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Brief Communication

# An evaluation of *MGMT* promoter methylation within the methylation subclasses of glioblastoma

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**Methylation analysis is a new powerful tool for the classification of brain tumors. Capper et al. classified glioblastoma (GBM) into K27, G34, RTK I, RTK II, RTK III (pediatric tumors), MYCN (enriched with MYCN amplification), mesenchymal, and midline (sharing epigenetic similarities with H3K27M but lacking this mutation). Here, we illustrate *MGMT* methylation distribution among each of the before mentioned methylation subclasses of GBM. We observe that most MYCN and RTKIII cases, as well as H3K27M midline gliomas, lack *MGMT* promoter methylation. More tumors were methylated within RTKI, RTKII, G34, and MID subgroups, whereas more tumors were unmethylated within the MES subgroup.**

Capper et al.[1] provided a comprehensive classification of central nervous system tumors based on DNA methylation that demonstrated substantial diagnostic precision over pathologic evaluations alone. Methylation profiling also allows for determination of the methylation status of the *O⁶-methylguanine DNA methyltransferase (MGMT)* gene promoter that has been demonstrated to be prognostic and predictive of response to temozolomide in patients with glioblastoma (GBM).[2] Here, we illustrate *MGMT* methylation distribution among the methylation subclasses of GBM described by Capper et al.

The Cancer Genome Atlas (TCGA) generated detailed information on the genomic and epigenomic alterations leading to gliomagenesis.[3] At the DNA level, the most common alterations involve the receptor tyrosine kinase pathway (eg, amplification of *EGFR*), phosphatidylinositol 3-kinase pathway (eg, deletion of *PTEN*), cell cycle pathway (eg, mutations in *CDKN2A/B*), p53 pathway, and telomere length maintaining pathways (eg, *TERT* promoter mutations). Unsupervised hierarchical clustering of gene expression data from the TCGA network recognized 4 distinct molecular GBM subtypes: proneural, neural, classical, and mesenchymal.[4] This was later specified to proneural, classical, and mesenchymal in *IDHwt* GBM.[5] The proneural subtype was characterized by abnormalities in *IDH* or *PDGFR*, whereas the classical and mesenchymal subtypes were characterized by *EGFR* and *NF1* mutations, respectively. At the level of gene expression, *MGMT* promoter methylation was not characteristic of any of the 4 subgroups described above.[4]

DNA microarray techniques have been applied to study the GBM methylome using probes targeting many of the known CpG sites. The initial study described the Glioma-CpG Island Methylator Phenotype and found this to be tightly linked to *IDH1* mutations and the proneural subtype and to predict a better prognosis.[6] Additionally, DNA methylation clusters 2 and 3 correlated with the classical and mesenchymal gene expression groups, respectively. A later study integrated epigenetic, genetic, and expression analyses and established 5 epigenetic subgroups of IDHwt gliomas: 2 with H3F3A mutations (K27 and G34), RTK I (enriched with *PDGFR* amplification/proneural expression), RTK II (enriched with *EGFR* amplification/classical expression), and mesenchymal (low copy number variations).[7] Subsequently, a large study of 606 GBM patients from the TCGA cohort grouped IDHwt GBM into 3 methylation clusters: LGm4 (equivalent to RTK II), LGm5 (equivalent to mesenchymal), and LGm6 (enriched with H3K27M and pilocytic features).[8] Finally, Capper et al.[1] classified GBM into K27, G34, RTK I, RTK II, RTK III (pediatric tumors), MYCN (enriched with MYCN amplification), mesenchymal, and midline (sharing epigenetic similarities with H3K27M but lacking this mutation). Reifenberger et al.[9] reported *MGMT* promoter methylation percentage for each of the methylation subgroups described by Ceccarelli et al.[8]

Methylation array data used in this study were utilized as the reference cohort for GBM and H3K27M brain tumor classes in

