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What single-cell RNA sequencing taught us about *MGMT* expression in glioblastoma

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Abstract

Background. The promoter methylation status of O-6-methylguanine-DNA methyltransferase (*MGMT*p) is an important prognostic marker in GBM. Previous studies showed that the expression of *MGMT* based on immunohistochemistry did not correlate with survival. This is partly because nontumor cells express *MGMT*. Single-cell sequencing assesses gene expression in tumor cells specifically.

Methods. We used publicly available data from 3 recent single-cell/nucleus sequencing GBM studies that included *MGMT*p methylation status data for patients to evaluate *MGMT* expression at the single-cell level.

Results. In the CPTAC study, a median of 0.82% and 5.7% of tumor cells expressed MGMT in the *MGMT*p methylated group and *MGMT*p unmethylated group, respectively (*P*-value .001). In the Neftel study, a median of 0.59% and 14.01% of tumor cells expressed MGMT in the *MGMT*p methylated group and *MGMT*p unmethylated group, respectively (*P*-value .01). Three unmethylated samples (out of 16) had *MGMT* expression <2%. It is unclear if this is due to technical inaccuracies as the Neftel paper did not specify the detection method for *MGMT*p methylation. Alternatively, the percentage of GBM cells expressing *MGMT* as a continuous variable may be more relevant than the dichotomous *MGMT*p status. The Wang study confirmed that *MGMT* expression can increase or decrease between paired primary and recurrent samples. The gene set enrichment analysis shows that *MGMT* expressing and negative cells are enriched with mesenchymal and proneural genes, respectively.

Conclusion. Single-cell data suggest that *MGMT* expression falls on a continuous spectrum. A smaller percentage of tumor cells express *MGMT* when *MGMT*p is methylated.

Key Points

- The percentage of tumor cells expressing *MGMT* is significantly less when *MGMT* promoter is methylated in GBM.
- *MGMT* expressing cells are enriched with mesenchymal genes, whereas *MGMT* negative cells are enriched with proneural genes.

Astrocytomas in adults are classified into isocitrate dehydrogenase (IDH) mutant and IDH-wild-type (IDHwt) subtypes. Glioblastoma (GBM) represents IDHwt astrocytoma grade 4 and is associated with poor prognosis.[1] The standard of care for GBM includes maximal safe resection followed by concurrent radiotherapy with an oral alkylating agent (temozolomide) and adjuvant temozolomide.[2] The promoter methylation status of O-6-methylguanine-DNA methyltransferase (*MGMT*p) is the most established molecular predictive marker for response to temozolomide and accordingly impacts overall survival in GBM.[3] Previous literature suggests that the median overall survival (OS) for patients with unmethylated *MGMT*p GBM is 14.11 months with a median progression-free survival (PFS) of 4.99 months. In contrast, the median OS for patients with methylated *MGMT*p GBM is 24.59 months with a PFS of 9.51 months.[4]

Importance of the Study

Despite the significance of *MGMT*p methylation status on survival in GBM, previous studies showed that the expression of *MGMT* based on immunohistochemistry (IHC) in GBM samples was variable and lacked association with survival, in part because nontumor cells express *MGMT*. Advanced technologies such as single-cell RNA (scRNA) sequencing/single-nucleus RNA (snRNA) sequencing and spatial transcriptomics have helped to elucidate the cell type composition of cancer and its microenvironment. scRNA sequencing allows to assess gene expression in tumor cells specifically. In this study, we explore *MGMT* expression at the single-cell level by analyzing publicly available data from 2 recent scRNA/snRNA sequencing studies. We show that the median *MGMT* expression in tumor cells ranged from 0.59% to 0.82% in *MGMT*p methylated GBM samples and from 5.7% to 14.01% in *MGMT*p unmethylated GBM samples. *MGMT* expressing cells are enriched with mesenchymal genes, whereas *MGMT* negative cells are enriched with proneural genes.

