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Cerebrospinal fluid: The new frontier for methylome-based diagnostic classification of brain tumors

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Over the past decade, major advances in tumor genomics and diagnostic molecular neuropathology have let to improved diagnostic accuracy, prognostication, and treatment selection for patients with primary and metastatic CNS tumors. While DNA or RNA-based sequencing methods are well suited to identify clinically relevant genomic alterations, they may not be disease-specific (eg, *BRAF* mutations) and many brain tumor entities lack consistent disease-defining, recurrent oncogenic driver mutations (eg, non-Wnt/non-SHH medulloblastoma and posterior fossa ependymoma). Tumor classification based on genome-wide DNA methylation profiling and machine-learning algorithms has proven a highly reliable and powerful diagnostic tool in neuropathology, including frequent instances where histology and sequencing results leave diagnostic ambiguity.

For the majority of brain tumor patients, primary tumor resection at the time of diagnosis is both clinically warranted and will yield sufficient tissue for histopathology and comprehensive molecular testing. However, several clinical scenarios exist where there is an urgent need to develop minimally invasive "liquid biopsy" assays to ascertain the diagnosis prior to decisions about surgical intervention. One such scenario is in patients with Central Nervous System Lymphoma (CNSL), where rapid initiation of chemotherapy can prevent the development of irreversible neurologic deficits and is potentially curative.

To date, the vast majority of CSF-based liquid biopsy assays in clinical development are based on the detection of recurrent mutations in cell-free DNA.[1–3] PCR-based assays are highly sensitive, but generally limited to the detection of a known hotspot mutation, such as in *BRAF* or *IDH1/2*.[4,5] In contrast, assays deploying next-generation sequencing panels have broader diagnostic utility, but at the cost of lower sensitivity.[6–8]

In this issue, Zuccato and Patil et al.[9] address a major need, ie, minimally invasive diagnosis of three major intra-axial types of brain tumors in adults, including glioblastoma, CNSL, and brain metastases. This was accomplished through the development of a cell-free methylated DNA immunoprecipitation plus high-throughput sequencing (cfMeDIP-seq) protocol and diagnostic methylome classifier (Binomial GLMnet) for circulating tumor-derived DNA (ctDNA) from CSF. This classifier was optimized through fifty iterations of 80% discovery sets using CSF methylomes obtained from 57 patients with glioblastoma, CNSL, and brain metastases, as well as nonneoplastic-control patients. Validation and model optimization were based on a set of 197 publicly available tissue methylomes from the disease types of interest. Utilizing this assay and classifier, the authors were able to reliably distinguish patients with glioblastoma, CNSL, and brain metastases with a high level of accuracy.

This innovative effort is the first to apply a methylation-based classifier to CSF for the differential diagnosis of several tumor types, and does so with accuracy approaching that of current tissue-based methylation classifiers while out-performing plasma methylome-based classifiers. While this particular diagnostic method is currently limited to three entities, the paper provides important proof of concept that CSF-based DNA methylation profiling is feasible in patients with brain tumors. There is perhaps no better example of a group who could maximally benefit from this type of technology than pediatric primary brain tumor patients. In this population with a very broad spectrum of disease entities, tissue-based DNA methylation profiling has proven especially valuable diagnostically.[10]

Based on the novel and provocative work by Zuccato and Patil et al., it is exciting to imagine a clinical reality in which CSF ctDNA testing would provide both methylome-based tumor classification and comprehensive mutational profiling in the form of a minimally invasive, rapid, and cost-effective diagnostic modality in routine practice. In this context, it is important to recognize the limitations of the current technology described in this manuscript. CSF ctDNA yields in the patient cohort were relatively high, and CSF was obtained in all patients at the time of Ommaya reservoir placement for intrathecal therapy, limiting the generalizability of this approach. While studying patients with a high disease burden can facilitate proof-of-concept studies and assay development, successful wide-based clinical implementation of CSF methylome-based diagnostic classification will require further technological refinements to increase sensitivity while

maintaining specificity. Nonetheless, this paper reflects a critical first step in the right direction and holds promise for a future where we can provide all of our patients with a definitive molecular diagnosis rapidly, safely, and without the need for immediate surgical intervention.

Conflict of Interest

A.M.M. and M.A.K. have nothing to disclose.

Declaration

The text is the sole product of the authors and no third party had input or gave support to its writing.

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