Transcriptional signatures in histologic structures within glioblastoma tumors may predict personalized drug sensitivity and survival

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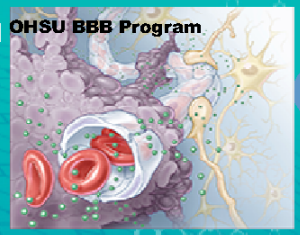

PURPOSE / OBJECTIVE(S)

- To assess whether differing tissue composition of GBM samples influences the results of known prognostic and diagnostic gene signatures.
- To determine if assessing a consistent intra-tumoral structure could provide more valid metrics for inter-tumoral comparisons.
- To create an improved prognostic gene signature.

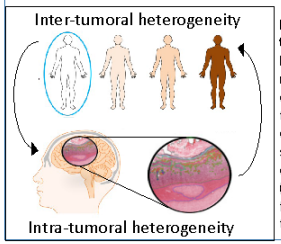

Figure 1. Intra- and inter-tumoral heterogeneity in GBM. Inter-tumoral heterogeneity refers to variance between different patients' tumors. Intra-tumoral heterogeneity describes variation within a single GBM, where one region of the tumor appears dissimilar (histologically and/or on MRI) from other regions of the same tumor. Image adapted from (1).

MATERIAL & METHODS

We analyzed RNA-sequencing (RNAseq) and corresponding clinical data from the open-source Ivy Glioblastoma Atlas Project (IvyGAP), to compare the transcription profiles of different histological structures (below) from 34 newly diagnosed GBM (http://glioblastoma.alleninstitute.org).

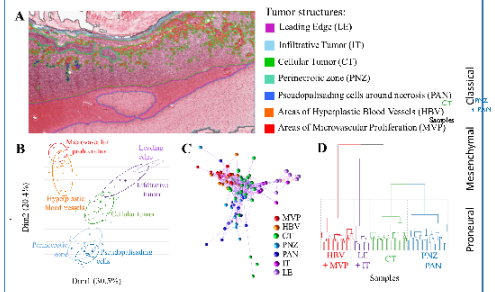

Figure 2. Histologic structures in GBM are molecularly distinct. (A) Structures were laser micro-dissected then subject to RNAseq. (B) Principle component analysis (PCA) of all samples from all structures shows majority of variance in this dataset is accounted for by structures. (C) Correlation network analysis corroborated PCA results. (D) 7 histologically-defined structures were collapsed to 4 regions applying the gap statistic and k-means clustering.

RESULTS

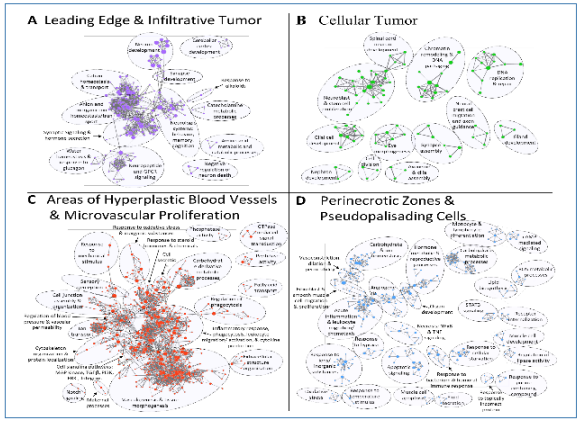

Figure 3. Gene set enrichment analysis (GSEA) revealed distinct biological processes enriched in each tumor structure.

(A) The tumor edge (LE/IT) had enrichment of normal central nervous system processes.

(B) The CT had enriched traditional cancer processes.

(C) Highly vascular areas (HBV/MVP) were associated with vascular development and integrity, as well as many immune processes, suggesting this is an inflammatory microenvironment.

(D) The PNZ/PAN areas appear to be a highly stressed microenvironment with hypoxia, immune activation, and necrosis.

