Epigenetic Classifiers for Precision Diagnosis of Brain Tumors

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ABSTRACT: DNA methylation profiling has proven to be a powerful analytical tool, which can accurately identify the tissue of origin of a wide range of benign and malignant neoplasms. Using microarray-based profiling and supervised machine learning algorithms, we and other groups have recently unraveled DNA methylation signatures capable of aiding the histomolecular diagnosis of different tumor types. We have explored the methylomes of metastatic brain tumors from patients with lung cancer, breast cancer, and cutaneous melanoma and primary brain neoplasms to build epigenetic classifiers. Our brain metastasis methylation (BrainMETH) classifier has the ability to determine the type of brain tumor, the origin of the metastases, and the clinical-therapeutic subtype for patients with breast cancer brain metastases. To facilitate the translation of these epigenetic classifiers into clinical practice, we selected and validated the most informative genomic regions utilizing quantitative methylation-specific polymerase chain reaction (qMSP). We believe that the refinement, expansion, integration, and clinical validation of BrainMETH and other recently developed epigenetic classifiers will significantly contribute to the development of more comprehensive and accurate systems for the personalized management of patients with brain metastases.

KEYWORDS: DNA methylation, brain metastasis, supervised machine learning, precision medicine, epigenetics

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Brain metastasis (BM) is a deadly complication that occurs in an estimated 10% to 20% of all cancer patients, most frequently originating from lung cancer, breast cancer, and cutaneous melanoma.[1] Often, it can be the initial presenting manifestation in patients with otherwise previously undiagnosed advanced stage cancer. Due to the dismal prognosis of this clinical complication, efficient and effective diagnosis and treatment are crucial.

Valuable information used to guide therapeutic approaches can be derived from the status of the systemic disease, patient's performance status, number, size and location of BM, and direct BM profiling, which can provide actionable alterations and aid in both diagnosis and prognosis of the disease. Exemplifying the importance of profiling of BM specimens, a recent study found that 53% of BMs have clinically informative molecular alterations not detected in the matched primary tumor.[2] Furthermore, in some cases, the accurate diagnosis of BM may be challenging for pathologists and clinicians, especially when the availability of BM surgical specimens is limited and/or when a BM is poorly differentiated. These challenges highlight the need to profile BM tissue directly to properly determine an accurate therapeutic approach.

In recent years, innovative studies have demonstrated the ability of DNA methylation profiling to aid in the diagnosis of cancers from unknown primary sites,[3] primary central nervous system (CNS) tumors,[4–6] and other primary neoplasms.[7–9] Given the potential clinical utility of DNA methylation profiling and leveraging on the findings of our seminal studies,[10–12] we recently aimed to complement these disease-specific epigenetic classifiers by first generating unique DNA methylation signatures of the most frequent types of metastatic brain tumors, followed by developing an efficient system to aid in the diagnosis of challenging cases by using a relatively small portion of BM tissue.[13] With this goal in mind, we focused on identifying informative combinations of genomic regions whose DNA methylation level could predict the type of brain tumor, the tissue of origin, and therapeutically relevant subtypes of metastatic brain tumors.[13] To generate high-quality genome-wide DNA methylomes that could be easily compared with data from other studies, we analyzed genomic DNA derived from micro-dissected formalin-fixed paraffin-embedded (FFPE) BM specimens with the Infinium HumanMethylation 450K (HM450K) microarray.[14] In our study, we included specimens from lung cancer brain metastases (LCBMs), breast cancer brain metastases (BCBMs), and melanoma brain metastases (MBMs), the three most frequent types of BM. In addition, we included BM specimens from patients with uncertain or incomplete histopathology diagnosis. We further integrated publicly available large-scale data from a variety of brain and extracranial primary tumors and employed machine learning algorithms to build robust BM classifiers.

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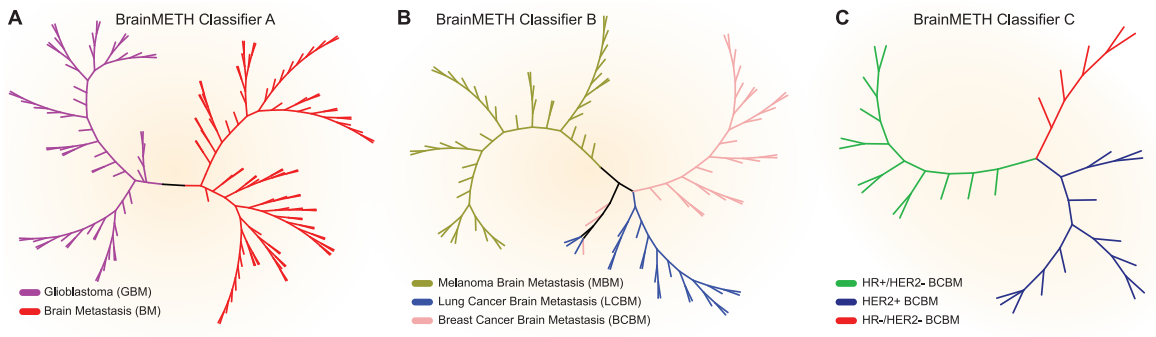

