MGMT PROMOTER METHYLATION DETECTION USING ENTROGEN METHYLATION-SPECIFIC REAL-TIME PCR

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INTRODUCTION

- *MGMT* promoter methylation status is a critical prognostic and predictive biomarker in high grade gliomas particularly glioblastoma, *IDH*-wildtype.
- Methylated *MGMT* promoter is associated with favorable response to alkylating agents, such as temozolomide.
- *MGMT* promoter contains two differentially methylated regions, DMR1 and DMR2. DMR2 spans CpG sites 73-90 and DMR2 methylation status is strongly associated with epigenetic silencing of *MGMT*.
- Timely reporting allows appropriate clinical decision making and is critical for clinical trial enrollment.
- Here, we report the implementation of a methylation-specific real-time PCR assay to detect the methylation status of the *MGMT* DMR2 promoter region at our institution.

MATERIALS AND METHODS

- DNA was extracted from formalin-fixed paraffin-embedded glioma tissue from 59 cases with known *MGMT* promoter methylation status determined by previous outside testing at reference or academic clinical laboratories (methylated n=33, unmethylated n=26).
- Real-time PCR was performed on bisulfite-treated DNA using fluorescent hydrolysis probes specific to methylated CpG sites 75-86 using the MGMTX-RT44 kit from Entrogen (9 runs, 2 medical technologists).
- Percent methylation is calculated in reference to the internal actin B quantity and normalized using a standard 5% methylation control.
- Accuracy, precision, sensitivity, specificity, lower limit of detection, and reportable range were determined.
- Turnaround time was defined as the interval between date of surgery (DOS) and *MGMT* result reporting. Only cases for which testing was ordered within 12 days after the DOS were included.

RESULTS

Figure 1: Validation results and quantitative cut-off value

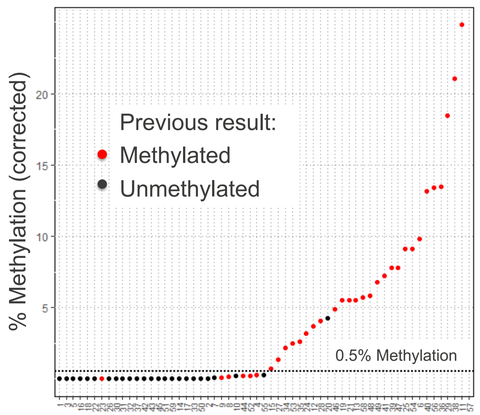

Figure 2: Detection of samples with low-level MGMT promoter methylation

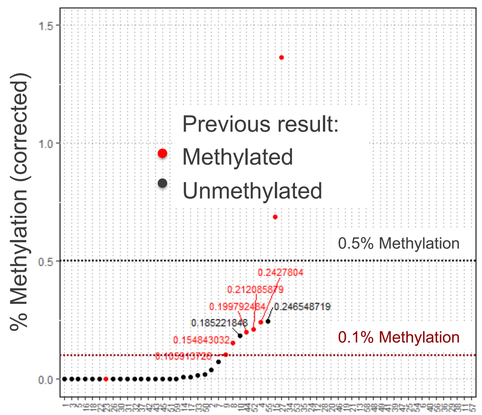

Figure 3: In-house testing expedited turnaround time

- The results demonstrated the optimal cut-off value of 0.5% to distinguish between tumors with methylated (red) and unmethylated (black) *MGMT* promoter (Figure 1). Using a quantitative cut-off value of 0.5%, the analytical accuracy was 84.5%.
- Several samples (n=7) demonstrated methylation levels slightly below the 0.5% cut-off value, between 0.1% and 0.5% (Figure 2), suggesting that these samples harbor low-level *MGMT* promoter methylation, as previously reported in the literature. Note that these samples were previously reported as either methylated (n=5, 71.4%) or unmethylated (n=2, 28.6%) by reference laboratories. When a cut-off value of 0.1% methylation was used, the analytical accuracy of the assay was 93.1%. For reporting purposes, cases with methylation values more than 0.1% but less than 0.5% were reported as “indeterminate”.
- Additional analytical statistics for the current assay using a cut-off value of 0.5% methylation are as follows: precision - 96.4%, sensitivity - 96.4%, and specificity - 95.6%.
- In-house testing significantly improved turnaround time (Figure 3). A Mann-Whitney U test indicated that the median turnaround time (between DOS and date of report) of the in-house assay (8 days ± 2) was significantly faster compared to send-out tests (12 days ± 3) (***p*** < 0.0001)

CONCLUSIONS

- This *MGMT* promoter methylation validation demonstrated high concordance between the current assay and previous results.
- Implementing the *MGMT* promoter methylation testing in our laboratory significantly decreased the turnaround time between sample collection and results reporting.
- The advertised turnaround time of this assay (from sample receipt in our lab to report date) is 14 days, although results can be reported as soon as 2-3 days after specimen receipt, expediting treatment decisions and clinical trial enrollment.
- Results are reported categorically based on the corrected percent methylated as methylated (≥ 0.5%), unmethylated (<0.1%), or indeterminate (0.1%-<0.5%), the latter suggesting the presence of low-level methylation.
- Cases with discordant methylation status between the current assay and previous results were likely due to differences in methodologies and interrogated CpG sites.

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