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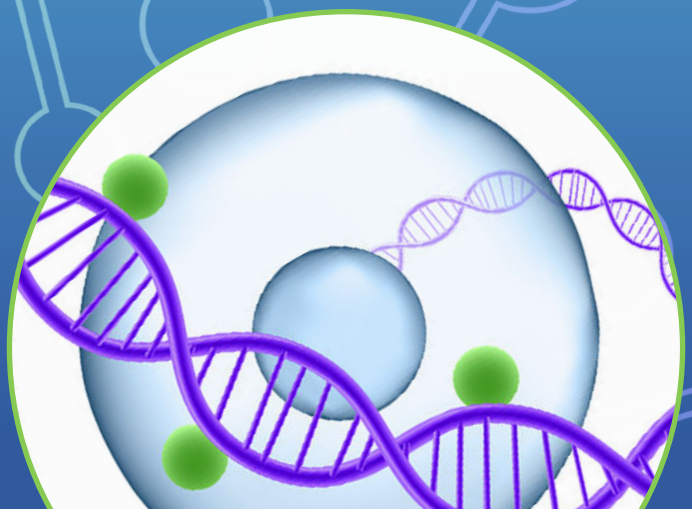
Single-Cell Methylome Profiling of Human Glioma



Overview

Alterations in DNA methylation patterns are a hallmark of various cancers and are closely associated with tumorigenesis, progression, and therapeutic responses.[1] Gliomas represent a highly diverse set of cancers and particular subtypes, such as isocitrate dehydrogenase (IDH) mutant tumors, are associated with hypermethylation of CpG sites.[2] Elucidating IDH-driven epigenetic changes are critical to better understand this complex disease and aid novel clinical discoveries.

Previous studies analyzing DNA methylation patterns have largely used bulk methods, which may mask critical insights due to the cellular heterogeneity of tumors. Although advances in single-cell analysis have revolutionized our understanding of tumors, single cell DNA methylation protocols remain technically challenging, time consuming and expensive to optimize, and typically offer limited cell throughput.

To address this challenge, we leveraged the highly parallelized barcoding technology in ScaleBio's Single Cell DNA Methylation kit to analyze an IDH mutant glioma tumor. This method utilizes the cell itself as a compartment to perform 2 or 3 rounds of sequential barcoding in a plate-based workflow, eliminating the need for complex instrumentation. Compared to other single cell methylation workflows, this technology enables analysis of tens of thousands of cells simultaneously and robust detection of hundreds of thousands of CpG sites.

Highlights

Quality data at scale

High quality methylomes were generated using ScaleBio's Single Cell DNA Methylation workflow from 8,049 glioma nuclei

Single cell resolution

Single cell DNA methylation enabled detection of tumor- and non-tumor cell types

High sensitivity

Differential methylation analysis revealed cancer-specific hyper- and hypomethylated regions, including known prognostic biomarkers

Methods

Flash frozen tumor tissue, which had tested positive for IDH1 and p53 mutations (IDH1:R132H, TP53:R273C), was obtained from an oligodendroma surgically removed from a 31yo female. Single nuclei were isolated and fixed using the ScaleBio Single Cell Methylation Nuclei Preparation Kit (product code 940554). The ScaleBio Single Cell Methylation Kit (product code 955253) was used to prepare single-cell methylation libraries **(Figure 1)**. Briefly, following nucleosomal depletion cells were stained with YOYO-1 and counted. 5000 nuclei/well were loaded into each well of 3 indexed-tagmentation plates, providing the first level of indexing via in-situ tagmentation. Nuclei were then pooled, stained with DRAQ5, and sorted into 4x96-well plates (24 nuclei/well). Following bisulfite conversion and clean-up libraries were prepared, adding the second level of indexing via PCR indices. Libraries were sequenced on a NovaSeq6000 S4 flowcell and analyzed with the ScaleBio Seq-Suite pipeline. Manual curation for cell type identification was done using hypomethylation in marker genes **(Figure 2B).**

Results

QC

Using metrics output from the ScaleBio Seq-Suite Methylation Pipeline we saw a median of 2.396 million reads/cell with >92% of reads found in cells, showing good coverage and high sequencing quality despite the challenging sample type. Across these reads we found a median of 146K CGs covered of which 84.18% were methylated, and a median of 3.73M CH covered of which 0.53% were methylated. Together these results show evidence of good bisulfite conversion and effective detection of methylation sites **(Table 1)**.Cell type annotation

Table 1. Sequencing summary of single cell DNA methylation library from a glioma sample.

