Robust detection, classification, and monitoring of pediatric brain tumors from cell-free dna methylomes

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Abstract

Introduction

Pediatric brain tumors are the leading cause of cancer-related mortality in children, representing a heterogeneous group of malignancies. DNA methylation-based classification has revolutionized pediatric neuro-oncology, providing a robust molecular fingerprint for tumor diagnostics. However, current diagnostic pipelines rely on tissue specimens, which are often inaccessible due to high-risk tumor locations. Moreover, longitudinal tissue sampling is rarely feasible, limiting disease monitoring.

Liquid biopsies (LBs) offer a minimally invasive alternative, capturing circulating tumor DNA (ctDNA) in cerebrospinal fluid (CSF). While blood-based LBs have transformed systemic cancer diagnostics, CNS tumor detection via plasma remains challenging due to the blood-brain barrier. CSF, however, serves as a valuable reservoir of tumor-derived material.

Despite this potential, existing ctDNA-based assays require high input levels and often rely on copy number variations (CNVs) or targeted mutation detection, limiting their sensitivity. To address these challenges, we developed M-PACT (Methylation-based Predictive Algorithm for CNS Tumors), a cfDNA methylation-based classifier optimized for ultra-low-input CSF samples.

Methods

We applied enzymatic methylation sequencing (EM-seq) to cfDNA from 255 patients (n = 338 samples). Our workflow preserved genome-wide methylation data from picogram-level cfDNA inputs. A deep learning-based classifier was trained using CNS tumor and non-oncological reference datasets, incorporating methylation imputation and tumor deconvolution to enhance detection sensitivity in low tumor burden samples. Additionally, serial CSF samples were analyzed to assess the potential of M-PACT in tracking tumor evolution.

Results

M-PACT demonstrated high classification accuracy (AUC = 0.9) across diverse pediatric CNS tumors. It correctly identified tumors with balanced genomes undetectable by CNV-based methods and accurately distinguished between primary and secondary malignancies. Longitudinal analysis of serial CSF samples revealed its utility in tracking clonal evolution and might help to detect emerging resistance mechanisms early.

Conclusions

M-PACT provides a sensitive and specific cfDNA-based methylation classifier for pediatric CNS tumors, enabling non-invasive diagnostics and minimal residual disease (MRD) monitoring. Its ability to classify tumors independent of CNV burden and track disease progression positions it as a valuable tool for clinical implementation. Future studies will focus on prospective validation to further assess its utility in patient stratification and therapeutic monitoring.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.