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Routine Diagnostic Medulloblastoma Subgrouping Using Low-Cost, Low-Input DNA Methylomics: Application to Trials Cohorts Previously Refractory-to-Analysis.

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Routine diagnostic medulloblastoma subgrouping using low-cost, low-input DNA methylomics: Application to trials cohorts previously refractory-to-analysis

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The subclassification of medulloblastoma into its four consensus molecular subgroups is rapidly informing disease treatment and risk-stratification. DNA-methylation microarrays have been essential for subgroup discovery and assignment in research studies, however their clinical utility is limited by expense, platform-specificity, sample number/quality requirements and practicality. Here, we aimed to (i) develop a rapid and robust, low-cost, array-independent, subgrouping assay suitable for routine diagnostic sub-classification of single samples, and (ii) assess its application to scant, poor-quality and historical samples, including the previously unassignable HIT-SIOP-PNET4 trial cohort of standard-risk medulloblastomas.

Using a cross-validated classification model, a minimal, multiply-redundant, 17-locus signature was derived to assign subgroup from 220 medulloblastomas profiled using Illumina 450K DNA-methylation arrays. We next adapted Agena's iPLEX assay to interrogate DNA methylation at these loci following bisulfite treatment. After *in-vitro* validation, the assay was applied to 101 independent DNA extracts from tumour material (fresh-frozen, FFPE and nuclear preparations (<30,000 nuclei)), representing all subgroups. Subgroup assignments from an optimised machine learning model were compared against gold-standard 450K calls for this validation cohort. 95/101 samples passed QC thresholds (technical, subgroup assignment confidence); all 95 recapitulated their corresponding 450K subgroup call. Assay turnaround (DNA extraction to result) was <5 days.

Following validation, subgrouping was attempted in 153 HIT-SIOP-PNET4 samples, previously unclassifiable due to sample limitations (e.g. scant FFPE material, nuclear preparations, <50ng dsDNA (>250ng required, 450K/EPIC array)). High-confidence calls were made for 70% (107/153) of samples. MB_{Group4} predominated (62/107) in this standard-risk cohort and subgroups showed predicted relationships to clinical, pathological and molecular demographics.

In summary, single medulloblastoma samples can be routinely, rapidly and robustly subgrouped using low-cost minimal DNA-methylation signatures and technologies (Agena) transferrable to many diagnostics laboratories. Importantly, the assay is applicable to limited and low-quality samples refractory to array-based analysis, allowing the retrospective unlocking of subgroup status in historical trials cohorts.

Resources:
Online classifier: insert address