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Tumor-Infiltrating Lymphocytes in Glioblastoma are Associated with NF1, TP53, and RB1 Mutations and the Mesenchymal Transcriptional Subtype

Caleb Rutledge

Emory University School of Medicine

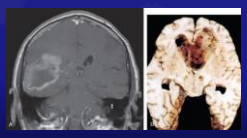
MD, MSCR Candidate

Emory University School of Medicine, Center for Comprehensive Informatics, Department of Biomedical Informatics, Department of Pathology and Laboratory Medicine, Winship Cancer Institute, Emory University

Glioblastoma (GBM)

Highly malignant brain tumor (WHO Grade IV astrocytoma)

Average survival of 15 months



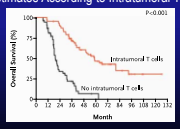
Stupp R, et al.; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005 Mar 10;352(10):987-96.

Tumor-Infiltrating Lymphocytes (TILs)

TILs inhibit tumor growth and prolong survival in many human cancers

Tumor antigens are targets for a T cell mediated adaptive immune response

Kaplan-Meir Survival Estimates According to Intratumoral T cells in Ovarian Cancer



Zhang L, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003 Jan 16;348(3):203-13.

Molecular Changes Associated with TILs

Lymphocytic infiltrates are associated with microsatellite instability and methylation aberrations in colorectal cancer

Molecular alterations within a tumor may be immunogenic and predict response to immunotherapy

Nosho K, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol. 2010 Dec;222(4):350-66. Review.

TILs in GBM

Tumor-infiltrating lymphocytes are present in GBM

Biologic and clinical significance are not fully defined

Evidence of cancer mediated immune suppression

Hypotheses

Tumor-infiltrating lymphocytes in GBM are correlated with specific molecular alterations

Contribute to host immune response

Related to patient outcome

The Cancer Genome Atlas (TCGA)

Catalogue of major cancer-causing genome alterations

DNA copy number, gene expression, DNA methylation, and nucleotide sequence aberrations

Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008 Oct 23;455(7216):1061-8. Epub 2008 Sep 4.

Methods

Publicly available molecular, histologic, and clinical data from TCGA

Histologic features including lymphocytes were annotated as 0 (absent), 1+ (present), and 2+ (abundant) in 171 cases

Chi-square, Fisher's exact, and Kaplan-Meir log-rank tests

Association Between Lymphocytes and other Histopathologic Features

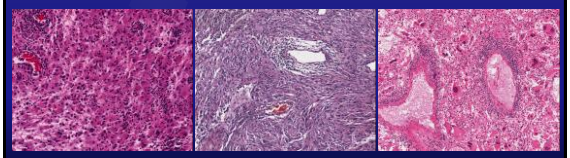
	Prob	Correlation
Inflammation	**<0.0001**	**0.9258**
Endothelial hyperplasia	0.0536	-0.1480
Epithelial metaplasia	**0.0361**	**0.1608**
Gemistocytes	**0.0047**	**0.2169**
Giant cells	**0.0188**	**0.1808**
Sarcomatous metaplasia	**0.0042**	**0.2194**
Microvascular hyperplasia	0.5949	-0.0408
Oligodendroglial cells	**0.0141**	**-0.1883**
Small cells	**0.0018**	**-0.2406**
Satellitosis	0.2023	-0.0981

Histopathologic features coded as 0, 1+ and 2+
Mantel-Haenzel Chi-square test
Spearman correlation

Negative Correlation
Positive Correlation

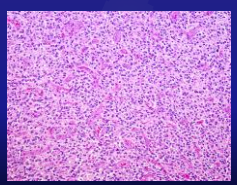
Lymphocytes Correlated with Specific Histologies

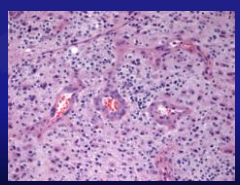
Positively correlated with gemistocytes, sarcomatous cells, epitheliod cells, and giant cells ($p<0.05$)



Lymphocytes Correlated with Specific Histologies

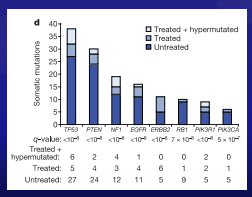
Negatively correlated with small cells and oligodendroglial cells ($p<0.05$)





Associations between Lymphocytes and Known Nucleotide Sequence Aberrations

Significantly mutated genes in GBM



Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008 Oct 23;455(7216):1061-8. Epub 2008 Sep 4.

