Radiographically Localized Biopsies Reveal Subtype Specific Differences in the Molecular and Cellular Composition at the Infiltrative Margins of Glioblastoma

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Pupose

- What are the molecular features of the non-enhancing (NE) region of glioblastoma?
- How does the cellular composition of the NE region of glioblastoma influence its gene expression signature?
- How do the molecular features and cellular composition of the NE region vary with respect to the subtype of glioblastoma?

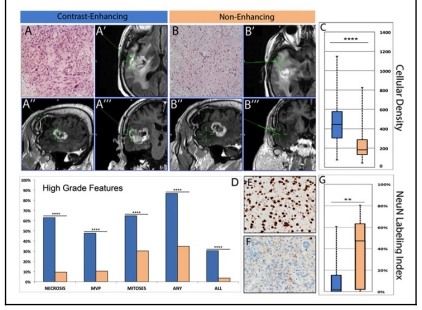
Background

Glioblastomas infiltrate the brain making complete removal by surgical resection impossible. Current analyses are predicated on biopsies taken from within the CE region of GBM without radiographic localization of the sample. However the mixture of tumor and non-neoplastic neural cellls that remain after the surgery in the NE FLAIR positive region of the lesion, forms the biological context for tumor recurrence and adjuvant therapies. We propose a method of sequential stereotactically guided biopsies, allowing for specimen localization, intra-tumoral comparative analysis the systematic study of infiltrated borders beyond regions of contrast enhancement.

Methods

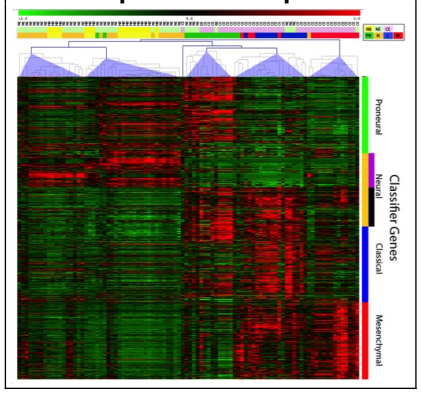
In 69 patients with high grade glioma, multiple radiographically-localized biopsies were obtained from the CE core and NE infiltrative edge of the tumor prior to debulking. RNA-seq was performed on 75 high grade glioma samples from 27 different patients. 39 samples were from the CE region and 36 samples were from the NE region. RNA seq analysis was also performed on 17 non-neoplastic normal brain samples acquired from 11 patients with no neuro-oncological history. A computational approach was used to deconvolve the RNA-seq dataset and estimate the expression profiles of six different cell types. The cell types included for this analysis were Olig2+/oligodendrocyte progenitor cells (OPCs), CD44+ astrocytes, CD44- astrocytes, microglia, oligodendrocytes and neurons.

Fig 1. Radiographic Localization and Histological Correlates of Contrast Enhancing and Non Enhancing Samples



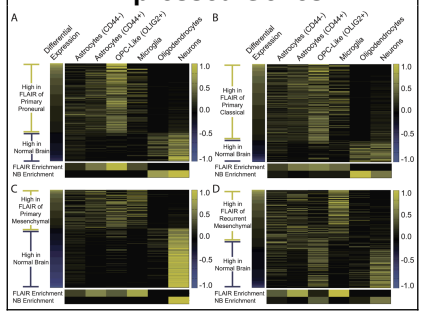
MRI screen captures show the radiographically localized sampling of CE (A'-A''') and NE (B'-B''') regions of GBM, and micrographs of the corresponding biopsies show the histological features of the highly cellular core (A) and the infiltrative margin (B) of the tumor (stained with Hematoxylin and Eosin). A' and B' show the axial FLAIR, A'' and B'' show the sagittal T1 with contrast, A''' and B''' show the coronal T1 with contrast. The green crosshairs mark the biopsy sites. Quantitative analysis of CE (blue bars) and NE (tan bars) samples shows significant differences in cellular density (C) and presence of histopathological hallmarks of high-grade glioma (D). Immunohistochemical analysis for NeuN shows numerous positive neurons in samples from NE regions (E) and only rare entrapped neurons in samples from the CE regions (F). Quantitative analysis of theses stains shows significant differences in the fractional abundance of NeuN+ neurons in NE vs. CE samples (G). In panels C, D and G, ** = p-value < 0.001; *** = p-value < 0.0001; **** = p-value < 0.00001.

Fig 2. Heat Map Showcasing the Subtype and Gene Expression Data for Sequenced Samples.



RNA-seq based expression profiling showing the expression of the Verhaak classifier genes across 84 samples (39 CE, 36 NE, and 17 NB). The samples were clustered using Spearman correlation into 2 major clusters. One cluster is predominantly composed of NE and normal brain (NB) samples, and the other is predominantly composed of CE samples. The CE cluster is further divided into 3 sub-clusters, with samples correlating with Proneural, Classical and Mesenchymal. The NE cluster contains the majority of Neural samples. The colored bars above the heatmap show the sample origin (upper bar; NB=yellow, NE=green, CE=pink) and the subtype classification (lower bar; Neural=brown, Proneural=green, Classical=blue, Mesenchymal=red). The heatmap shows high levels of expression as red and low levels as green.

Fig 3. Heatmaps Showcasing the Deconvolved Cellular Distribution of Gene Expression for Differentially Expressed Genes



Heatmaps showing the deconvolved cellular distribution of gene expression for differentially expressed genes (p<0.05) comparing normal brain to A) NE of Proneural GBMs, B) NE of Classical GBMs, C) NE of Primary Mesenchymal GBMs, and D) NE of Recurrent Mesenchymal GBMs. For each gene (rows) the expression level is normalized across cell types (columns) so that the value in the heat map reflects its fractional abundance in a given cell type. To obtain these cellular distributions, we deconvolved the NE and NB samples in aggregate and obtained a single average cellular distribution estimate for each gene. Although differential expression information was not provided to the deconvolution algorithm, all four heatmaps show a sharp transition in cellular composition between genes that are expressed at higher levels in the NE tumor tissue vs. genes that are expressed at higher levels in normal brain. The small heat maps that appear underneath each panel represent the fraction of the total number of differentially expressed genes in each sample group are predominantly expressed in each of the six cell types.

Summary

- Tissue samples obtained from the CE region had a greater number of histological features of glioblastoma in comparison to those samples from the NE region.
- These two regions display different patterns of gene expression including genes expressed in the four glioma subtypes. The distribution of subtypes seen in the CE is significantly different to those seen in the NE region.
- The differentially expressed genes in the NE tissue are distributed across multiple cell types and display a GBM subtype specific pattern. The NE regions of proneural gliomas are enriched in genes which are expressed by Olig2+/OPC-like cells. In comparison the NE regions of mesenchymal gliomas are enriched in genes which are expressed by microglia and CD44+ cells.
- Despite their different expression patterns, gene ontology analysis of the deconvolved expression data showed that both the Olig2+and CD44+ cells are highly enriched in cellular proliferation. This suggests that these cells may represent two distinct types of neoplastic glioma cells which lie witin the NE region of the tumor.

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