PATH-04. OPTIMIZING CELL-FREE DNA ASSAYS FOR LOW INPUT LIQUID BIOPSY SAMPLES FROM PEDIATRIC BRAIN TUMOR PATIENTS

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BACKGROUND: Genetic and epigenetic profiling have transformed biological understanding, diagnostics, and therapeutic approaches for pediatric central nervous system (CNS) tumors. Obtaining tumor tissue may be associated with neurosurgical risk, may not be feasible in some cases, and is not routinely performed during follow-up or at relapse. Liquid biopsies (LBs) have emerged as a minimally invasive, serially collectable source of tumor-derived DNA. Here, we collected cerebrospinal fluid (CSF) and serum LBs from a large, unselected, cross-entity pediatric CNS tumor cohort (n=55 patients) for method optimization. METHODS: Cell-free DNA (cfDNA) isolated from CSF and serum samples was analyzed 1) by low-coverage whole genome sequencing for copy number variation (CNV)-based tumor detection and 2) by enzymatic conversion (EC)-based DNA methylation profiling using the EPIC DNA methylation array and the Heidelberg Brain Tumor Classifier. All library protocols were optimized for LB samples with low cfDNA amounts in the picogram range. Matched tumor samples were used as reference. RESULTS: Our wLGS-based cfDNA profiling protocol had a high technical success rate across the whole cohort (>95%), despite very low cfDNA amounts for most CSF samples. The majority of CSF LBs captured tumor-derived CNVs and displayed a high fraction of tumor-derived cfDNA (>5%, range 0-78%). For serum LBs, only few samples showed detectable CNVs. Proof-of-concept methylation analyses leveraging a new EC-based workflow allowed molecular classification using CSF, particularly for embryonal brain tumors (1/13 samples). Clinical workup of selected cases illustrates the potential of LBs to aid as diagnostic tool, monitor disease, and characterize tumor evolution. CONCLUSIONS: Tackling a major roadblock of clinical translation of LBs, this study delineates new methods tailored to low input cfDNA samples. Results highlight the clinical utility of LBs for the management of brain tumor patients, providing a strong rationale for prospective validation within pediatric neuro-oncology trials.