Clinical and molecular analyses of long-term survivors of glioblastoma

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Abstract

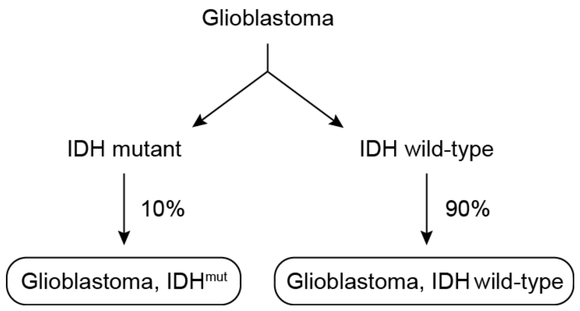
Background: Glioblastoma (GBM) is a uniformly fatal disease with standard treatments conferring a median overall survival of only 14.6 months. Because of limited local control with these frontline therapies, less than 10% of patients will survive five years. The molecular mechanisms that promote improved therapeutic responses remain unknown.

Objective and Methods: The objective of this work was to identify features that render these typically refractory tumors highly sensitive to radiation (RT) and/or temozolomide (TMZ). GBM patients who survived more than five years (long-term survivors; LTS) and who had tissue available for molecular analysis were identified using the Mayo Clinic Neuro-Oncology Registry. These patients were frequency matched to patients who survived less than two years (short-term survivors; STS) by IDH mutation, TERT promoter mutation and 1p/19q codeletion. Subsequent analyses focused on patients who were TERT mutant only or triple-negative as these would be given the same WHO classification. Molecular classification was accomplished using the following technologies: Illumina EPIC methylation array, RNA sequencing, Oncoscan copy number array, and DNA mutation sequencing.

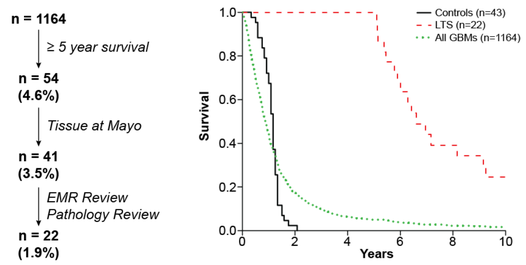
Results: Assessment of methylation patterns, gene expression, and copy number alterations revealed no striking differences between STS and LTS. As expected, patients with IDH mutant GBM were younger at diagnosis than those with IDH wild-type tumors. Despite receiving RT/TMZ regimens at lower rates, LTS demonstrated better survival. Moreover, these LTS retain classic features of typical GBM including gain of chromosome 7, EGFR amplification, PTEN deletion, and partial deletion of chromosome 9. However, more terminal deletion events are observed in STS as are deletions on chromosomes 13-15.

Conclusions: There exists many similarities in both the methylation patterns and gene expression profiles of STS and LTS. Despite possessing many prominent GBM-related aberrations, genome wide copy number analysis suggests LTS are more genomically quiet than STS particularly in regard to the abundance of terminal amplification and deletion events.

WHO Classification



Cohort Establishment



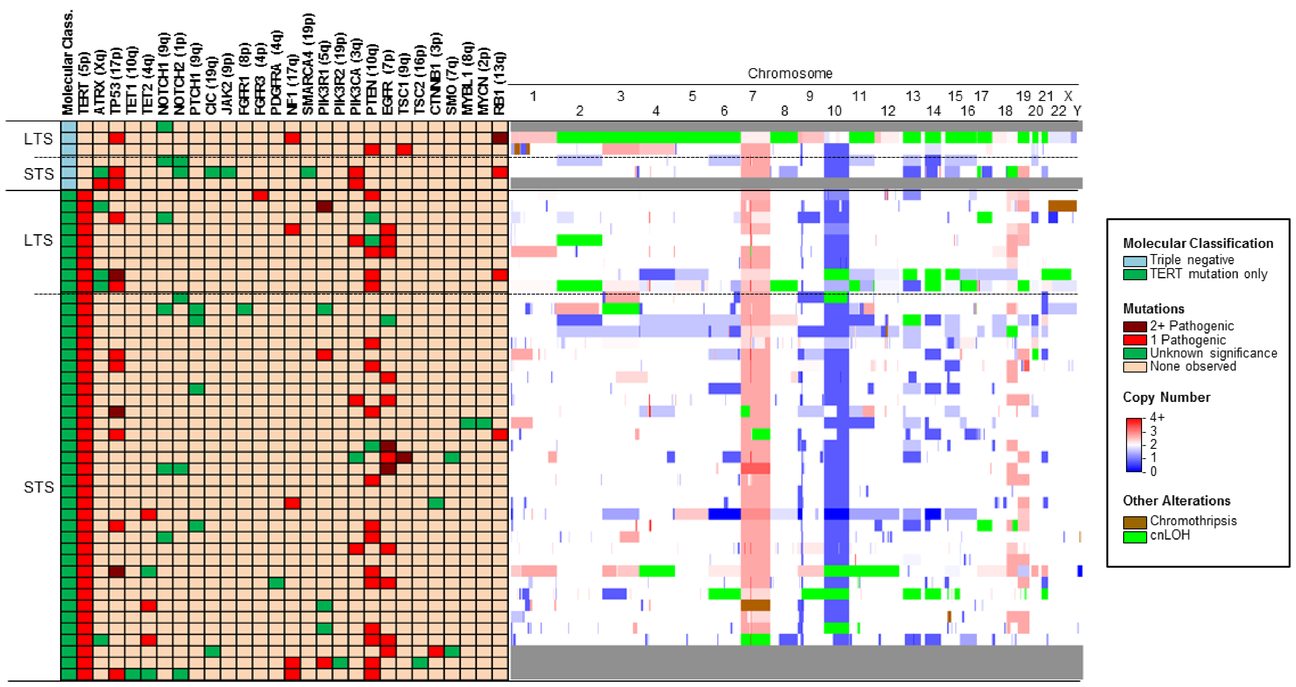
Patients diagnosed with GBM were found using Mayo Clinic's Neuro-Oncology Registry. Twenty-two patients were selected based off survival, pathology, and tissue availability (left). The survival of frequency matched controls was representative of all comers (right).