Despite the significance of *MGMT*p methylation status on survival in GBM, previous studies showed that the expression of *MGMT* based on immunohistochemistry (IHC) in GBM samples was variable and lacked association with survival.[5,6] This is in part because nontumor cells, including endothelial cells and macrophages, can express *MGMT* limiting accurate interpretations of the IHC stains. Interestingly, heterogeneity in *MGMT*p methylation status has been reported even within the same tumor.[7–9] Furthermore, it has been observed that the *MGMT*p methylation status can change between paired primary and recurrent samples in 19%–24% of cases, more commonly from methylated to unmethylated status but also the other way around. It appears that the *MGMT*p methylation status in the recurrence setting has a less significant impact on survival.[10,11]

Advanced technologies such as single-cell RNA (scRNA) sequencing and spatial transcriptomics have helped to elucidate the cell type composition of cancer and its microenvironment. Recent scRNA/single-nucleus RNA sequencing (snRNA-seq) studies have demonstrated that GBM cells exhibit a high degree of heterogeneity and plasticity and seamless transitions between cellular states.[12,13]

In this article, we use publicly available data from 3 recent scRNA/snRNA IDHwt GBM studies[12–14] to explore and uncover details about *MGMT* expression in GBM in light of *MGMT*p methylation status at the single-cell level. We also use publicly available data from one spatial transcriptomics study[15] to visualize *MGMT* expression in methylated and unmethylated GBM samples.

Methods

We looked for publicly available scRNA/snRNA GBM studies that included *MGMT*p methylation status data for patients. We first used snRNA data from 18 treatment-naive GBM patients prospectively collected by the Clinical Proteomic Tumor Analysis Consortium.[14] The cohort is well annotated and includes information about the *MGMT* promoter methylation status for samples as determined by the *MGMT*-STP27 model from DNA methylation data. Data were downloaded from the GDC Data Portal. Details about the files used in this analysis can be found in the Supplementary Document. Cell type annotation (tumor cells versus microenvironment) was applied per the original paper's annotation.

We then used the dataset by Neftel et al.[12] The study performed scRNA sequencing on 20 adult IDHwt GBM samples. The Supplementary Table was downloaded from the original paper and included the clinical characteristics for the cohort including the *MGMT* promoter methylation status. However, the *MGMT*p determination method was not specified in the paper. The preprocessed matrix file was downloaded from the 3CA database.[16] The 3CA database houses 77 scRNA datasets where the quality control, filtering, and cell type annotation were all consistently applied to all the datasets and made available to download.

Subsequently, we applied the findings to the study by Wang et al.[13] The study profiled 86 primary-recurrent patient-matched paired GBM specimens with snRNA sequencing. Seventy-six of these samples were IDHwt GBM. The study did not include *MGMT*p methylation data for the samples. However, we were interested in the change of *MGMT* expression between the primary and recurrent samples. The data were downloaded from GEO using the accession number GSE174554.

We finally used data from 2 IDHwt GBM samples from a recent spatial transcriptomics study[15] to visualize *MGMT* expression in tumor spots specifically. The study included several IDH mutant and IDHwt samples, including different regions from the same tumor. The *MGMT*p methylation status was not available for all samples. We excluded "partially methylated" samples to limit obscurity. We then chose ZH1019_T1 (*MGMT*p methylated) and ZH1007_inf (*MGMT*p unmethylated) as they had the clearest pathology images. The processed spatial data were downloaded from GEO using the accession number GSE237183. *MGMT*p designation was obtained from Supplementary Table S1 from the manuscript. Metadata pertaining to tumor spot annotation were obtained by contacting the authors of the manuscript.

We only used de-identified data. Under the federal regulations for human subjects (45 CFR Part 46), research involving publicly available datasets would not require institutional review board review.

We used R 4.3.1 to analyze the scRNA/snRNA datasets. All code used is available on GitHub (https://github.com/iyadalnahhas/scRNA_*MGMT*/blob/main/scRNA_*MGMT*.Rmd)

Seurat objects were created for the above studies per the Seurat V5 workflow.[17] For the CPTAC and Wang et al. studies, quality control was completed as follows: cells were selected for further analysis after excluding potential empty droplets (less than 200 genes per cell) and too few unique molecular identifiers (UMIs < 1,000) and doublets or multiplets (cells with more than 10,000 genes per cell). Low-quality or dying cells were excluded by selecting cells with less than 10% mitochondrial genes. The data were then normalized and highly variable features were selected. The data were then scaled and dimensionality reductions were applied.

