

Genomic Heterogeneity of Glioblastoma: a Comparison of the Enhancing Tumor Core and the Brain Around Tumor

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Abstract

Glioblastoma Multiforme (GBM) is a highly aggressive brain tumor and comprised of heterogeneous genetic profiles making it difficult to target treatment due to non-uniform responses and the recurrence of the tumor after treatment.¹⁻³

A current obstacle with using conventional MRI to guide a surgical biopsy and resection is the primary focus being on the enhancing tumor core, leaving behind brain around the tumor (BAT). BAT is rarely biopsied, but is known to contribute to a recurrence of the tumor⁴⁻⁶. BAT areas will be the study's focus of genomics. This project is a smaller portion of a large R21 grant, an NIH-funded project, with an overarching goal to determine the genomic expression profiling of BAT and core areas of GBMs, the correlation of MRI imaging to areas of likely recurrence and thereby be able to provide specialized patient treatment by providing a set of image-based criteria (phenotypically) that predict the genomic signatures of treatment resistance.

The experiment conducted in this sub-project, identified genomic characteristics of GBM specimens from the BAT and core regions. TGEN received the biopsied BAT areas of GBM tissue samples, isolated the DNA, performed the array-based Comparative Genomic Hybridization (aCGH), and analyzed the data received from the aCGH. The primary goal is to assess the differences in the genotypes of tumor cells within brain around tumor (BAT) and the cells residing in the core of the tumor. We hypothesized: (1) The BAT genotype will be different from the core of the tumor (2) and we will be able to identify what genomic alterations may explain for the variants/recurrent tumor. Anticipated results were expected to demonstrate genetic heterogeneity within a patient's GBM comprised of more than one, as well as, genomic differences in the brain tumor core compared to the BAT³⁶.

Within the constraint of this sub-project, I completed the genomic analysis of the tumors from three GBM patients. Considering the small sample size, I focused on the genomic analysis of a single patient that yielded the best results via aCGH, Patient 8. Our results demonstrated GBM heterogeneity, biopsy among the spatial location of the tumors to be important, specifically with BAT samples showing only amplification of chromosomal regions harboring genes implicated in cell invasion and migration via the Wnt/Beta-catenin, CKLF and NFKB pathways.

Introduction

Glioblastoma (GBM) is a diffuse grade IV astrocytoma that comprises around 40% of primary cerebral neoplasms, and is very aggressive with a short life expectancy following diagnosis⁴⁷. After aggressive surgical resection and chemo/radiation therapy, the average life span is only 14 months². The heterogeneity of the composition of this tumor (also seen in other cancers), makes it difficult to treat and indistinguishable during surgery and biopsy due to surrounding inflamed brain tissue^{1, 5-9, 34, 36}. Currently, practiced surgical methods do not project the phenotypic variations of the tumor well, forcing resection of the tumor's enhancing core (enh core) but leaving the recurrent rim⁴⁸. This leaves behind brain around tumor (BAT) that is resistant to therapy and the source of the recurrence⁵⁻⁹. Hence, this area will likely lead to future therapy targeting and I sought to assess the differences in the genotypes of cells within brain around tumor (BAT) and the enh core.

Specific Aim: Conducting an array-based Comparative Genomic Hybridization (aCGH) of the BAT and enh core samples, we hypothesize: (1) The BAT genotype will be different from the enh core (2) and we will be able to identify what genomic alterations may explain for the variants/recurrent tumor.

Rationale: Medicine overall is moving towards patient personalized treatment based on genomics. This is likely realistic for treatment of GBM patients as well. This study will give insight to better understanding current known genetic pathways and the discovery of new key genetic pathways that have potential to be exploited as personalized therapeutic targets^{32, 37}. In the future this could increase patient survival by lowering rates of treatment resistance and tumor recurrence.

Methods

Study Design: Twelve GBM patients were enrolled by St. Joseph's IRB approved protocol prior to their surgical resection for pre-operative multi-parametric MRI and for stereotactic MRI guided biopsies of the enhancing core and BAT regions, with the final number of biopsies determined during the time of surgery by the neurosurgeons. Due to time and specimen constraint of this smaller sub-project, I was only able to complete the analyses of the GBM tumors from three patients. A neuropathologist checked for tissue quality and tumor cellularity of each specimen.

TGEN acquired the samples from St. Joseph's and did not have access to the master patient list, making this a retrospective cohort case study. The tumor DNA was isolated from multiple frozen biopsies of the three patients and Agilent® SurePrint G3 Human High-Resolution Discovery Microarray Kit was used to determine CNV (Copy Number Variations)^{8, 9, 15}. The control for the aCGH is Agilent's® commercially available pooled reference DNA. CNV were calculated using log change relative to an internal reference. CNV were conducted using software that included DNA Analytics and GeneSpring GX9.

