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Molecular neuropathology: The times they are a-changin'

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When I was a neuropathology fellow at Massachusetts General Hospital from 2007 to 2009, all that was needed for brain tumor diagnostics were H&E slides. Occasionally, immunohistochemistry such as an INI-1 stain was necessary, but even in the department where the 1p19q discovery was made, brain tumor diagnosis was almost entirely based on morphology in which interpretation was often subjective. At the time, I wrote that "loss of chromosomal arms 1p and 19q is observed in ~80% of oligodendrogliomas, 50% to 60% of anaplastic oligodendrogliomas, and 30% to 50% of oligoastrocytomas." Primitive neuroectodermal tumor (PNET) was also considered to represent a single disease entity, distinguished only by anatomic location and distinguishing it from a glioblastoma was often impossible. Neurooncologists sometimes joked that if a brain tumor was sent for review to three neuropathologists, at least four different diagnoses would be rendered. New tumor entities were usually established based on morphological features of a handful of cases collected at a single institution.

Beginning with the description of molecular glioblastoma[1] and medulloblastoma[2] subtypes, our understanding of brain tumors has changed. The importance of molecular subclasses was initially considered prognostic rather than diagnostic. It was only after that analysis of brain tumors using whole-genome DNA methylation profiling by 450k and then EPIC array along with machine learning that exposed not only the vast heterogeneity of brain tumors, but also our limits in diagnosing them based on morphology alone.[3] Numerous new subtypes of brain tumors were discovered using DNA methylation signatures and subsequent DNA and RNA sequencing. Molecular diagnostics has changed our understanding of and how we define known and new brain tumors. While the 2021 WHO Classification of Tumors of the Central Nervous System does not endorse any specific molecular technique, it acknowledges the unique role of methylome analysis for the diagnosis of many WHO brain tumor subtypes.[4]

The main value of DNA methylation profiling, however, is not simply the discovery of new tumors or prognostication. By using DNA methylation, we can now quantify our diagnostic error rate and have a reproducible, albeit imperfect, molecular benchmark for our diagnoses and clinical trial inclusion criteria. In their landmark paper, Capper et al estimated that 12%-14% of cases could significantly benefit from DNA methylation classification.[3] Hwang et al further showed a truly catastrophic impact on clinical trials using histopathology alone; DNA methylation-based re-analysis of the Children's Oncology Group ACNS0332 CNS-PNET Trial showed that 71% of histologically confirmed PNET actually represented other molecularly defined brain tumor entities that should have been excluded from trials, leading to trial failure.[5]

In this issue, Wu et al provide additional support for the clinical utility of DNA methylation profiling.[6] In their cohort, approximately 46% of cases benefited from additional information obtained by DNA methylation profiling improving diagnostic accuracy, including over 25% resulting in a new diagnosis. For cases with descriptive diagnoses due to ambiguous morphology, almost half of them could be correctly diagnosed using DNA methylation profiling. While most of their cohort consisted of consults suggesting selective bias toward diagnostically challenging tumors, the data confirm that descriptive diagnoses are no longer clinically relevant, and the diagnostic evaluation of such cases would benefit from DNA methylation profiling. In light of recently failed CNS clinical trials and poor diagnostic reproducibility particularly for high-grade pediatric brain tumors, it is imperative that all future clinical trials include DNA methylation analysis as an initial molecular screening method.

DNA methylation analysis has some important limitations which Wu et al address. DNA methylation profiling requires relatively high tumor cell content, preferably over 70%, for accurate classification. The authors show that additional deconvolution strategies may resolve cases with low tumor cell purity. Wu et al also show that DNA methylation results need to be closely integrated with morphologic assessment and additional ancillary studies, particularly in the setting of discrepant methylation class with low confidence score. Lastly, while DNA methylation provides a useful tool for the discovery of novel molecular subtypes these methylation subtypes need to be also analyzed by other techniques to identify relevant molecular drivers to identify the underlying mechanism of each unique methylation signature.

From the beginning, the DNA methylation profiling effort has benefited from data sharing and an open-source philosophy. Building on the work of Capper et al, Wu et al also embrace the community-based approach in the analysis and discovery of new brain tumor subtypes. Brain cancers are rare, therapeutic opportunities limited, and clinical trials are failing. It is therefore critical that the neuropathology community continues to share data to improve diagnostics and facilitate therapeutic discovery. While the current academic environment and funding system tend to encourage individual institution-based studies, new entities, and classifiers, that practice is detrimental to science and our patients. Once data sharing, inter-institutional validation, and open access to diagnostic classifiers are fully embraced, then we will know that times have truly changed.

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