MGMT expression was determined using the FetchData command from Seurat for all studies, including the spatial transcriptomic study. *MGMT*+ was defined as feature expression values > 0. *MGMT* expression was compared between *MGMT*p methylated and unmethylated samples using the Wilcoxon rank sum test. Cell-cycle scores were calculated, and cell-cycle classification predictions (G2M, S or G1 phase) were applied per the Seurat workflow. We then used Seurat's FindMarkers function to find the differentially expressed genes between the cellular groups of interest. As a default, Seurat uses the nonparameteric Wilcoxon rank sum test to perform this analysis. Gene set enrichment analysis (GSEA) was performed using clusterProfiler.[18] The C2 curated gene collection set was used from the Molecular Signatures Database (MSigDB) using the msigdbr package in R.

Results

Percentages of MGMT Expression per MGMTp Status

 # CPTAC study.—*MGMT*p methylation data for the CPTAC snRNA cohort were available for 13 patients (7 patients with unmethylated *MGMT* and 6 patients with methylated *MGMT*) whose samples were included in this analysis. The number of tumor cells per sample after quality control ranged from 1,121 to 10,996 (median 5,447 cells). *MGMT* expression data were extracted by Seurat and cells were classified into *MGMT* expressing (*MGMT*+) and *MGMT* not expressing (*MGMT*–).

Positive (nonzero) feature expression values ranged from 0.69 to 3.15. Based on this, the number of cells expressing *MGMT* ranged from 0.19% to 1.43% in the *MGMT*p methylated group (median 0.82%), and from 2.17% to 28.36% in the *MGMT*p unmethylated group (median 5.7%) (Wilcoxon rank sum test *P*-value = .001). Table 1 shows the percentage of *MGMT* expressing cells for each of the 13 samples in this cohort. It therefore appears that all MGMTp methylated GBM samples had < 2% cellular expression of *MGMT* in this snRNA expression dataset.

 # Neftel et al. study.—*MGMT* promoter methylation data for the Neftel scRNA cohort were available for 20 patients (16 patients with unmethylated *MGMT* and 4 patients with methylated *MGMT*). The number of tumor cells per sample after quality control ranged from 121 to 435 (median 221 cells). *MGMT* expression data were extracted by Seurat and cells were classified into *MGMT* expressing (*MGMT*+) and *MGMT* not expressing (*MGMT*–).

Positive (nonzero) feature expression values ranged from 0.012 to 2.39. Based on this, the number of cells expressing *MGMT* ranged from 0% to 1.26% in the *MGMT*p methylated group (median 0.59%), and from 0.3% to 29.13% in the *MGMT*p unmethylated group (median 14.01%) (Wilcoxon rank sum test *P*-value = .01). Table 2 shows the percentage of *MGMT* expressing cells for each of the 20 samples in this cohort. Three unmethylated samples (out of 16) had *MGMT* + cells < 2% (0.3%, 0.68%, 0.73%).

 # Wang et al. study.—Of the 76 IDHwt samples, 48 were paired (primary/recurrent) and passed the above-specified quality control. We excluded samples having fewer than 10 malignant cells after quality control. This left 42 samples (21 pairs). The number of tumor cells per sample ranged from 12 to 5,868 (median 666.5 cells). *MGMT* expression data were extracted by Seurat and cells were classified into *MGMT* expressing (*MGMT*+) and *MGMT* not expressing (*MGMT*-). Positive (nonzero) feature expression values ranged from 0.69 to 3.38.

Of the 21 paired samples, *MGMT* expression decreased at recurrence in 11 pairs and increased in 10 pairs. By using the 2% cutoff from the CPTAC study findings, *MGMT*p methylation status changed from unmethylated to methylated in 4/21 pairs (19%) and from methylated to unmethylated in 4/21 pairs (19%). Figure 1 shows a ladder plot demonstrating the change in *MGMT* expression percentage between primary and recurrent samples.

Cell Cycling per *MGMT* Expression

By applying cell-cycle scores to the CPTAC cohort per Seurat methods, 80.6% of *MGMT* + cells were noncycling whereas 56.8% of *MGMT*- cells were noncycling. The difference is smaller in the Neftel et al. study: 63.7% of *MGMT*+ cells were noncycling and 69.98% of *MGMT*- cells were noncycling.