Data Analysis: TGEN analyzed the data of the aCGH and assessed the CNV that appeared to be aberrant. Hierarchical clustering was used to show relations between samples based on similarity³⁹⁻⁴⁰. We conducted genetic pathway analysis, looking for a driver of GBM³⁹⁻⁴⁰. Agilent® Oligonucleotide Array-Based CGH for Genomic DNA analysis from DNA isolation to feature extraction of the array data onto our genomic workbench version 7 was used for analysis. We analyzed the array data in Genomic Workbench 7.0 for Copy Number Variations (CNV).

Results

Described below are the data from the analyzed specimens of three different patients through an array aCGH that I performed. The number of BAT and enhancing core tumor (enh) biopsies that were analyzed are given: Patient 2 had 3 samples (2 Enh, 1 BAT), Patient 4 had 5 samples (3 Enh, 2 BAT), and Patient 8 had 14 samples (7 Enh, 7 BAT).

Considering the small sample size, I focused on analyzing a single patient, Patient 8. Patient 8 yielded the best results of the three patients to analyze, due to sample quality and number of biopsies. In total, 14 specimens from patient 8 were acquired and 7 were BAT, 7 were enh core, and 1 marginal (not included in analysis). Two of the BAT samples were determined inadequate to the neuropathologist, meaning there was predominantly too much normal brain parenchyma. Two enh core biopsies were excluded from analysis to balance BAT and enh samples; otherwise, the results would have been falsely, drastically skewed.

Table 1. Patient 8's BAT Samples CNV Commonalities: Only Amplifications Observed.

GENE SYMBOL	GENE NAME
ARR-19 (also referred to as CMT2E)	Cytoskeleton remodeling of various actin-binding proteins, androgen receptor inhibitor
VE-cadherin	Vascular endothelial cadherin
CKLF	Chromokine-like factor
CDY12L4	CKLF-like factor 12, transmembrane domain containing protein 1-4
DPP-10	Dipeptidyl peptidase
GAT	Carbonic Anhydrase 7
APP-191 (also referred to as NAL1)	NEDD8 activating enzyme E1 regulatory subunit
BEAN1	Protein BEAN1/brain-expressed protein according to RefSeq
TRAP25 (also referred to as MED30)	Mediator of RNA Polymerase II transcription subunit 30
PFKP2	Pyruvate Dehydrogenase Phosphatase 2
ORNL3L2	Cytoskeletal dynein 3 light intermediate chain 2, cytosolic
CECC79	Coiled-coil domain-containing protein 79
TK2	Thymidine kinase 2, mitochondrial
SLC39A8	Zinc transporter 8

Table 1. Summary of Patient 8's CNV amplifications or loss in the BAT samples. Interestingly, only CNV amplifications were observed. The gene symbol and name are given of the amplifications.

Figure 3. MRI Images of Patient 8's GBM, Locations of Biopsies, and Type of Tissue Retrieved.

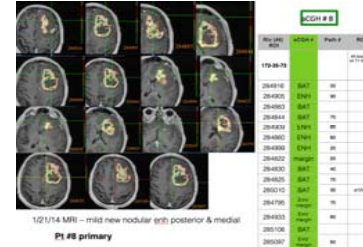


Figure 3. MRI images of a left frontal lobe GBM with the various biopsy locations depicted by the intersection of the green lines. The table: ROI (regions of interest) column are the biopsy locations coded to correlate with the MRI that will be sent to ASU to incorporate into their machine learning algorithm, aCGH column identifies if the sample is BAT or enh, and the path column displays percent tumor of the sample.

Figure 4. Displaying a Comprehensive View of our Samples from Patient 8.

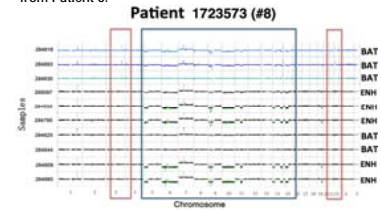


Figure 4. Gross genomic view of Patient 8's samples. Chromosomes 3 and 22 display focal amplifications in BAT samples and Chromosomes 5-15 demonstrate similar changes evident in both BAT and enh samples.

Discussion

When one thinks about GBM it is best to take a landscape view starting from 20,000-feet away and where we are readily able to identify an amplification of chromosome 7 and a deletion of chromosome 10, leading to EGFR gene amplification and PTEN loss, respectively. Although GBM is heterogeneous when comparing different portions like enh and BAT, there is no major rearrangement seen in this chr 7 and 10 pattern throughout most GBM patients.