Patient Demographics

	STS IDH-wildtype (n=38)	LTS IDH-wildtype (n=12)	STS IDH-mutant (n=5)	LTS IDH-mutant (n=10)
Ave. age at diagnosis (SD)	59.8 (8.9)	48.3 (15.4)	47.6 (13.1)	40.4 (15.2)
Sex	10 F / 28 M (26.3% / 73.7%)	7 F / 5 M (58.3% / 41.7%)	2 F / 3 M (40.0% / 60.0%)	5 F / 5 M (50.0% / 50.0%)
Surgery	19 GTR / 19 STR (50.0% / 50.0%)	7 GTR / 5 STR (58.3% / 41.7%)	5 GTR / 0 STR (100.0% / 0.0%)	8 GTR / 2 STR (80.0% / 20.0%)
RT/TMZ treatment	19 (50.0%)	4 (33.3%)	3 (60.0%)	2 (20.0%)

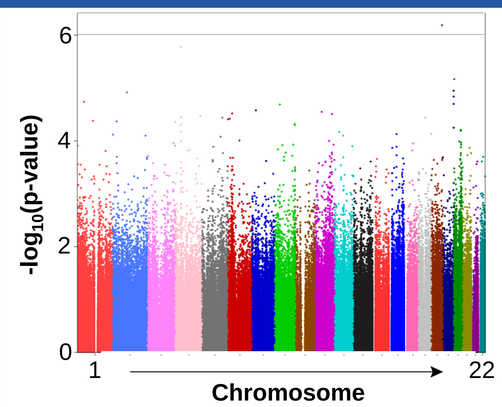
Notes: [1]Kruskal-Wallis rank sum test, [2]Pearson's Chi-squared test

Genomic profile of IDH^{wt} GBM



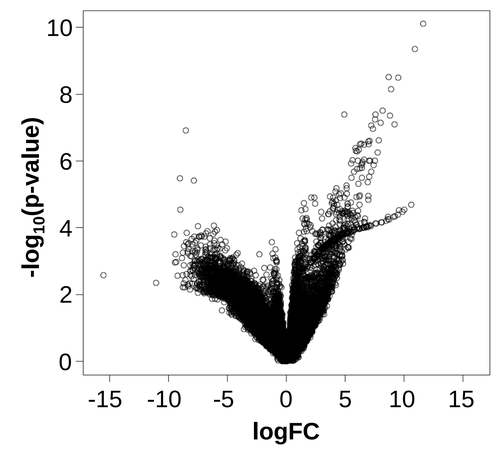
DNA mutation sequencing (*left*) and Oncoscan copy number analysis (*right*) were performed on DNA extracted from both STS and LTS. Mutations were detected by targeted next generation sequencing of coding regions in 50 genes associated with central nervous system tumors. Variants were only assessed if they had ≥200x coverage and ≥10% allele frequency. Genome wide copy-number changes and loss of heterozygosity were identified by manual examination of OncoScan array data using Chromosome Analysis Suite.

Differential Methylation Profile of IDH^{wt} GBM



Global methylation patterns were assessed using the Infinium MethylationEPIC 850K methylation platform. Differentially methylated CpG loci between STS and LTS were plotted by the $-\log_{10}$(p-value). Probes near single nucleotide polymorphisms were removed.

Differential Gene Expression of IDH^{wt} GBM



Transcriptome capture data from STS and LTS was processed using Map-Rseq and normalized using trimmed mean normalization. EdgeR was run to perform differential expression using a negative binomial model.

Conclusions

- Differential analysis of methylation and gene expression data revealed no striking differences between STS and LTS.
- LTS retain many prominent GBM-associated aberrations.
- LTS demonstrate fewer terminal amplifications and deletions than STS.

Acknowledgments

This project was supported by funding from Mayo Clinic Cancer Center Eagles Fellowship and the NIH R01CA184320.