Going beyond the atlas – mapping the tumor landscape with complete genomic signatures

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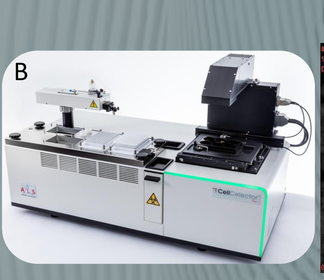
Abstract

Glioblastoma multiforme (GBM) is an aggressive malignancy with dismal outcome. Despite advances in tumor biology, limited data capable of linking tumor landscape with genomic signatures to improve patient outcomes exist. Current atlas methods identify cellular location and phenotype in sections, however, woefully underrepresent the totality of the genomic and transcriptomic landscape, frequently focusing pre-defined panels of genes / transcripts. One powerful combination capable of revealing such data is the CellCelector platform paired with ResolveOME™ technology, which provides complete genomes & transcriptomes of single cells. We present an unbiased approach that simultaneously allows for complete assessment of the genome and transcriptome in a highly heterogenous and deadly tumor type.

GBM tissues were digested *in-situ* & regions of interest (ROI) were selected for analysis using the CellCelector. A total of 24 ROI, >200µM apart were evaluated across the tumor. Importantly, no pre-defined stains were utilized to ensure random cell sampling. ROI were processed using ResolveOME, and sequencing was performed targeting a minimum of 2M paired end (PE) reads for both the genome and transcriptome. Data were processed using BaseJumper™ platform to assess genomic structure, and gene expression in relation to genomic heterogeneity. We observe heterogeneity between ROI including genomic alterations on chromosomes 2, 8 and 10. With ResolveOME™ technology, we are empowered to understand the heterogeneity of this deadly disease, and importantly, observe key insights into immune infiltration of these tumor regions.

Workflow & Methods

Workflow



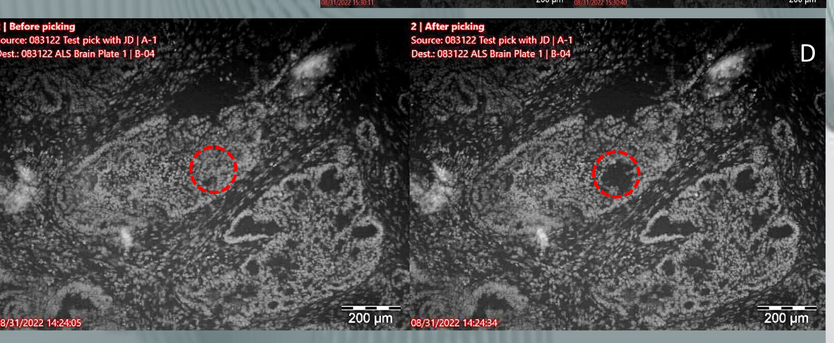


Figure 1: Workflow summary Cell Celector & ROI selection –

A. **Workflow:** Cryopreserved COT embedded tissues were sectioned onto slide. These were thawed and subjected to 1hour collagenase digestion with neutral protease. After digestion, ROIs were identified, and deposited into plates prior to being subjected to ResolveOME™ chemistry, sequencing and analysis

B. Cell Celector Instrument

C. Representative image of selected ROI: 10x magnification of ROI pre/post selection via 50µM capillary. Red circle indicates region of interest that was selected, with few cells selected (SC24).

D: Representative image of ROI selected – 10x magnification of ROI pre/post selection via 50µM capillary. Red circle indicates ROI selected, with moderate number of cells selected (SC02).

Methods Continued

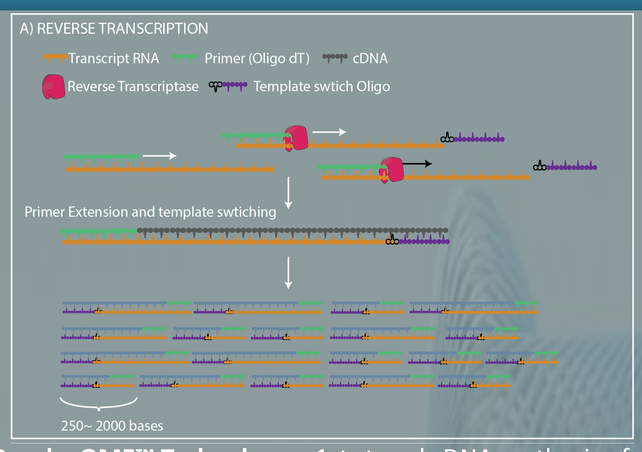


Figure 2A: ResolveOME™ Technology – 1st strand cDNA synthesis of mRNA was performed using a template switching-based reverse transcription process on the complete polyA+ transcriptome. Each set of transcripts remained in well during nuclear lysis & subsequent genomic amplification.