If one goes from a 20,000-foot view to an up-close view, we can begin to appreciate on the minute and subtle changes. These minor, almost quiet genetic changes can potentially be the inciting alteration leading to invasion and migration observed in BAT. Common to all three patients, hence observed in patient 8's samples, is amplification of genes involved in the Wnt and NFKB pathway. Somatic changes/mutations signify permanent changes in DNA; whereas, epigenetic changes result in subtle changes (not mutations) that may modify gene expression. Genome alterations hold an important role in explaining cell invasion and migration.

Looking at specific commonalities and/or differences in the BAT samples of patient 8, only gene gains were found when comparing BAT samples. Again to focus on these similarities listed in Table 1, we can derive a story:

Arr-19 inhibits androgen receptor (AR)⁵⁰. AR is thought to compete with Wnt signaling by way of beta-catenin⁵¹. Therefore, if you block AR it yields more beta-catenin for the Wnt canonical pathway⁵¹. The increased levels of Wnt canonical pathway beta-catenins are able to escape ubiquitosis degradation and can translocate into the nucleus to undergo transcription. Beta-catenin also binds VE-cadherin, aiding in the process of invasion into the vascular

endothelium⁵². Beta-catenin binds to VE cadherins, allowing the cadherins to bind together at the cytoskeleton junction⁵³. As the endothelial cells contract, these newly formed cell-cell adhesions will be the root of creating a permeable barrier⁵⁴. CKLF, as well as being involved in chemotaxis (migration), acts as actin polymerization⁵⁵. CKLF and CMTM1-4 are essential agents involved in tumor and stromal chemotaxis during progression and metastasis of tumor dissemination⁵⁶. The more readily available actin and beta-catenin are, the more gaps that can be formed, thus, paving the way for invasion to occur under chemotactic guidance.

Many proteins undergo ubiquitination via the Neddylaton pathway; neither NFKB nor its inhibitor are exempted from this inevitable fate. NFKB's inhibitor is bound to it⁵⁷. When NFKB and its inhibitor go through Neddylaton, NFKB is freed from its inhibitor; allowing NFKB to be involved in cell survival, invasion/metastases (NFKB turns on transcription of VE-Cadherin), and inflammation⁵⁷⁻⁶¹. NEDD4 is an E3 ubiquitin-protein ligase that transfers ubiquitin to the ubiquitin-targeted substrate marking it for imminent degradation⁶²⁻⁶³. BEAN1 binds to the WW domains of NEDD4 and an increase in both proteins leads to an escalation in neddylaton, indirectly increasing NFKB activity, which promotes cell migration⁶²⁻⁶⁴.

Another appreciable amplification alludes to migration/metastasis. Studies have shown that mRNA of TRAP25 isoform is present only in circulating cells, but is not expressed in cultured adherent cells⁶⁵.

One clear observation is that BAT gene expression is amplified to favor invasion and migration. Although we were not able to elucidate a driver, we can appreciate the gain in gene expression of BAT tissue to favor invasion and metastasis in the epithelial-mesenchymal transition.

Conclusions

GBM is the most common primary central nervous system tumor carrying a poor prognosis. As mentioned above, invasive GBM is currently resistant to therapy, and is the cause of recurrence.

Our results demonstrated GBM heterogeneity and importance of the biopsy among the spatial location of the tumors, specifically with BAT samples. An increase in amplification of genes involving the Wnt/B-catenin, CKLF and NFKB pathways were observed; which promote invasion and migration, also known as, mesenchymal-endothelial transition. Currently, a biopsy can miss the portion of the tumor that is not susceptible to the specific treatment, especially in regions of BAT, which are regions rather difficult to visualize during surgery. Subsequently, this portion continues to grow uninhibited. As such, the future direction of this study is aimed to non-invasively identify the genotypic profile of patients GBM phenotypically via MRI. This is of profound importance in patient specific care, treatment, and outcome.

Heterogeneity and the invasive characteristics of glioblastoma are challenging in treatment resulting in low survival rates. The heterogeneity of GBMs also leads to different mechanisms of invasion, thus, will likely require different therapeutic approaches. Further analysis is needed to determine the genetic drivers of invasion, migration, and survival in GBM as these are probable therapeutic targets; thus, could have significant, translational impact for individualized patient care.

Dedication & Acknowledgements

Dedication: Dedicated in the memory of my loving grandmother, Wanda Ford. She is one of many who lost their life too young due to GBM. I would like to thank Dr. Tran and his colleagues for the opportunity to work with them on a project that carries a heavy sentimental value for myself. May this R21 study, TGEN's future studies, and to all researching GBM significantly impact patient care and provide a longer, meaningful life for patients/family suffering from GBM.

Acknowledgements: I want to thank my mentor Dr. Nhan Tran and his colleagues at TGEN's Brain Tumor Unit Lab. I wish Dr. Tran and his collaborators the best of luck with the continued R21 project.