Analysis of Genomic Alterations in Tumor Subclones in a Patient with a Multifocal Glioblastoma

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Introduction

Glioblastomas remain the most common primary malignant brain tumors with dismal prognosis and average survival of 15 months. Gliomas were thought of as being clonal; however, multiple lines of evidence support a complex heterogeneous view of gliomas with the presence of multiple genetically different subclones. Radiation and chemotherapy introduce further variability and resistance by inducing genetic alterations allowing for selective expansion. Specifically of interest are multifocal glioblastomas, where multiple tumor foci could represent subclones arising independently, or derived from one another

Methods

Two multifocal glioblastoma patients presented with GTCs, one found to have a contrast enhancing multifocal lesion, and another having progressed to one. Patients underwent GTR. Tumor samples were obtained from different foci and sent for whole exome sequencing to Yale Center for Genome Analysis. Coding sequences were captured using xGEN v1.0, followed by Illumina DNA sequencing. Reads were mapped to Hg19 with BWA-MEM, processed using GATK with mean coverage of >95X blood, >190X tumor tissue. SNVs/InDels were called with HapotypeCaller, annotated using ANNOVAR, dSNP, 1000Genomes, NHLBI, ExAC. Somatic SNVs/InDels were identified using Mutect v2.7.1 and Indelocator v36.3, CNVs calculated using tumor/blood coverage, normalized by total, segmentation performed with DNACopy R, MetaSVM8 used to predict deleteriousness

Results

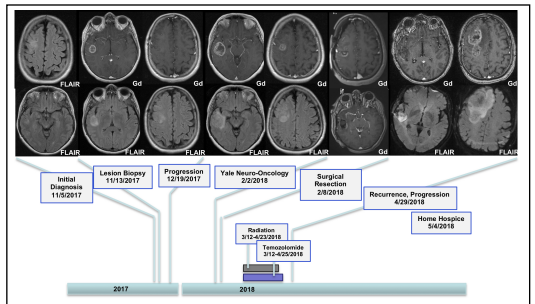

Figure 1. Glioma progression and aggressive clinical course of Patient 1 with multifocal right frontotemporal glioblastoma.

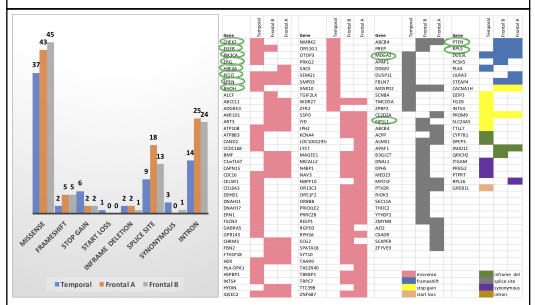

Figure 2. Single nucleotide variations, insertions and deletions (SNVs/InDels) identified in Patient 1 glioblastoma foci.

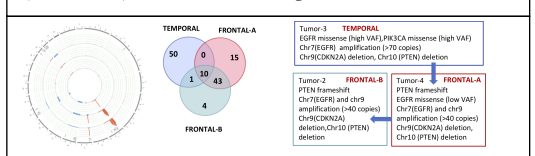

Figure 3. CNVs (copy number variations) in Patient 1 glioblastoma.

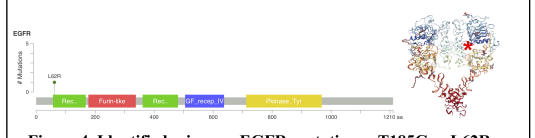

Figure 4. Identified missense EGFR mutation, c.T185G, p.L62R.

Results (continued)

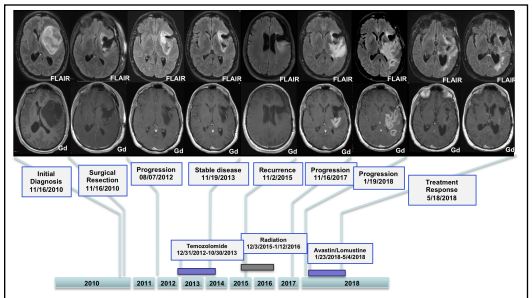

Figure 5. Glioma progression and slower clinical course in Patient 2 with left frontotemporal multifocal glioblastoma.

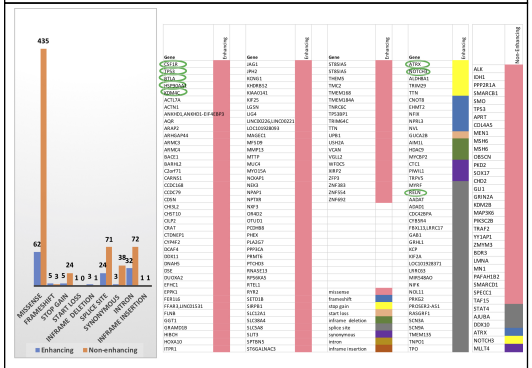

Figure 6. Single nucleotide variations, insertions and deletions (SNVs/InDels) identified in Patient 2 glioblastoma foci.

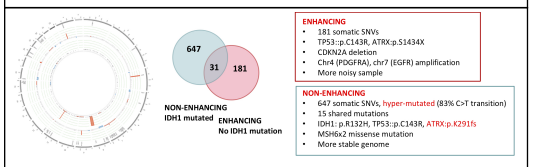

Figure 7. CNVs (copy number variations) in Patient 2 glioblastoma.

Conclusions

Whole exome sequencing of tumor foci in multifocal glioblastomas and multi-region sampling of gliomas in general can help understand genetic basis of tumor heterogeneity, detect genetic alterations amenable to targeted therapies for individual glioma subclones, and shed light onto glioma evolution. Understanding sequence of derivation of glioma foci from original cell populations can identify proper radiologic readouts for tracking treatment efficacy and aid in monitoring resistant glioma subclones as they arise

Future Directions

Clonal analysis using R MClust. Determine whether clinical behavior of glioblastoma foci correlates with *in vitro*, trial chemotherapy agents. *In vitro* and *in vivo* essays to validate genetic drivers and assess their contribution to gliomagenesis

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of using whole exome sequencing to understand genetic basis of glioma, 2) Appreciate glioma heterogeneity as related to tumor evolution, 3) Identify potential applications to glioma treatment

References

Erson Omay et al Longitudinal analysis of treatment~induced genomic alterations in gliomas. Genome Med. 2017 Feb 2; 9 (1):12 -- Harmanci et al Integrated genomic analyses of de novo pathways underlying atypical meningiomas. Nat Commun 2017 Feb 14; 8: 14433 -- Clark et al. Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. Nat Genet 2016 Oct: 48 (10): 1253~9 -- Gunel et al. Molecular mechanisms underlying malignant progression of low grade IDH1 mutant meningiomas. Neurosurgery 2016 Aug; 63 Suppl 1:199 -- Erson Omay et al Somatic POLE mutations cause an ultra~mutated giant cell high grade glioma subtype with better prognosis. Neuro Oncol 2015 Oct 17 (10): 1356~64