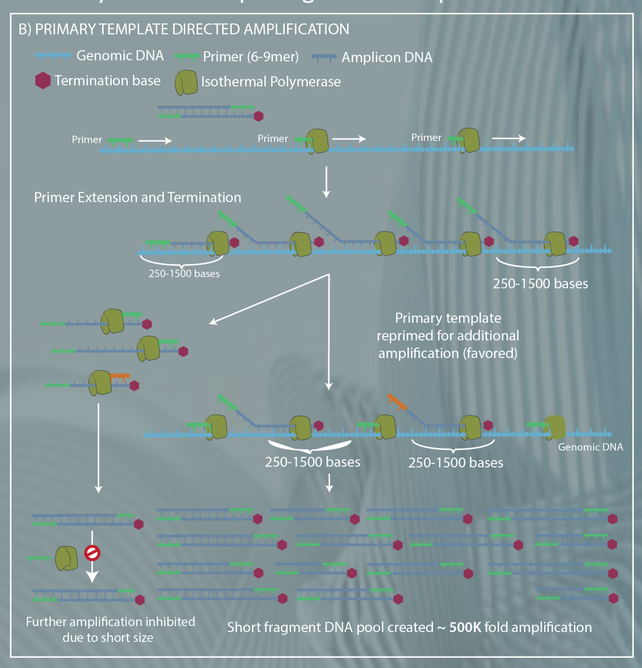


Figure 2B: ResolveOME™ Technology Continued – after cDNA generation, genomes were amplified using Primary Template-directed Amplification (PTA)[1]. After amplification, affinity purification separated amplified genomic DNA and full length cDNAs. Distinct libraries were generated after transcriptome enrichment for downstream sequencing and analysis with BaseJumper™ software. Samples were sequenced using Illumina 2x50 PE chemistry targeting a minimum of 2 million PE reads. All samples were then uploaded to the BaseJumper software for downstream analysis. A total of two pipelines (PreSeq v 1.6.2 and RNAseq version 1.4.2) were run on the DNA and RNA fractions respectively.

Results

Copy number aberrations suggest clonal evolution in GBM ROIs

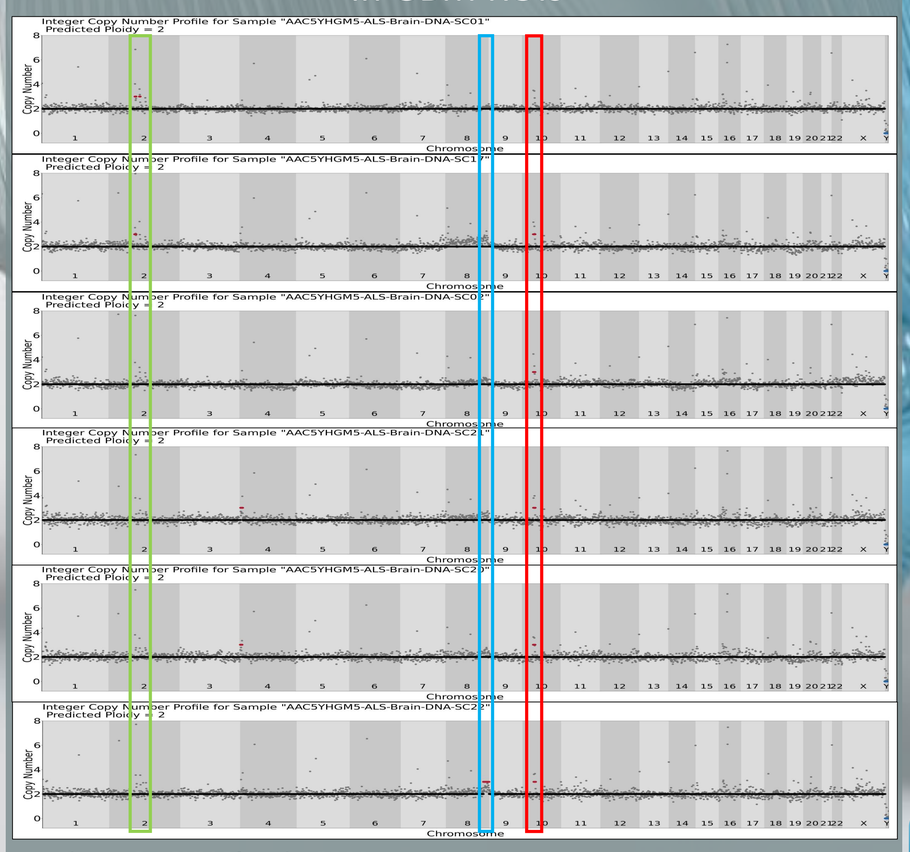


Figure 3: CNV Plots in GBM

CNV plots demonstrate conserved regions of gains in distinct foci within the genome. Regions of interest have differing and sequentially similar foci suggesting clonal evolution within the sample. Green box: focal gain on chromosome 2, Blue box: Increasing copy number alterations on 8, some below limit of detection (SC17, SC20), and Red box: Gains on chromosome 10, encompassing the *RET proto-oncogene*.

