IMPLEMENTATION OF DNA METHYLATION PROFILING FOR BRAIN TUMOR DIAGNOSIS: THE FIRST BRAZILIAN EXPERIENCE

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Introduction: DNA methylation profiling has revolutionized diagnostic neuropathology since its implementation in Heidelberg. It associates a high throughput methylation array data with a machine learning based classifier in order to predict the accurate diagnosis of several brain tumor entities. This approach allows not only appropriate classification of difficult-to-diagnose cases, but also to subclassification within tumor classes, such as Medulloblastomas, Ependymomas and Meningiomas, with prognostic and therapeutic significance. Despite its use became widespread in several countries, methylation profiling for brain tumor classification has never been performed in Brazil.

Objective: To implement and validate a DNA methylation array and the Heidelberg Brain Tumor classifier to aid in the diagnosis of brain tumors in Brazil.

Methods: Eight cases with known or highly suspected diagnosis and a normal brain cortex tissue sample were selected for this validation. DNA samples were extracted from FFPE specimens and evaluated after bisulfite conversion using the BeadChip HumanMethylation450 (8 samples) or Infinium MethylationEPIC 850 (1 sample) arrays. The raw data for each sample was uploaded in the Heidelberg Neuropathology Website and evaluated in the classifier versions 11b4, 12.3 and 12.5. The results were interpreted together with all other clinical, histopathological, immunohistochemical and molecular data available in order to render an integrated diagnosis according to the 2021 WHO Classification of Central Nervous System Tumors.

Results: Two samples were not suitable for classification due to bad fixation and poor DNA quality. Six cases received a high calibrated score for known methylation classes, matching with the original or expected diagnosis. One specimen had a low calibrated score for methylation class Glioblastoma, RTK2 subtype, but this information was considered for the final diagnosis of Glioblastoma, IDH-wildtype. The integrated diagnosis for the other samples were Astroblastoma, *MN1*-altered; Diffuse hemispheric glioma, H3 G34-mutant; Anaplastic Pleomorphic Xanthoastrocytoma; Diffuse high grade glioma, H3 K27-altered, Medulloblastoma, SHH-activated, subclass 4 and Control tissue, hemispheric cortex. Representative images of selected cases are illustrated in figure 1. The whole cohort is summarized in table 1.

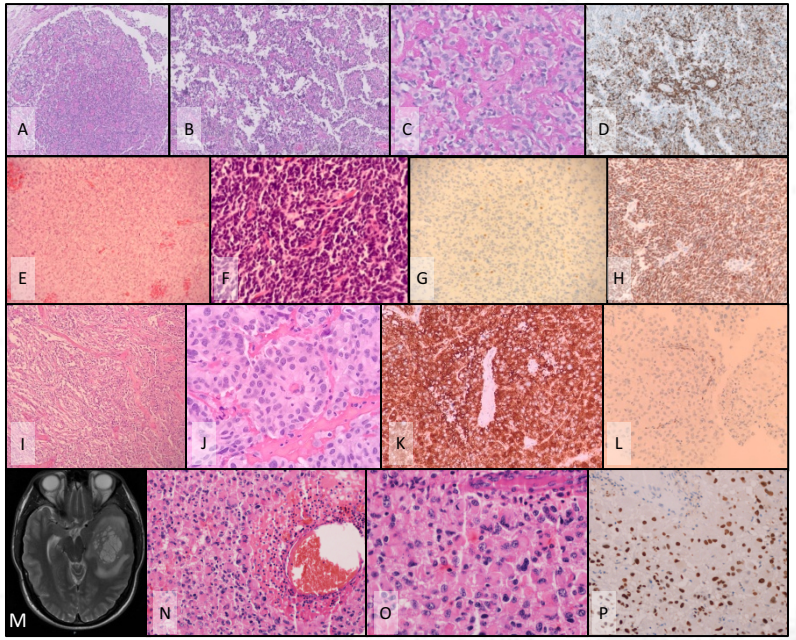

Figure 1. Representative images of selected cases. A-D: Astroblastoma, *MN1*-altered, with relative well circumscription (A), astroblastic rosettes (B) and stromal hyalinization (C). GFAP highlights the perivascular rosettes (D). E-H: Diffuse hemispheric glioma, H3 G34-mutant, displaying diffuse glial (E) and embryonal-like (F) patterns. There was loss of ATRX expression (G) and strong positivity for H3 G34R antibody (H). I-L: Anaplastic pleomorphic xanthoastrocytoma, showing compact fascicular areas (I) and astroblastic-like pseudorosettes (J). This case was diffusely positive for BRAF VE1 antibody (K), with complete absence of p16 expression (L). M-P: High grade hemispheric gljoma, H3 K27M-mutant. This tumor was located in temporal lobe, unrelated to midline structures (M). It was a high grade glioma with gemistocytic-like cells, perivascular inflammation and high mitotic activity (N and O). H3 K27M antibody was strongly positive in tumor cells (P).

Table 1. Summary of the clinicopatological data, methylation class and integrated diagnosis for all the evaluated samples.

Sex	Age	Site	Methylation Class	Integrated Diagnosis
F	12	Temporal	Methylation class control tissue, hemispheric cortex	Normal brain parenchyma
M	20	Temporal	Diffuse midline glioma, histone 3 K27-mutant	High grade hemispheric glioma, H3 K27M-mutant
F	67	Temporal	Glioblastoma, RTK2 subtype	Glioblastoma, IDH-wildtype
F	62	Frontal	(Anaplastic) pleomorphic xanthoastrocytoma	Anaplastic pleomorphic xanthoastrocytoma
F	22	Temporal	High grade neuroepithelial tumor with BEND2:MN1 fusion	Astroblastoma, *MN1*-altered
M	5	Parietal	No match	Ependymoma, NOS
M	16	Frontoparietal	Methylation class glioblastoma, IDH wildtype, H3.3 G34 mutant	Diffuse hemispheric glioma, H3 G34-mutant
M	15	Parietal	No match	High grade SEGA-like astrocytoma, NEC
M	34	Cerebellum	Medulloblastoma, SHH-activated, subclass 4	Medulloblastoma, SHH-activated

Conclusions: DNA methylation profiling was able to confirm and refine the diagnosis of the majority of this first set of cases, with a relatively simple workflow and interpretation. Poor fixation may interfere in test accuracy, so it is crucial to maintain good practices in specimen handling. Applying the array to a higher number of cases will allow us to get more experience with this tool and benefit more patients with a precise neuropathological diagnosis.

References:

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