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EDITORIAL

Unlocking diagnostic potential: DNA methylation profiling in cerebrospinal fluid for central nervous system tumors

Celeste Antonacci, Luana Abballe, Sara Patrizi and Evelina Miele

Onco-Hematology, Cell Therapy, Gene Therapies and Hemopoietic Transplant, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

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1. Introduction

Central nervous system (CNS) tumors encompass an exceptionally heterogeneous group of neoplasms and remain among the leading causes of cancer-related morbidity and mortality, particularly in children. For decades, histopathological evaluation has represented the cornerstone of CNS tumor diagnosis, progressively complemented by molecular characterization of key biomarkers identified through DNA and RNA sequencing technologies [1]. Nevertheless, the marked histological complexity and overlapping morphological features of many CNS tumors, together with the absence of recurrent oncogenic driver mutations in certain entities, frequently result in diagnostic ambiguity, underscoring the need for more robust and integrative diagnostic tools.

Over the past decade, the introduction of DNA methylation (DNAm) profiling has substantially advanced our understanding of CNS tumor biology. By capturing genome-wide epigenetic signatures, DNAm-based classifiers have demonstrated superior diagnostic accuracy and reproducibility compared with conventional histopathology and sequencing approaches, thereby contributing to major revisions in the World Health Organization (WHO) classification of CNS tumors [1]. Despite these advances, the simultaneous acquisition of sufficient tumor material for comprehensive histological and molecular analyses is often not feasible. The invasiveness of biopsy procedures and the limited surgical accessibility of certain lesions continue to pose significant challenges for tissue sampling.

In this context, liquid biopsy, and in particular cerebrospinal fluid (CSF) analysis, has emerged as a promising alternative source of tumor-derived material. Given that many CNS tumors arise within or in close proximity to the ventricular system, tumor-derived DNA is frequently detectable in the CSF, both as circulating tumor DNA (ctDNA) and within circulating tumor cells (CTCs). Accordingly, DNAm profiling of CSF-derived tumor DNA offers a minimally invasive strategy for the detection, classification, and molecular characterization of CNS tumors in both adult and pediatric patients [2–4].

The aim of this editorial is to critically examine the strengths and current limitations of CSF DNA methylation profiling in the diagnosis, longitudinal disease monitoring, and therapeutic stratification of CNS tumors. We provide illustrative examples of its application in tumor characterization, both as a standalone approach and in integrative multi-omics frameworks. Finally, we discuss how advances in next-generation methylation technologies, coupled with artificial intelligence driven analytical tools, may further expand its diagnostic accuracy and translational utility, paving the way toward a new era of precision diagnostics in neuro-oncology.

2. From tissue to liquid biopsy: the rise of DNA methylation profiling of CNS tumors

In 2018, Capper and colleagues introduced a DNAm – based classification of CNS tumors, enabling the definition of more than 80 CNS tumor entities [1]. This powerful machine learning approach has markedly enhanced the biological understanding of CNS neoplasms and fundamentally reshaped their diagnostic landscape. Importantly, besides the information about the somatically acquired DNA changes in cancer cells, DNAm profiling reflects the characteristics of the cell of origin, enabling biologically meaningful stratification of CNS tumors beyond morphology or genetic mutations.

Although initially tissue-based, in the last decade, several groups have explored the feasibility of applying DNAm profiling to liquid biopsy, including both plasma [5,6] and CSF [7,8]. Using low-input methylation techniques, such as cell-free methylated DNA immunoprecipitation sequencing (cfMeDIP-seq), nanopore sequencing, or miniaturized Infinium arrays, early studies have demonstrated that DNAm profiling of liquid biopsies can successfully reconstruct the tumor methylome from minimal DNA quantities [5,9,10].

Within the context of CNS tumors, CSF has emerged as a particularly advantageous source of tumor-derived DNA compared with plasma. Owing to its anatomical proximity to the tumor and direct contact with the central nervous system, CSF typically contains higher concentrations of circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) [11]. DNAm profiling of CSF has been successfully applied across a broad spectrum of CNS tumor entities, including glioblastoma (GBM),

CONTACT Evelina Miele ✉ evelina.miele@opbg.net Onco-Hematology, Cell Therapy, Gene Therapies and Hemopoietic Transplant, Bambino Gesù Children's Hospital, IRCCS, Viale di San Paolo 15, Rome 00146, Italy

medulloblastoma (MB), ependymoma (EPN), and pineoblastoma (PB) [7,12–14], consistently demonstrating high concordance with matched tissue-derived methylation profiles. Collectively, these findings underscore the potential of CSF-based DNAm profiling as a reliable and minimally invasive approach for CNS tumor classification [15,16].

By faithfully capturing the epigenetic identity of the tumor, CSF liquid biopsy expands the diagnostic armamentarium for CNS neoplasms and offers new opportunities for accurate diagnosis in settings where tissue sampling is limited or infeasible.

3. Diagnostic and prognostic applications

Given its high concordance with tissue-derived DNAm signatures, CSF-based DNAm profiling represents a valuable complement to conventional diagnostic approaches. This is particularly relevant in pediatric neuro-oncology, where repeat biopsies are rarely feasible and tissue availability is often limited. In such settings, DNAm profiling of CSF can inform clinical management by providing real-time insights into tumor classification and molecular evolution. Recent studies, including our own experience with pineoblastoma [14], have shown that CSF DNAm profiling can precisely establish diagnosis and molecular subgrouping even in cases with limited or ambiguous tissue material. These data further support the growing evidence that CSF-based DNAm profiling provides a diagnostic accuracy comparable to conventional tissue-based methods while significantly reducing patient risk. Notably, this approach has proven effective even in the absence of detectable CTCs, as shown in MB [13], and in CNS lymphoma [3] where tumor-derived DNAm signals were identified despite negative CSF cytology.

