Analysis of differential methylation patterns associated with long-term survival in glioblastoma

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BACKGROUND

- With no major advances in treatment in the last 15 years, glioblastoma (GBM) remains a malignancy with poor prognosis - median overall survival (mOS) roughly 12-14 months after diagnosis
- Some patients with GBM have extended survival for unclear reasons
- Beyond MGMT methylation (6-month mOS benefit on temozolomide[1]) and IDH mutation status (41-month mOS benefit[2]), there are no other established major tumor factors associated with long-term survival.
- We designed a single-center exploratory study to evaluate for methylation patterns that may be associated with long term survival in GBM.

METHODS

- Study included patients with histopathologically confirmed GBM and tumor biopsy or resection specimens available at University of Iowa Hospitals and Clinics, treated between 2007 and 2017
- Patients designated short term survivors (STS) if post-diagnosis survival < 12 months; long term survivors (LTS) if post-diagnosis survival >= 34 months
- Specimens on initial resection/biopsy and at recurrence (if available) underwent Illumina Infinium EPIC microarray methylation analysis
- IDH mutation and MGMT methylation detected via methylation profiling classifier
- Samples between LTS and STS compared for differences in methylation patterns

RESULTS

Short Term Survivors

SampleID	Age	Sex	Extent of Surgery	ECOG at Tx Start	Survival Time (mo)	MGMT (PCR)	MGMT Array Testing	IDH Mutation Array Testing
BDG2	62	F	Gross total resection	1	6.63	Unknown	Unmethylated	Wild-type
BDG5	65	F	Gross total resection	1	9.63	Unknown	Unmethylated	Unknown
BDG7	38	F	Gross total resection	1	10.43	Unknown	Methylated	Mutated
BDG9	50	F	Gross total resection	1	8.40	Unknown	Unmethylated	Unknown
BDG18	61	M	Gross total resection	0	10.50	Unknown	Unmethylated	Wild-type
BDG22	64	M	Gross total resection	1	6.63	Unknown	Methylated	Wild-type
BDG24	64	M	Gross total resection	1	6.63	Unknown	Unmethylated	Unknown
BDG25	57	M	Gross total resection	1	9.87	Unmethylated	Unmethylated	Wild-type
BDG28	57	M	Gross total resection	1	9.87	Unmethylated	Unmethylated	Wild-type
BDG31	61	M	Gross total resection	0	5.20	Methylated	Methylated	Wild-type
BDG34	61	M	Gross total resection	0	5.20	Methylated	Unknown	Unknown
BDG13	65	F	Subtotal resection	1	6.57	Unknown	Unmethylated	Unknown
BDG17	51	M	Subtotal resection	1	3.43	Unknown	Unmethylated	Wild-type
BDG11	55	M	Biopsy	1	3.47	Unknown	Unmethylated	Unknown

Long Term Survivors

SampleID	Age	Sex	Extent of Surgery	ECOG at Tx Start	Survival Time (mo)	MGMT (PCR)	MGMT Array Testing	IDH Mutation Array Testing
BDG1	33	M	Gross total resection	0	222.83	Unknown	Methylated	Mutated
BDG12	65	F	Gross total resection	1	35.20	Unknown	Methylated	Wild-type
BDG14	67	M	Gross total resection	1	67.77	Methylated	Unmethylated	Wild-type
BDG16	65	F	Gross total resection	1	35.20	Unknown	Methylated	Wild-type
BDG19	68	F	Gross total resection	1	34.53	Methylated	Methylated	Wild-type
BDG20	54	M	Gross total resection	0	68.30	Indeterminate	Methylated	Wild-type
BDG23	68	F	Gross total resection	1	34.53	Methylated	Methylated	Wild-type
BDG27	67	M	Gross total resection	1	67.77	Methylated	Methylated	Wild-type
BDG29	67	M	Gross total resection	1	67.77	Methylated	Methylated	Wild-type
BDG6	53	M	Subtotal resection	1	137.40	Methylated	Methylated	Wild-type
BDG10	38	F	Subtotal resection	1	72.37	Unknown	Methylated	Mutated
BDG15	61	M	Subtotal resection	1	45.23	Unknown	Methylated	Wild-type
BDG21	38	F	Subtotal resection	1	72.37	Unknown	Methylated	Mutated
BDG26	61	M	Subtotal resection	1	45.23	Unknown	Unmethylated	Unknown
BDG33	53	M	Subtotal resection	1	137.40	Methylated	Unmethylated	Unknown

Top Differentially Methylated Genes in LTS vs STS

Methylated Diagnostic	Unmethylated Diagnostic	Methylated Recurrent	Unmethylated Recurrent
PTPRN2	PTPRN2	DLG2	PTPRN2
PRDM16	C7orf50	TCF4	FOXP1
MAD1L1	SLC6A12	TRAPPC9	CAMTA1
DIP2C	CDH4	ANKS1B	RPTOR
MGMT	COL9A3	GPM6A	CHST11
INPP5A	TNXB	MTUS1	MAD1L1
AGAP1	BCL11A	SORBS2	TP73
CTBP2	PDE1C	TNXB	SEPTIN9

- 15 LTS and 14 STS samples identified and compared (n = 29)
- In total, methylation arrays identified 696 differentially methylated CpG sites significantly associated with long-term survival
- Ingenuity Pathway Analysis (IPA) was used to identify pathways enriched for by top differentially methylated genes

FUTURE DIRECTIONS

- Preclinical studies to evaluate the role of differentially methylated genes, including highly affected phosphatase genes, in GBM:
  - growth,
  - migration,
  - proliferation, and
  - therapeutic resistance
- Evaluate for possible contribution of CpG shores and open-seas regions with respect to GBM survival

REFERENCES

1. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005;352(10):997-1003.
2. Christians A, Adel-Horowski A, Banan R, et al. The prognostic role of IDH mutations in homogeneously treated patients with anaplastic astrocytomas and glioblastomas. *Acta Neuropathol Commun.* 2019;7(1):156.

Questions?
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Top Ingenuity Pathway Analysis Results:

Methylated in Long Term Survivor Diagnostic Specimens
- cAMP-mediated signaling
- GPCR signaling
- Protein Kinase A signaling

Unmethylated in Long Term Survivor Diagnostic Specimens
- Axonal Guidance Signaling
- Endocannabinoid Neuronal Synapse Pathway

Methylated in Long Term Survivor Recurrence Specimens
- CREB Signaling in Neurons
- GPCR signaling

Unmethylated in Long Term Survivor Recurrence Specimens
- ESC Transcriptional Network
- Synaptogenesis Signaling

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