Find Differentially Expressed Markers between *MGMT*± and *MGMT*− Cells

To find the differentially expressed genes between cells expressing *MGMT* and cells not expressing *MGMT*, we used the unmethylated cases in the CPTAC cohort since the unmethylated cases include a higher proportion of *MGMT* expressing cells. Supplementary Table 1 includes the results of this analysis.

Functional Enrichment Analysis of Differentially Expressed Genes

GSEA of the differentially expressed genes between *MGMT*+ and *MGMT*– cells was applied. Interestingly, the VERHAAK_GLIOBLASTOMA_PRONEURAL set had the highest normalized enrichment score (NES) in the *MGMT* negative cells (NES 3.49, adjusted *P*-value 2.173636e-08). On the other hand, the VERHAAK_GLIOBLASTOMA_MESENCHYMAL set had the highest NES in the *MGMT*+ cells (NES –3.2, adjusted *P*-value 2.173636e-08). The WP_DNA_REPAIR_PATHWAYS_FULL_NETWORK (genes: *MGMT*,

Table 1. Percentage of *MGMT* Expression per Sample in the CPTAC Cohort

Case ID	MGMTp status	MGMT expression (%)
C3N-03184	Methylated	0.19
C3N-02181	Methylated	0.71
C3L-03968	Methylated	0.77
C3L-02705	Methylated	0.88
C3N-02769	Methylated	1.35
C3L-03405	Methylated	1.43
C3N-01798	Unmethylated	2.17
C3N-02784	Unmethylated	4.01
C3N-02783	Unmethylated	4.89
C3N-03186	Unmethylated	5.7
C3N-03188	Unmethylated	8.37
C3N-02190	Unmethylated	9.43
C3N-01816	Unmethylated	28.36

Table 2. Percentage of *MGMT* Expression per Sample in the Neftel et al. Cohort

Case ID	MGMTp status	MGMT expression (%)
MGH152	Methylated	0
MGH66	Methylated	0.23
MGH100	Methylated	0.96
MGH128	Methylated	1.26
MGH125	Unmethylated	0.3
MGH121	Unmethylated	0.68
MGH143	Unmethylated	0.73
MGH101	Unmethylated	4.58
MGH115	Unmethylated	4.38
MGH124	Unmethylated	8.26
MGH105	Unmethylated	10.35
MGH106	Unmethylated	13.97
MGH151	Unmethylated	14.05
MGH129	Unmethylated	16.84
MGH110	Unmethylated	20.93
MGH104	Unmethylated	22.82
MGH122	Unmethylated	26
MGH113	Unmethylated	21.5
MGH136	Unmethylated	24.45
MGH102	Unmethylated	29.13

POLE4, FANCF, CETN2, DDB2) set was enriched in the *MGMT*+ cells (NES –1.71, adjusted *P*-value .042) (Figure 2). Markers of "stem cells" such as CD133 and CD15.[19] do not appear to be more expressed in *MGMT*+ cells.

Visualization of *MGMT* Expression Using Spatial Transcriptomics

Figure 3 visualizes *MGMT* expression within tumor spots specifically for 2 samples[15]: a) ZH1019_T1 (*MGMT*p methylated) and b) ZH1007_inf (*MGMT*p unmethylated). *MGMT* expression is less prevalent in tumor spots when *MGMT*p is methylated (5.6% vs. 19.7% of tumor spots when *MGMT*p is methylated vs. unmethylated, respectively). Of note, 10× Visium was used for the spatial analysis of these samples. 10× Visium has a resolution of 100 µm and therefore does not reflect single-cell resolution.[20]

Discussion

The *MGMT* promoter methylation status is the most established predictive marker of response to temozolomide

