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Validation of Real-Time Methylation-Specific PCR to Determine O⁶-Methylguanine-DNA Methyltransferase Gene Promoter Methylation in Glioma

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Epigenetic silencing of the DNA repair protein O⁶-methylguanine-DNA methyltransferase (MGMT) by promoter methylation predicts successful alkylating agent therapy, such as with temozolomide, in glioblastoma patients. Stratified therapy assignment of patients in prospective clinical trials according to tumor MGMT status requires a standardized diagnostic test, suitable for high-throughput analysis of small amounts of formalin-fixed, paraffin-embedded tumor tissue. A direct, real-time methylation-specific PCR (MSP) assay was developed to determine methylation status of the *MGMT* gene promoter. Assay specificity was obtained by selective amplification of methylated DNA sequences of sodium bisulfite-modified DNA. The copy number of the methylated *MGMT* promoter, normalized to the β -*actin* gene, provides a quantitative test result. We analyzed 134 clinical glioma samples, comparing the new test with the previously validated nested gel-based MSP assay, which yields a binary readout. A cut-off value for the *MGMT* methylation status was suggested by fitting a bimodal normal mixture model to the real-time results, supporting the hypothesis that there are two distinct populations within the test samples. Comparison of the tests showed high concordance of the results (82/91 [90%]; Cohen's kappa = 0.80; 95% confidence interval, 0.82–0.95). The direct, real-time MSP assay was highly reproducible (Pearson correlation 0.996) and showed valid test results for 93% (125/134) of samples compared with 75% (94/125) for the nested, gel-based MSP assay. This high-throughput test provides an important pharmacogenomic tool for individualized management of alkylating agent chemotherapy.

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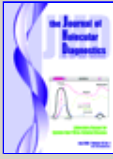
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