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

Schwalbe, E.C.1,2, Hicks, D.1, Gohike, H.1, Rafiee, G.1, Enshaei, A.1, Potluri, S.1, Matthiesen, J.1, Mather, M.1, Chaston, R.1, Crosier, S.1, Smith, A.J.1, Williamson, D.1, Bailey, S.1, Clifford, S.C.1

1 Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, U.K.
2 Northumbria University, Newcastle upon Tyne, U.K.
3 Sequenom, Hamburg, Germany
4 NewGene, Newcastle upon Tyne, U.K.

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.