Beyond its diagnostic utility, CSF DNAm profiling opens new possibilities for disease monitoring and treatment guidance. Longitudinal changes in CSF DNAm patterns can serve as sensitive biomarkers of treatment response or minimal residual disease, often preceding radiological evidence of progression. For example, in MB, CSF DNAm signature can be exploited to identify MB subtype, and its changes can be used to assess disease status, including treatment response and recurrence [13]. Similarly, in GBM, a prognostic and diagnostic model based on CSF DNAm profiling of 8 hub genes has been proposed as a strategy to monitor disease recurrence [17]. In glioma, the O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation in CSF can be used as a tumor-specific biomarker to define diagnosis and treatment and to monitor recurrence and prognosis [18]. Moreover, integrating DNAm profiling with complementary cfDNA analyses including mutation detection (such as *IDH1/IDH2*, *TERT* promoter mutations, *H3K27M/H3G34R*, *BRAF* in gliomas, *TP53* in MB); copy number variationand deletions (such as 1p/19q codeletion, +7/-10, *CDKN2A/B* deletion in gliomas, *MYC* amplification in MB), gene fusions/rearrangements (such as KIAA1549-BRAF in gliomas, C11orf95-RELA, *YAP1* fusion in EPN) – can further enhance diagnostic and prognostic confidence. For instance, in brainstem gliomas, the combined detection of genetic mutations and DNAm features in CSF-derived ctDNA has proven useful for monitoring minimal residual disease [19].

4. Multi-omics profiling of CSF: a powerful approach for improving CNS tumor understanding

Although DNAm profiling has proven to be effective in supporting diagnosis and prognosis, the use of a single-omic approach may represent a limitation in understanding the complexity of CNS tumor biology. To address this, recent studies have explored transcriptomics, proteomics, and metabolomic features of CSF across various malignancies [20–23] including CNS tumors [24,25]. Integrating these orthogonal molecular layers has the potential to provide a more holistic view of tumor biology, capturing complementary aspects of tumor behavior and microenvironmental interactions.

To date, only one study has merged CSF methylomic and proteomic data to enhance CNS tumor classification performance [23]. Although this integrative approach yielded optimal results for CNS lymphoma, improvements were modest for GBM and brain metastases, underscoring the need for further methodological refinement and optimization of multi-omics integration strategies.

Given the cost and technical complexity of such analyses, coordinated efforts among academic institutions, bioinformatics consortia, and clinical laboratories will be essential to enable scalable and clinically meaningful multi-omics profiling. Ultimately, these advances may facilitate the development of CSF-based “digital biopsies,” allowing real-time monitoring of therapeutic efficacy, early detection of relapse, and refined patient stratification for precision clinical trials.

5. Technical and clinical challenges

Despite encouraging results, several technical and clinical challenges currently limit the widespread implementation of CSF-based DNAm profiling. CSF can only be collected a limited number of times during a patient’s lifetime and the yield of tumor-DNA in CSF is typically low and depends on lesion size, proximity to CSF spaces, and prior therapy, which can influence detection rates and increase variability among patients.

At the same time, bisulfite treatment followed by next-generation sequencing (NGS), the gold standard method for DNAm evaluation, usually induces severe DNA damage, highlighting the need for optimized extraction methods and highly sensitive analytical workflows to increase the applicability in clinical practice For example, the use of the Illumina platform to perform low-pass whole-genome sequencing (LP-WGS) could enable a comprehensive molecular profiling of the primary tumor, reducing the required cfDNA input from nanogram to picogram levels as already demonstrated in CNS embryonal tumors [26].

In addition, standardization is urgently needed from pre-analytical handling to bioinformatics pipelines. Existing classifiers trained on tissue data may not fully capture the specific noise and fragmentation patterns of circulating DNA, necessitating tailored models for liquid biopsy samples.

Moreover, for recently defined entities such as high-grade astrocytoma with piloid features (HGAP) there are no open-source DNAm datasets available, which complicates the identification of these entities.

Clinically, questions remain regarding how to interpret discordant findings between CSF and tissue or how to integrate DNAm data with imaging, histopathological evaluation, and molecular results. Moreover, prospective validation in large, multicenter cohorts will be critical to establish the real clinical utility. Finally, ethical and logistical considerations, including pediatric sampling, storage, and data sharing, must be addressed to enable equitable implementation across healthcare systems.

6. Conclusion

CSF-based DNAm profiling represents a transformative step toward minimally invasive, accurate, and dynamic diagnosis of CNS tumors. By bridging histopathology and molecular precision, it offers clinicians a powerful tool to classify, monitor, and understand tumor biology across patient populations. In pediatrics, where CNS tumors account for a substantial proportion of cancer-related mortality, CSF-based DNAm profiling holds promise as a safe, robust, and informative diagnostic tool. The rapid evolution of methylation technologies and computational approaches are expected to make CSF-based diagnostics more accessible. Nanopore sequencing and single-molecule methylation calling may soon allow same-day results with minimal DNA input and explore the full spectrum of epigenetic changes [4,27,28]. Artificial intelligence – driven classifiers are being trained to interpret low-coverage data, offering robust predictions even from fragmented circulating DNA [29]. Future methodological innovations and clinical validations will unlock the full potential of CSF-based DNAm profiling, ushering in a new era of epigenetic diagnostics for both pediatric and adult neuro-oncology.

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Author contributions

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