Routine molecular subgrouping of medulloblastoma: Bridging the divide between research and the clinic using low-cost DNA methylomics

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Background

DNA-methylation patterns allow the subclassification of medulloblastoma, the most common childhood malignant brain tumour, into four molecular subgroups (WNT, SHH, MBGrp3 and MBGrp4). These subgroups have distinct molecular and clinico-pathological features, and their distinction is now informing future treatments and risk-stratification. Whilst microarrays to assign subgroup are suitable for research purposes, they are limited by expense, platform-specificity, sample quality requirements and practicality. Here, we aimed to develop a low-cost, array-independent, robust subgrouping assay suitable for routine quality-controlled subclassification, including scant and poor-quality samples.

Methods

A minimal, multiply-redundant, 19-locus methylation signature was derived to assign subgroup, using Illumina 450k DNA-methylation array data and subgroup calls from 225 medulloblastomas. A cross-validated machine-learning classifier was developed to assign subgroup using these loci. We next investigated whether bisulfite treatment of DNA could induce methylation-dependent SNPs suitable for multiplexed interrogation of methylation status, using an adaptation of Sequenom's iPlex assay. Multiplexed primer-mixes were designed and quantitation validated using molar-ratios of bisulfite-treated methylated:unmethylated DNA. Subsequently, the assay was run on 101 DNA extracts from fresh-frozen, FFPE and cytospin (<30,000 nuclei) tumour material, representing all subgroups. Subgroup assignments by Sequenom assay were compared to gold standard 450k array calls.

Results

Validation using molar-ratios of methylated:unmethylated DNA demonstrated close concordance between methylation-ratios and Sequenom methylation estimates at all loci. Subsequently, 101/101 (99/101 with high confidence) medulloblastomas tested were assigned to the same subgroup by both Sequenom and 450k assays.

Conclusions

Medulloblastomas can be routinely subgrouped using minimal DNA-methylation signatures. The assay is suitable for reliable, robust subgroup assignation from poor-quality, degraded samples using 100ng of DNA. The assay's low-cost, rapidity (3 days from extraction to result) and application to single samples demonstrate its potential for routine use. This first demonstration of multiplexed, methylation-based Sequenom subtyping holds rich promise for future molecular subclassification and prognostication across diverse tumour types.