Lymphocytes Correlated with Mutations in NF1, TP53, and RB1

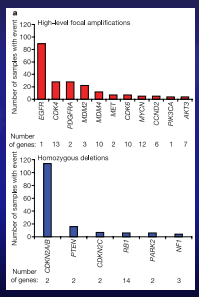
	Lymphocytes		
	0, 1+, 2+	0 versus 1+, 2+	0, 1+ versus 2+
TP53	**0.0023**	**0.0054**	0.0562
PTEN	0.0981	0.0994	0.4455
NF1	0.5083	0.7777	**0.0406**
EGFR	0.6198	0.5759	0.3122
ERBB2	--	--	--
RB1	**0.0425**	**0.0395**	0.2658
PIK3R1	0.3855	0.4448	1.0000
PIK3CA	0.7452	1.0000	1.0000
IDH1	0.7071	1.0000	0.3522

Mutations coded as 0, 1
Mantel-Haenzel Chi-square test (MH exact test for cell counts <5)
Chi-square test (Fisher's exact test for cell counts <5)

Associations between Histologic Subtypes and Molecular Alterations

Gemistocytic, sarcomatous, epithelioid, and giant cell histologic subtypes are characterized by mutations in NF1, TP53, and RB1

Associations between Lymphocytes and Known Copy Number Alterations



Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008 Oct 23;455(7216):1061-8. Epub 2008 Sep 4.

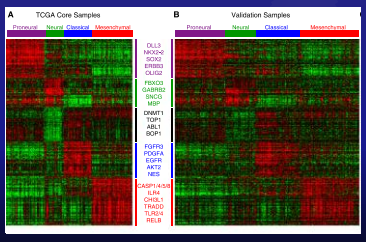
Lymphocytes Correlated with NF1 Deletion and EGFR Amplification and were Depleted in CDKN2A-deleted GBMs

	Prob	Correlation
CDKN2A deletion	**0.0030**	**0.2895**
EGFR amplification	**0.0030**	**-0.2899**
NF1 deletion	**0.0013**	**-0.3133**
PDGFRA amplification	0.6714	-0.0414

Lymphocytes coded as 0, 1+ and 2+
Mantel-Haenzel Chi-square test
Spearman correlation

Gene Expression Subtypes in GBM

TCGA defined Proneural, Neural, Classical, and Mesenchymal transcriptional subtypes



Verhaak RG, et al.; Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell. 2010 Jan 19;17(1):98-110.

Lymphocytes Enriched in the Mesenchymal Subtype

71% of cases classified as having abundant (2+) lymphocytes were in the mesenchymal subtype

Verhaak Classification	Lymphocytes 0	1+	2+	Total
Classical	31	10	1	42
Mesenchymal	27	17	**12**	56
Neural	13	9	2	24
Proneural	19	18	2	39

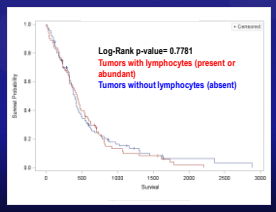
Mantel-Haenzel Chi-square test
MH exact test for cell counts <5

Mutation/deletion of NF1 defines the Mesenchymal subtype

Effect of Lymphocytes on Survival

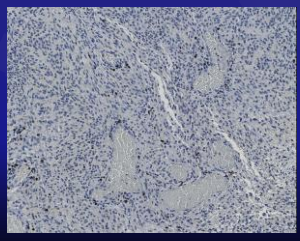
Lymphocytes were not associated with prolonged survival

Kaplan-Meir Survival Estimates According to Lymphocytes (0 versus 1+, 2+)



Ongoing Studies

Define subpopulations of TILs in GBM using immunohistochemistry for further correlative analyses



Conclusions

TILs have biologic significance in GBM

Enriched in the mesenchymal transcriptional subtype

Associated with mutations in NF1, TP53, and RB1; with deletions in NF1; and with histologic subtypes characterized by mutations in NF1, TP53, and RB1

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