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Letter to the Editor

Satisfying your neuro-oncologist: a fast approach to routine molecular glioma diagnostics

Diffuse gliomas are the most common primary brain tumors in adults. Discrimination between astrocytic and oligodendroglial lineage differentiation has been subject to substantial intra- and interobserver variability, impairing prognostic and predictive stratification. Therefore, the 2016 WHO Classification of Tumors of the Central Nervous System introduced molecular markers, notably isocitrate dehydrogenase (IDH) mutation and loss of heterozygosity 1p/19q status, to reduce diagnostic bias.[1] Nevertheless, standardized protocols for diagnostic (eg, IDH1/2 mutation, loss of heterozygosity 1p/19q) and predictive markers (eg, O^6-methylguanine-DNA methyltransferase [MGMT] promoter methylation) are lacking. In addition, practical implementation of routine molecular workup of these tumors may be compromised by impractically long turnaround times and economic restraints, including lack of required equipment.

To overcome these shortcomings, we established a fast, robust, and easy-to-implement strategy for routine molecular diagnostics of diffuse gliomas by integrating two techniques, multiplex ligation-dependent probe amplification (MLPA P088; IDH mutations, 1p/19q, cyclin-dependent kinase inhibitor [CDKN]2a/b) and MGMT promoter bisulfite sequencing (PyroMark), and compared results with DNA methylation/copy number variation (CNV) profiling.[2]

DNA extracts were prepared from cryosections or formalin-fixed paraffin embedded blocks (Maxwell DNA FFPE kit, Promega) and concentration was measured spectrometrically (Nanodrop, Thermo Fisher). Typically, 125 ng DNA were used for MLPA[3] performed on a robotic thermocycler system (RoboAmp 4200, ALS Jena) using oil seals (LCS, Ventana). MLPA reaction products were examined by capillary gel electrophoresis (ABI GA 3500) and quantified by Coffalyser (MRC Holland). For MGMT promoter analysis, at least 100 ng of DNA were bisulfite converted (EpiTect Kit, Qiagen) and sequenced by PyroMark Q24 (Qiagen, 6 cytosine-phosphate-guanine [CpG] islands; primers: MGMT_F:GTTTYGGATATGTTGGGATAGT, MGMT_R:AATAAAAACRCCTACAAAACCACTC, MGMT_Rn:biotin–AAAACCACTCRAAACTACCACC, MGMT_seq:GGATATGTTGGGATAGTT); averages <10% were considered unmethylated.[4] For methylome analysis, 500 ng DNA were bisulfite converted and tested using the Infinium Methylation EPIC Microarray (Illumina), and data were analyzed by BrainTumor Methylation Classifier v11b4.[2]

Our MLPA validation set comprised 3 nonneoplastic brain tissue references and 8 tumors previously examined by IDH sequencing, 1p/19q fluorescence in situ hybridization (FISH), and OncoScan (Affymetrix): 2 glioblastomas, IDH-wildtype; 4 diffuse astrocytomas, IDH-mutant; 1 oligodendroglioma, IDH-mutant–1p/19q codeleted; and 1 lung adenocarcinoma brain metastasis, KRAS[p.G12A]. All CNVs were confirmed with OncoScan matched MLPA P088 data. With the exception of IDH1[p.R132L], which is not covered and therefore not detectable by P088, there was complete concordance of MLPA to sequencing and FISH. In 3 of 8 cases analyzed by the OncoScan assay, point mutations were missed: 1xTP53[p.R273H], 1xIDH1[p.R132H], and falsely identified IDH1[p.R132L] as IDH1[p.R132H]; therefore, this array is no longer included in our diagnostic workup.

Overall match between fast assays MLPA/PyroMark and BrainTumor Methylation Classifier in consecutive brain tumor biopsies was high (Fig. 1A). IDH mutations and CNVs (1p/19q; CDKN2a/b[5]) were reliably detected by MLPA. MGMT status mainly exhibited threshold-related and CpG island–specific discrepancies (Fig. 1B).