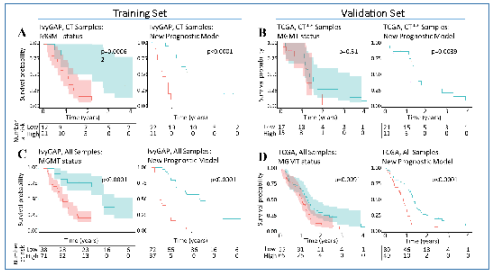

Figure 6. Novel prognostic gene signature created utilizing solely cellular tumor (CT) sample gene expression data. Kaplan-Meier survival analysis of (A) IvyGAP CT samples, (B) CT-predicted** TCGA GBM samples, (C) all IvyGAP samples, and (D) all TCGA samples dichotomized into high and low risk groups based on MGMT promoter methylation status (left) and using our novel model (right).

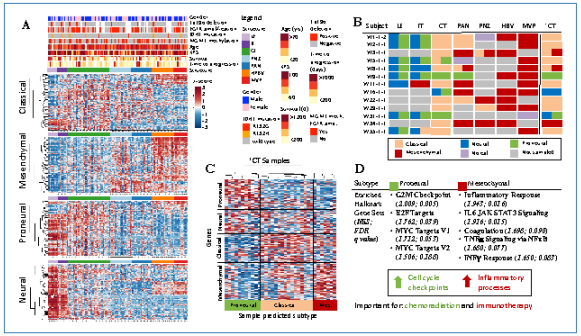

Figure 4. Molecular subtype classification depends on structure, with CT able to distinguish biologically distinct subtypes

A) Expression of GBM molecular subtype gene sets (ref 2) in IvyGAP samples from each region showed that structure is a main contributor to expression of subtype gene signatures.

B) Subtype classification for samples corresponding to subjects with ≥4 samples from different regions. A single subject was classified as multiple subtypes.

C) Unsupervised hierarchical clustering of only CT samples, z-score normalized across CT samples.

D) Biologically important hallmark gene sets enriched in the GBM subtypes (stratified based on the CT sample analysis). NES: normalized enrichment score.

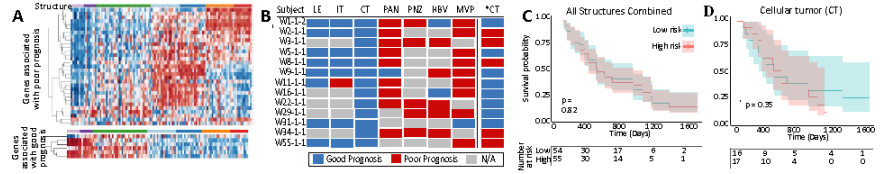

Figure 5. Established prognostic gene signature expression is driven by tumor structure. (A) An established survival prediction gene set (ref 3) shows differential expression based on tumor structure. (B) A single patient was predicted to be either high or low risk depending on the structure analyzed. (C-D) Kaplan-Meier survival analysis of all samples, and CT samples. Neither stratified groups with significantly different overall survival.

SUMMARY / CONCLUSION

- Histologic structures within a GBM are molecularly distinct, with unique biological processes enriched in each structure.
- Molecular subtype classification established by The Cancer Genome Atlas depends on structure.
  - CT sampling allows the ability to distinguish between biologically distinct subtypes.
- Expression patterns of established prognostic gene signature are driven by tumor structure.
  - Variations in histology may confound results of gene signatures created from mixed-structure samples.
- Focusing specifically on the CT structure improves:
  - GBM subtyping into biologically distinct cohorts.
  - Patient risk stratification.
- Novel gene signatures were created to identify:
  - Unique histological structures in a GBM.
  - Highest-risk GBM patients.
- These advances will help guide the future development of personalized medicine approaches for GBM and enhance prognostics to identify patients with the highest-risk of rapid progression.

REFERENCES / ACKNOWLEDGEMENTS

(1) N.R. Parker et al (2015) Frontier in Oncology. 5:55.

(2) R. G. Verhaak et al (2010) Cancer Cell. 17:98-110.

(3) H. Colman et al. (2010) Neuro Oncol. 12:49-57.

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