QC Metric	
Number of Passing Cells	8,094
Percent Reads in Passing Cells (%)	92.29
Median Total Reads	2,396,578
Median Unique Reads	439,649
Median of CG Methylation Percent (%)	84.18
Median of CH Methylation Percent (%)	0.53

Next we generated a UMAP projection using the CG methylation data **(Figure 2A).** Using 50kb non-overlapping bins we generated a list of differentially methylated regions (DMR) within the different clusters and used specific marker genes to annotate the cell types in which we had high confidence **(Figure 2B)**. Examination of the %CH methylation showed the expected pattern, with low %CH methylation in all cell types other than neurons, where ~6% was observed, providing orthogonal confirmation that these annotations are correct **(Figure 2C)**.

Single-cell methylation analysis

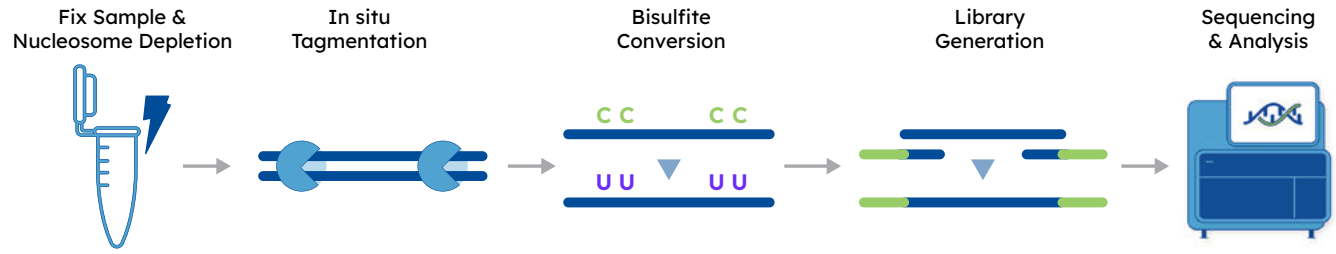
To begin investigating the patterns revealed in single-cell methylation profiling that may be masked in bulk sequencing we then analyzed hypomethylated and hypermethylated DMRs within each cluster to identify cell type specific marker genes **(Figure 3A)**. Notably we found that both the specific genes and the extent of hypo/hypermethylation changed substantially from cluster to cluster, with extensive hypermethylation observed in the cancer stem cells, IDH mutant marker cells, and tumor associated microglia, and extensive hypomethylation observed in tumor-associated microglia **(Figure 3A)**.

Using the single-cell data we were also able to identify specific methylation signatures salient to gliomas in the cancer stem cell population, such as hypermethylation of MGMT, which is associated with better response to specific therapeutic agents[3,4] **(Figure 3B)**. Hypermethylation of HOXD3, which has also been shown to be a potential prognostic indicator[5], was observed, as well as hypermethylation of TET and DNMT genes, providing global dysregulation of methylation in these cancer cells **(Figure 3B)**. Notably many of these hypermethyation patterns were also observed in the tumor-associated microglial cells **(Figure 3C)**.

Conclusions

These data show the ScaleBio Single Cell DNA Methylation workflow generates high quality data from tumor tissue. Single cell methylome analysis resolves the challenge of tumor heterogeneity and enables the detection of cell types and identification of cancer-associated differentially methylated regions, which may be obscured by bulk analysis. In addition, single cell analysis detected known prognostic biomarkers for glioma specifically in cancer cell types, validating our approach. Compared to bulk DNA methylation approaches, single cell DNA methylation analysis offers a comprehensive, cellularly resolved view of tumor-associated methylomes and provides insights into cellular heterogeneity and gene regulation.

Figure 1.



Overview of the ScaleBio Single Cell DNA Methylation workflow. Nuclei samples are fixed and barcoded in situ using tagmentation, enabling sample multiplexing. Bisulfite conversion is then performed, followed by cleanup and the addition of adaptors and a second barcode to complete library construction. Libraries are sequenced and data is analyzed using the ScaleBio Seq Suite.

Figure 2A. Annotated UMAP showing cell types recovered.

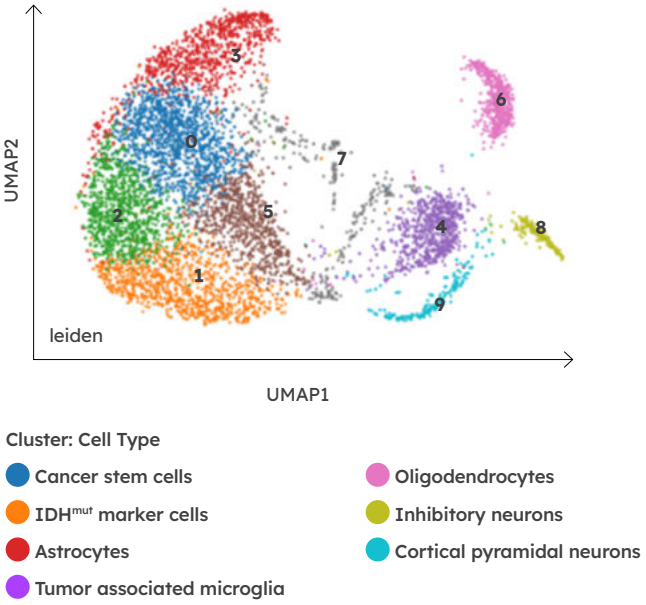


Figure 2B. Markers used for cell type annotation.