Results Continued

Expression profiles are similar among ROIs

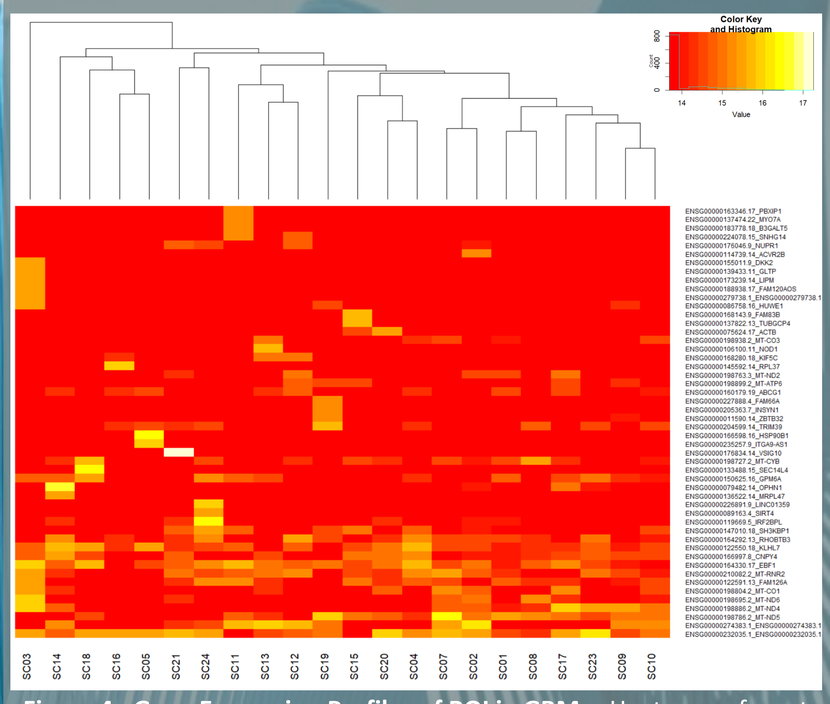


Figure 4: Gene Expression Profiles of ROI in GBM -- Heat map of most variable genes between individual ROI. Some of the most highly differentially expressed genes include *Hyccin PI4KA lipid Kinase complex Subunit 1 (HYCC1), EBF1 (EBF Transcription Factor 1),* & *KLHL7 (Kelch Like Family Member 7). HYCC1* is likely involved in the B-catenin/Lef signaling, with regulation linked to myelination, and is closely associated with oligodendrocyte formation. The resultant protein is a component of *PI4K* that allows localization to the plasma membrane. *EBF1* is more closely related to immunoregulation and suggests that there are high levels of immune cell infiltrations within ROIs demonstrating increased expression. Finally, *KLHL7* is associated with protein degradation in neurons. Interestingly both, *PBXIP1 (PBX Homeobox Interacting Protein 1) & MYO7A* (*Myosin VIIa)* are minimally differentially expressed in these ROI. Both genes are typically associated in neuronal settings, with resultant proteins interacting with the PBX1 homeodomain protein (altering transcriptional activation, and associated with various brain tumors) and as a potential tumor suppressor in GBM respectively.

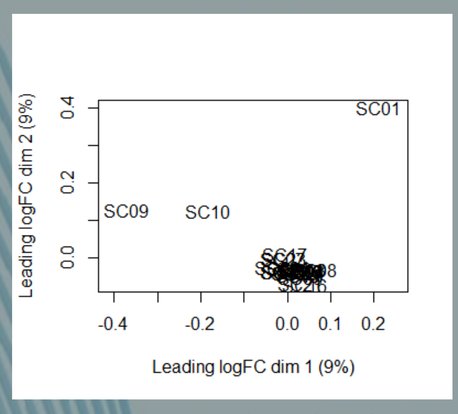


Figure 5: Principal Components Analysis Plot of ROI

As seen in the heat map above the majority of ROI appear closely related, with ROIs 1, 9, & 10 being most unique relative to the group.

Summary

A total of 24 independent regions of interest in OCT embedded non-fixed glioblastoma tissues sections were assessed using ResolveOME™ chemistry and BaseJumper software. These ROI were selected from a 5µM section using a Cell Celector with 50µM capillary after digestion with collagenase and neutral protease.

BaseJumper analysis demonstrated alterations in copy number profiles suggesting the potential for clonal evolution within this highly aggressive tumor type. Conserved chromosomal alterations include gains on chromosomes 2 and 10 including the *RET* proto-oncogene. Additional analysis of the whole transcriptome indicated that while overall the genomic profiles between the ROI were similar there were a few key genes that were differentiated between regions of interest. These include a series of genes associated with neuronal development, and regulation including *HYCC1* & *EBF1*, associated with immune cells and suggesting a high degree of tumoral infiltration.

References

1) Gonzalez-Pena V, Natarajan S, Xia Y, Klein D, Carter R, Pang Y, Shaner B, Annu K, Putnam D, Chen W, Connelly J, Pruett-Miller S, Chen X, Easton J, Gawad C. Accurate genomic variant detection in single cells with primary template-directed amplification. Proc Natl Acad Sci U S A. 2021 Jun 15;118(24):e2024176118. doi: 10.1073/pnas.2024176118. PMID: 34099548; PMCID: PMC8214697.