Whereas applicability of the fast tests is restricted by the fact that only 4 common IDH1/2 mutations are detectable (MLPA P088), and may also be compromised by low tumor cell content (PyroMark), methylomics detected IDH mutant gliomas even in samples with <30% tumor cellularity, while requiring longer turnaround times (Fig. 1C–E).

Based on our results, we advocate a stepwise approach, providing fast-track results obtained by MLPA/PyroMark for first-line therapy decisions within a week after surgery. Whenever possible, methylome profiling should be initiated simultaneously to independently secure diagnosis, detect rare entities (eg, diffuse midline gliomas), and identify potential therapeutic targets (eg, epidermal growth factor receptor amplification).

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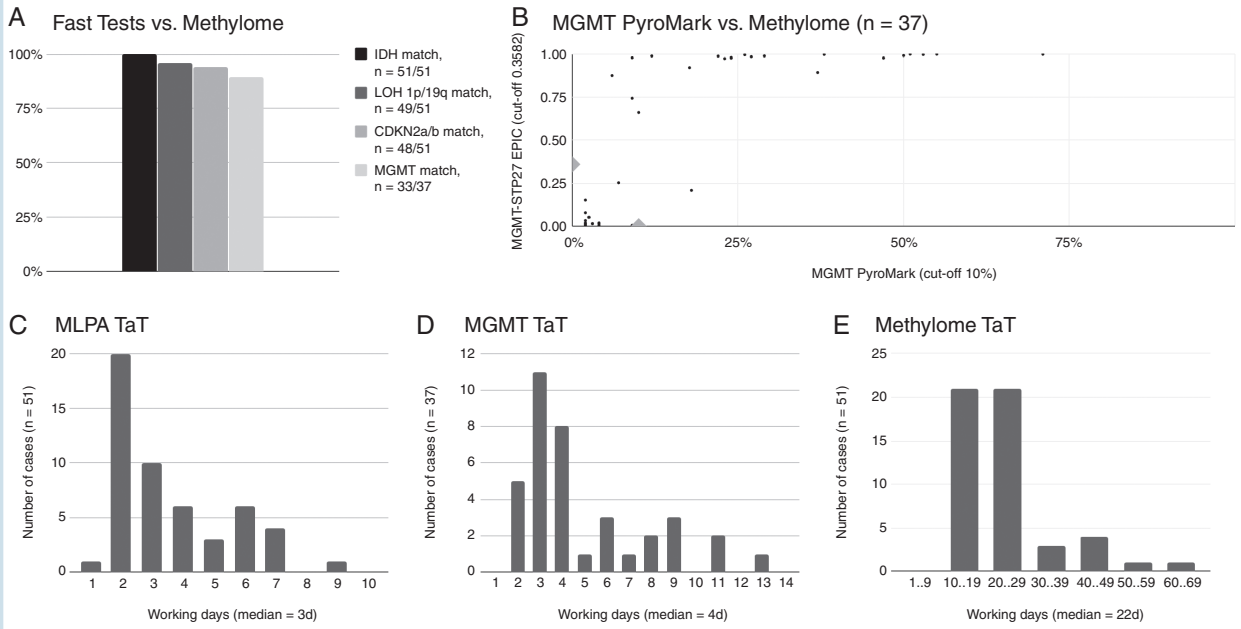

Fig. 1 (A) A cohort of 51 consecutive routine diagnostic samples examined with the fast tests (MLPA/PyroMark) and verified by Brain Tumor Methylation Classifier, delivering tumor type, CNVs, and MGMT promoter methylation status. (B) Comparison of MGMT promoter methylation values as determined by PyroMark and EPIC methylome array. Both techniques provide sufficient stratification based on published diagnostic cut-offs.[2,4] (C–E) Turnaround times (TaT) calculated from diagnostic reports and plotted as histograms, counting from the day of surgery. DNA extraction follows overnight proteinase K tissue dissection. Robotic MLPA requires 19 h 35 min walk-away and approximately 10 min hands-on time due to automated master mix preparation. PyroMark bisulfite sequencing requires an additional overnight procedure compared with robotic MLPA. For economic reasons, methylation profiling requires parallel analysis of 16 specimens per microarray; actual processing time is 3 working days.

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