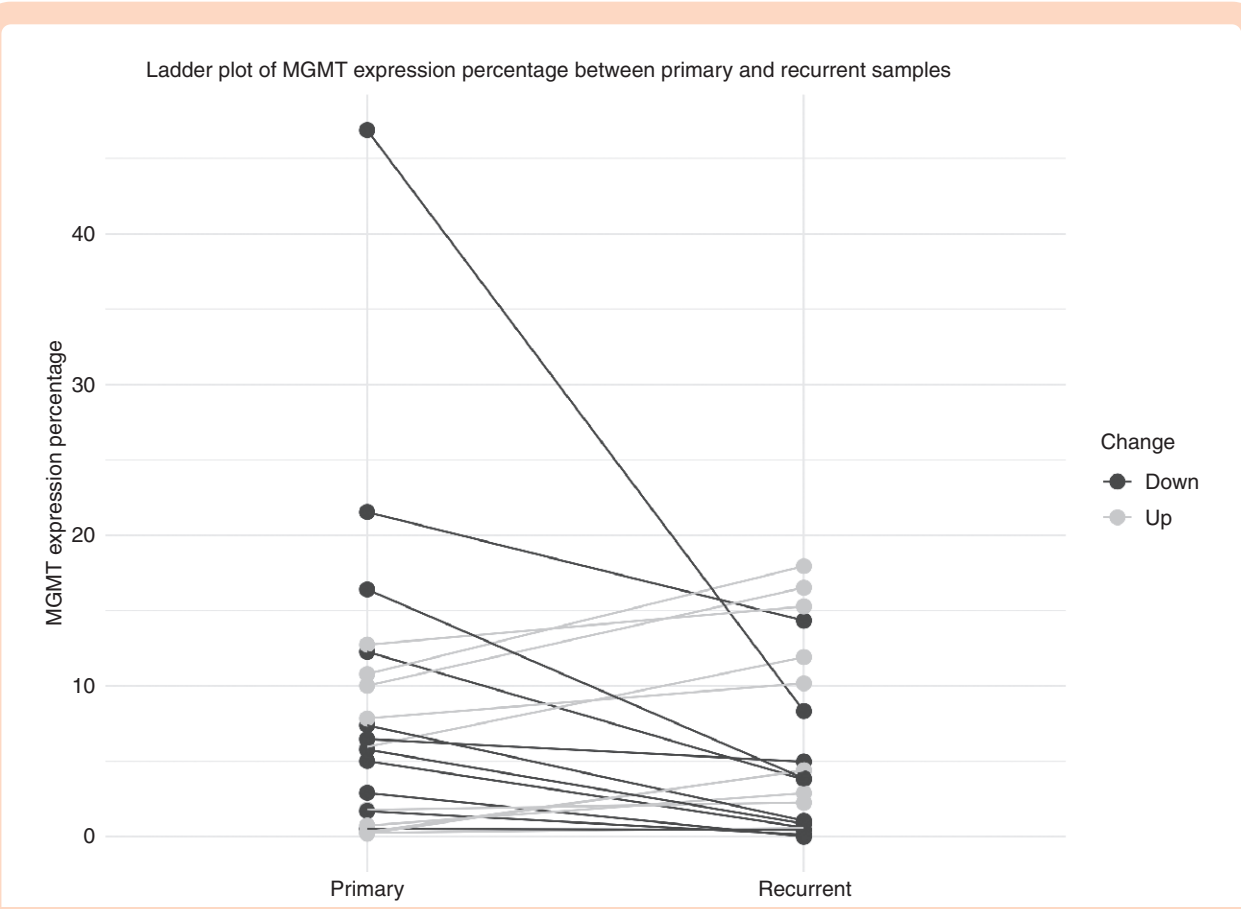

Figure 1. A ladder plot showing the change in *MGMT* expression percentage between primary and recurrent samples in the Wang et al. cohort.

in GBM. In this manuscript, we aimed to explore *MGMT* expression at the single-cell level considering *MGMT*p methylation status. We used publicly available data from 3 scRNA/snRNA sequencing studies. The CPTAC cohort is well annotated and includes information about *MGMT* promoter methylation status for samples as determined by the *MGMT*-STP27 model from DNA methylation data. In the CPTAC cohort, the median expression of *MGMT* was 0.82% in the *MGMT*p methylated group and 5.7% in the unmethylated group. *MGMT* expression was < 2% in *MGMT*p methylated group.

