treatment recurrent glioma were generated from MRI-localized biopsies that had combined RNA sequencing and histopathological data: Gene Set 1: recurrent Tumor, Gene Set 2: infiltrated cortex, Gene Set 3: reactive tissue and treatment effect.

• These gene sets can be applied to gene expression data from new tissue samples to categorize biopsies into one of three major tissue types that exist in the post-treatment recurrent setting.

• Future work will involve refining these gene sets by incorporating other histopathological markers and adding more samples.

• Further, this algorithmic approach of classifying heterogenous tissue from recurrent glioma patients is currently being used to build radiographic models of intratumoral heterogeneity.
References