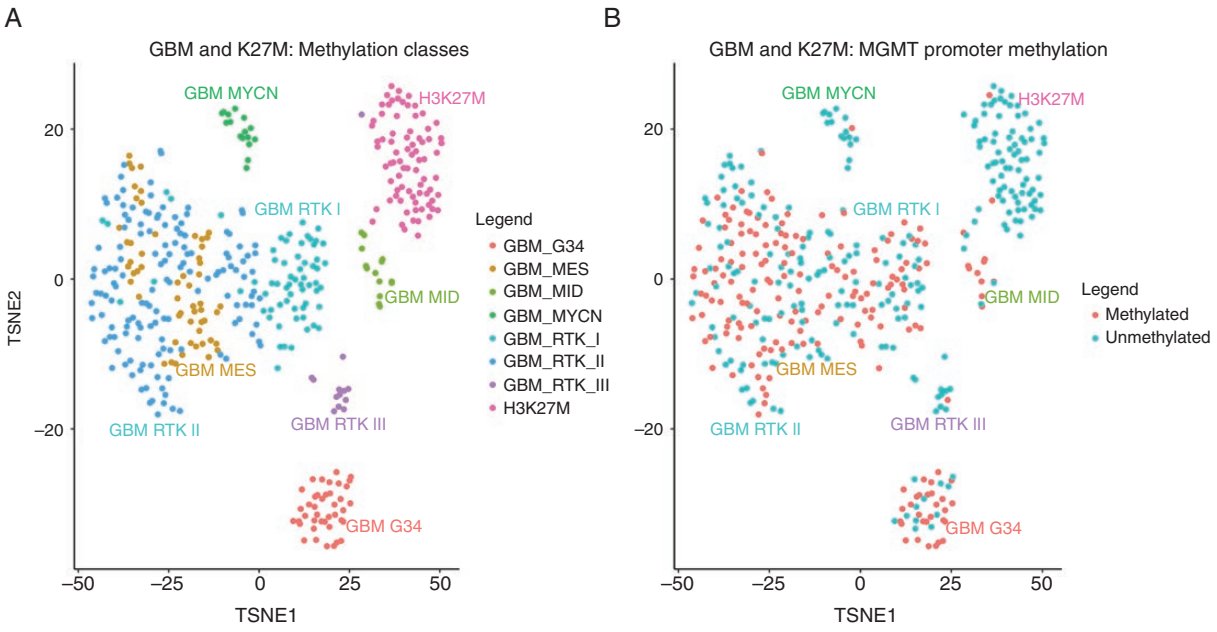


**Figure 1.** (A) Clustering of glioblastoma samples ($n = 425$) using t-SNE dimensionality reduction. Individual samples are color-coded by respective class color. (B) Glioblastoma samples colored by MGMT methylation status and labeled by class abbreviation.

the work of Capper et al.[1] IDAT files were downloaded from the National Center for Biotechnology Information Gene Expression Omnibus data repository, accession GSE90496. IDAT files were preprocessed and batch adjusted following methods described by Capper et al.[1] *MGMT* promoter methylation was performed utilizing the MNPpredict_MGMT function from the mnp.v11b4 Classifier R package. t-Distributed Stochastic Neighbor Embedding (t-SNE) analysis was performed using the R package Rtsne v0.15 (cran.r-project.org/web/packages/Rtsne).The following non-default parameters were utilized: perplexity = 20, theta = 0, eta = 100, exaggeration_factor = 20, num_threads = 2. To maintain reproducibility set.seed(1) was used. All analyses were performed with R-3.6.3.

Overall, 182/347 (52.4%) of GBM cases had methylated *MGMT* promoter, but only 2/78 (2.6%) of H3K27M midline gliomas had methylated *MGMT* promoter. Among the GBM subclasses, *MGMT* promoter methylation was present in GBM_G34: 29/41 (70.7%); GBM_MES: 24/56 (40.7%); GBM_MID 10/14 (71.4%); GBM_MYCN 1/16 (6.3%); GBM_RTK_I: 35/64 (54.7%); GBM_RTK_II: 82/143 (57.3%); GBM_RTK_III: 1/13 (7.7%).

We observe that most MYCN and RTKIII classes lack *MGMT* promoter methylation. Similarly, most H3K27M midline gliomas are *MGMT* promoter unmethylated as described previously.[10] More tumors were methylated within RTKI, RTKII, G34, and MID subgroups, whereas more tumors were unmethylated within the MES subgroup. However, no clear clustering based on *MGMT* promoter methylation status was observed as illustrated in Figure 1.

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