In the Neftel study, the median expression of *MGMT* was 0.59% in the *MGMT*p methylated group and 14.01% in the *MGMT*. Three *MGMT*p unmethylated samples had *MGMT* expression in < 2% of tumor cells. Noting that the Neftel study used scRNA sequencing and not snRNA sequencing, the < 2% observation from the CPTAC study may not apply to scRNA values. Moreover, the Neftel paper did not specify the method used to detect *MGMT*p methylation, and typos/inaccurate annotation cannot be excluded. Or could it be that truly *MGMT*p unmethylated samples can have a low *MGMT* expression status? Could this explain why some unmethylated *MGMT*p patients surpass the expected survival?

The possibility of *MGMT*p methylation status changing at GBM recurrence has been previously reported, more commonly from methylated to unmethylated status but also the other way around.[10,11] We confirm this finding by using snRNA data. Of the 21 paired primary and recurrent GBM samples in the Wang et al. dataset, *MGMT* expression decreased at recurrence in 11 pairs and increased in 10 pairs. By using the 2% cutoff, *MGMT*p methylation status changed from unmethylated to methylated in 4/21 pairs (19%) and from methylated to unmethylated in 4/21 pairs (19%).

We then identified differentially expressed genes between *MGMT* expressing and *MGMT* negative cells. Functional enrichment analysis using GSEA revealed that *MGMT* expressing cells are enriched with mesenchymal genes and *MGMT* negative cells are expressed with proneural genes. Unsupervised hierarchical clustering of bulk RNA data from the TCGA network recognized 3 distinct molecular IDHwt GBM subtypes: proneural, classical, and mesenchymal.[21] The mesenchymal subtype has been linked to aggressive behavior. The fact that *MGMT* expressing cells are enriched with the mesenchymal subtype genes support this notion. Moreover, *MGMT* expressing cells do not appear to be more cycling than *MGMT* negative cells. And markers of "stem cells" such as CD133 and CD15 do not appear to be more expressed in *MGMT*+ cells.