Figure 1. Application of the BrainMETH classifiers for the stratification of multiple brain tumors: (A) Distances between primary brain tumors (glioblastoma; purple branches; n = 60) and brain metastases (BMs; red branches; n = 94) using the DNA methylation levels of genomic regions included in the BrainMETH classifier A. (B) Distances between metastatic brain tumors from patients with primary lung cancer (LCBM; blue branches; n = 22), breast cancer (BCBM; pink branches; n = 28), and melanoma (MBM; brown branches; n = 44) using the DNA methylation levels of genomic regions included in the BrainMETH classifier B. (C) Distances between brain metastases from different breast cancer therapeutic subtypes including hormone receptor (HR)-positive/HER2-negative BCBMs (green branches; n = 13), HER2-positive BCBMs (blue branches, n = 13), and HR-/HER2- (a.k.a. triple-negative breast cancer) BCBM (red branches; n = 5) using the DNA methylation levels of genomic regions included in the BrainMETH classifier C. The phenetic trees were generated using the Pearson's Correlation as a distance metric, with average linkage as clustering approach in the FigTree, version 1.4.3, tool with radial tree layout.

Our study resulted in a three-step brain metastasis DNA methylation (BrainMETH) classification system capable of distinguishing (1) BM from primary brain tumors (BrainMETH classifier A), (2) the tissue of origin of the BM specimen (BrainMETH classifier B), and (3) the therapeutically relevant subtype of BM specimens in breast cancer patients (i.e. hormone receptor–positive/HER2-negative, HER2-positive and triple-negative breast cancer, BrainMETH classifier C; Figure 1).[13] These classifiers demonstrated an excellent ability to determine the origin and therapeutic subtype of metastatic brain tissues, even for cases with unknown or uncertain initial diagnosis, missing information, and patients with synchronous or asynchronous brain lesions. To further the translation of these findings into clinical practice, we selected the most informative genomic regions and designed specific targeted DNA methylation assays using quantitative methylation-specific polymerase chain reaction (qMSP). This cost-effective approach showed excellent efficiency in accurately diagnosing both primary and metastatic brain tumors of various origins.

To ensure the reproducibility of these findings and increase the utility of the data generated from this study, we deposited the raw methylation data to the National Center for Biotechnology Information (NCBI)'s Gene Expression Omnibus (GEO) repository (GSE108576 and GSE44661) and provided all the relevant clinical information for patients included in the study as part of a separate article.[14] This resource supports interoperability and wider use by the bioscience community by providing a manually curated data set in the Investigation-Study-Assay (ISA) framework that includes the clinical-demographic information for all the patients linked to the respective raw DNA methylation data (.idat) files. In addition to the standardization for downstream data analysis provided by the ISA framework, we believe that this resource will not only allow for the replication of each step of our study but will also facilitate the translation of these data into additional practical histomolecular applications.[14]

Additional expansion of the BrainMETH classifier would involve the evaluation of BM from less frequent origins, such as renal or colorectal carcinoma that were not covered in our studies. As recently demonstrated for glioblastoma, a potential limitation of these analyses is the presence of intra-tumor DNA methylation heterogeneity.[15] Perhaps this, as well as other disease-specific features, should be considered in future work to improve and expand the applicability of DNA methylation–based diagnostic signatures for clinical use.

The acquisition of samples for accurate diagnosis can be challenging in some patients with brain neoplasms due to several factors, such as anatomical location or extensive metastatic disease (making patients poor candidates for neurosurgery). Establishing a minimally invasive approach to identify therapeutic targets, diagnose, classify, and potentially follow-up BM lesions will significantly impact the management of patients with brain neoplasms. Recent next-generation sequencing-based approaches have shown that BM can be profiled using cell-free DNA (cfDNA) extracted from cerebrospinal fluid (CSF) without the need for surgical intervention.[16,17] We speculate that the adaptation of the BrainMETH classifier and CNS tumor DNA methylation classifiers to cfDNA derived from pre-surgical CSF specimens may significantly impact patient management, leading to a reduction of invasiveness, especially for those patients with uncertain diagnosis and inoperable brain tumors.

Given the impactful clinical applications of DNA methylation analysis, we believe that the clinical and epigenetic data generated in our study can be easily adapted to expand the applications of the BrainMETH classifier and to identify new epigenetic signatures involved in the BM etiology. Specifically, the adaptation and validation of these epigenetic classifiers to streamlined polymerase chain reaction (PCR)-based assays

may allow a wide range of laboratories to assay patient-derived tumors in a cost-effective manner.

Author Contributions

Author contributions: JIJO and AOM-P performed bibliographical search. JIJO, AOM-P, MPS and DMM designed the commentary content. MPS and DMM performed the data normalization and phenetic trees. JIJO and AOM-P wrote the manuscript. MPS and DMM provided guidance about the content. All authors read and approved the final manuscript before submission.

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