Cell Type	Marker gene(s)
Cancer stem cells	SOX2, GRIA1
Astrocytes	GFAP
Oligodendrocytes	MOG, PLP1
Inhibitory neurons	GAD1
Cortical pyramidal neurons	SATB2, TBR1
Tumor-associated microglia	CD163, TMEM119, CD68

Figure 2C. %CH methylation mapped onto the UMAP projection generated using CG methylation data.

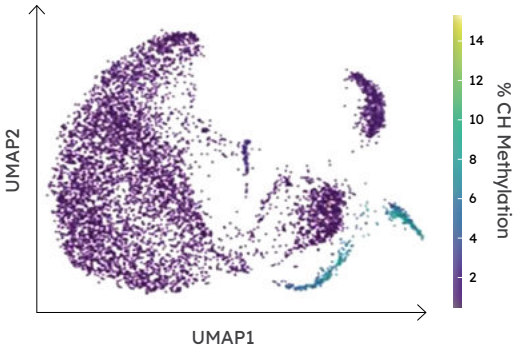
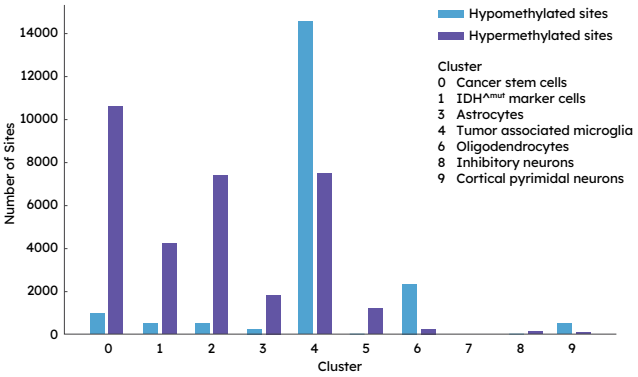
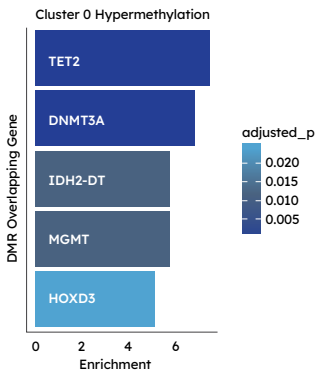


Figure 3A.



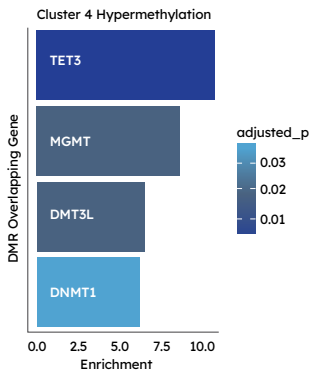
The number of hyper- (dark blue) and hypomethylated (light blue) CG sites found in different clusters (numbers correlate with those shown on the UMAP in figure 2A). Clusters 2, 5, 7 are unannotated populations.

Figure 3B.



Hypermethylation of specific genes observed in the cancer stem cell population.

Figure 3C.



Hypermethylation of specific genes observed in the tumor-associated microglial population.

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Product Code	Product Description
940554	ScaleBio™ Single Cell Methylation Nuclei Preparation Kit
955251	ScaleBio™ Single Cell Methylation Kit - Small
955253	ScaleBio™ Single Cell Methylation Kit - Large

References

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2. Han S, Liu Y, Cai SJ et al. IDH mutation in glioma: molecular mechanisms and potential therapeutic targets. 2020. Br J Cancer 122, 1580–1589.
3. Lam K, Eldred BSC, Kevan B et al. Prognostic value of O6-methylguanine-DNA methyltransferase methylation in isocitrate dehydrogenase mutant gliomas. 2022. Neurooncol Adv v.4(1): vdac030
4. Ozair A, Bhat V, Alisch RS et al. DNA Methylation and Histone Modification in Low-Grade Gliomas: Current Understanding and Potential Clinical Targets. 2023 Cancers (Basel) 15: 1342.
5. Mamatjan Y, Voisin MR, Nassiri F et al. Integrated Integrated molecular analysis reveals hypermethylation and overexpression of HOX genes to be poor prognosticators in isocitrate dehydrogenase mutant glioma. 2023 Neurooncol 25: 2028-2041.

To learn more about ScaleBio's Single Cell DNA Methylation Kit and download example datasets, visit: scale.bio/scalebio-single-cell-methylation-kit

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