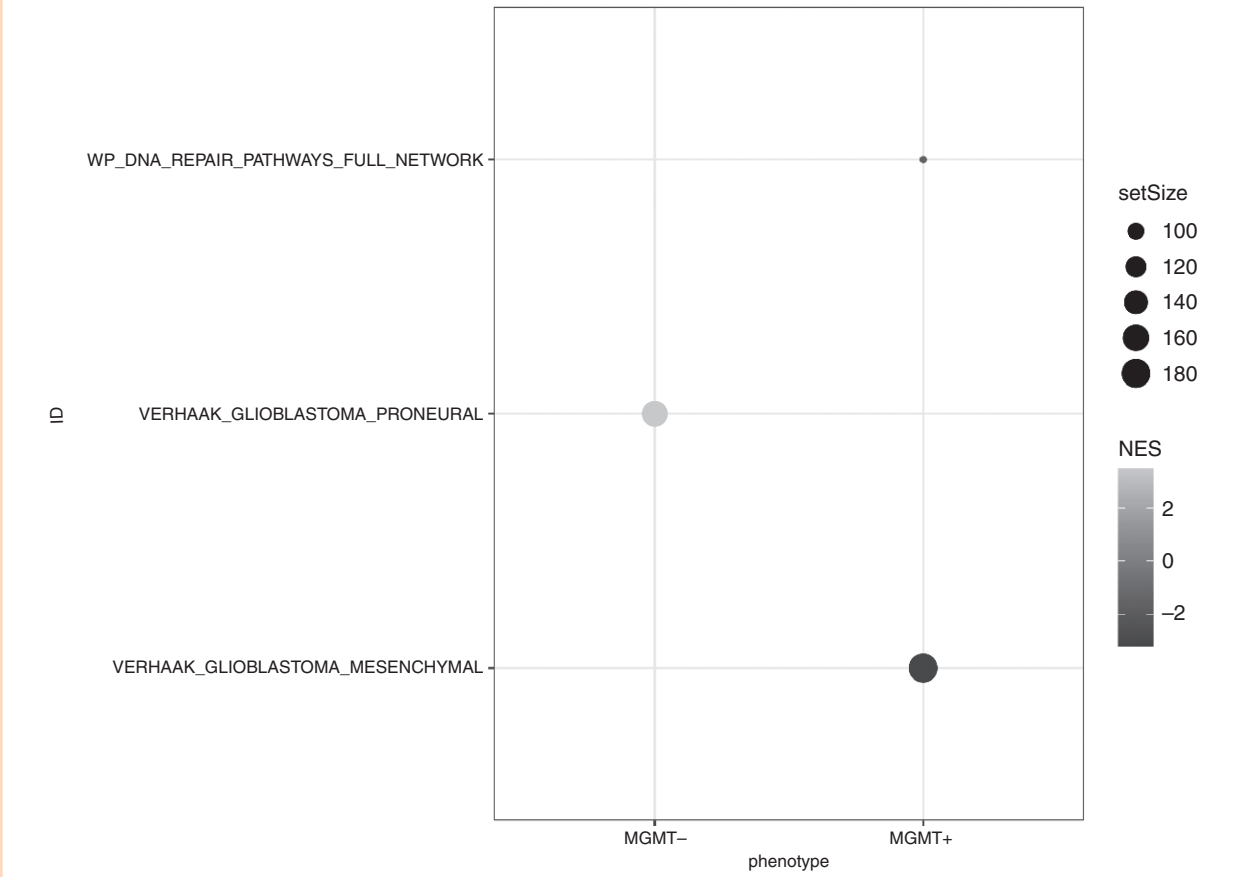

Figure 2. GSEA showing *MGMT*+ cells are enriched with DNA repair pathways and Verhaak mesenchymal signature, whereas *MGMT*– cells are enriched with Verhaak proneural signature.

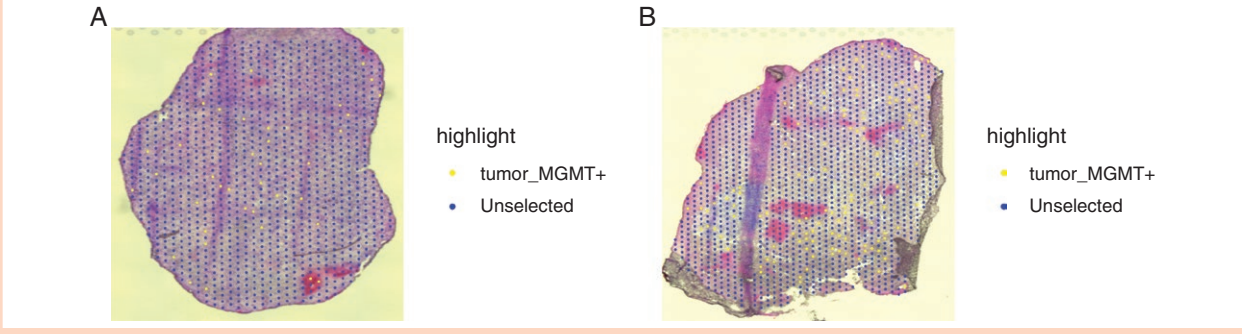

Figure 3. *MGMT* expression within tumor spots specifically for 2 samples: (A) ZH1019_T1 (*MGMT*p methylated) and (B) ZH1007_inf (*MGMT*p unmethylated).

Conclusion

*MGMT*p methylation status is clinically thought of as a dichotomous variable. scRNA sequencing data suggest that *MGMT* expression falls on a continuous spectrum. Bigger longitudinal studies that correlate *MGMT* expression—based on single-cell data—with survival are needed to determine whether the percentage of GBM cells expressing *MGMT* as a continuous variable within a sample is a more accurate prognostic marker than the dichotomous *MGMT*p status, and how this can be incorporated into clinical practice.

Supplementary material

Supplementary material is available online at *Neuro-Oncology Advances* (https://academic.oup.com/noa).

Keywords

glioblastoma | *MGMT* | single-cell sequencing | spatial transcriptomics

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Conflict of interest statement

The authors report no conflicts of interest

Authorship statement

Conception and design of the study: I.A. and W.S. Acquisition and analysis: I.A. Interpretation of data: I.A., M.K., and W.S. Drafting and revision of the written manuscript: I.A., M.K. and W.S. All authors discussed and reviewed the manuscript and approved the manuscript for publication.

Data availability

Publicly available data were used as per the Methods section.

Code availability

All code used is available on GitHub (https://github.com/iyadalnahhas/scRNA_*MGMT*/blob/main/scRNA_*MGMT*.Rmd)

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References

1. Ostrom QT, Price M, Neff C, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2015-2019. *Neuro Oncol*. 2022;24(Suppl 5):v1v1–vv95.
2. Stupp R, Mason WP, van den Bent MJ, et al; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987–996.
3. 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.
4. Alnahhas I, Alsawas M, Rayi A, et al. Characterizing benefit from temozolomide in MGMT promoter unmethylated and methylated glioblastoma: a systematic review and meta-analysis. *Neurooncol. Adv.*. 2020;2(1):vdaa082.
5. Preusser M, Charles Janzer R, Felsberg J, et al. Anti-O6-methylguanine-methyltransferase (MGMT) immunohistochemistry in glioblastoma multiforme: observer variability and lack of association with patient survival impede its use as clinical biomarker. *Brain Pathol*. 2008;18(4):520–532.
6. Burke E, Grobler M, Elderfield K, et al. Double-labelling immunohistochemistry for MGMT and a "cocktail" of non-tumourous elements is a reliable, quick and easy technique for inferring methylation status in glioblastomas and other primary brain tumours. *Acta Neuropathol Commun*. 2013 Jun 10;1:22.
7. Parker NR, Hudson AL, Khong P, et al. Intratumoral heterogeneity identified at the epigenetic, genetic and transcriptional level in glioblastoma. *Sci Rep*. 2016 Mar 4;6:22477.
8. Parkinson JF, Wheeler HR, Clarkson A, et al. Variation of O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in serial samples in glioblastoma. *J Neurooncol*. 2008;87(1):71–78.
9. Wenger A, Ferreyra Vega S, Kling T, et al. Intratumor DNA methylation heterogeneity in glioblastoma: implications for DNA methylation-based classification. *Neuro Oncol*. 2019;21(5):616–627.
10. Brandes AA, Franceschi E, Tosoni A, et al. O(6)-methylguanine DNA-methyltransferase methylation status can change between first surgery for newly diagnosed glioblastoma and second surgery for recurrence: clinical implications. *Neuro Oncol*. 2010;12(3):283–288.
11. Feldheim J, Kessler AF, Monoranu CM, et al. Changes of O(6)-methylguanine DNA methyltransferase (MGMT) promoter methylation in glioblastoma relapse-a meta-analysis type literature review. *Cancers*. 2019;11(12):1837.
12. Neftel C, Laffy J, Filbin MG, et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell*. 2019;178(4):835–849.e21.
13. Wang L, Jung J, Babikir H, et al. A single-cell atlas of glioblastoma evolution under therapy reveals cell-intrinsic and cell-extrinsic therapeutic targets. *Nat Cancer*. 2022;3(12):1534–1552.
14. Wang LB, Karpova A, Gritsenko MA, et al; Clinical Proteomic Tumor Analysis Consortium. Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell*. 2021;39(4):509–528.e20.
15. Greenwald AC, Darnell NG, Hoefflin R, et al. Integrative spatial analysis reveals a multi-layered organization of glioblastoma. *Cell*. 2024;187(10):2485–2501.e26.
16. Gavish A, Tyler M, Greenwald AC, et al. Hallmarks of transcriptional intratumour heterogeneity across a thousand tumours. *Nature*. 2023;618(7965):598–606.
17. Hao Y, Stuart T, Kowalski MH, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol*. 2024;42(2):293–304.
18. Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation*. 2021;2(3):100141.
19. Sloan AR, Silver DJ, Kint S, Gallo M, Lathia JD. Cancer stem cell hypothesis 2.0 in glioblastoma: where are we now and where are we going? *Neuro Oncol*. 2024;26(5):785–795.
20. Vahid MR, Brown EL, Steen CB, et al. High-resolution alignment of single-cell and spatial transcriptomes with CytoSPACE. *Nat Biotechnol*. 2023;41(11):1543–1548.
21. Wang Q, Hu B, Hu X, et al. Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. *Cancer Cell*. 2017;32(1):42–56.e6.