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METHYLATION ANALYSIS OF MGMT PROMOTER USING A BENCH-TOP LONG-READ SEQUENCER

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Background: Glioblastoma, IDH-wildtype (GBM), is associated with an extremely poor prognosis. Temozolomide (TMZ) is administered concurrently with radiotherapy and as maintenance therapy. The therapeutic efficacy of TMZ depends on the expression of MGMT (O⁶-methylguanine-DNA methyltransferase), a DNA repair enzyme, and is regulated by the methylation status of the MGMT promoter region. Recently, single nucleotide variants have been successfully analyzed using long-read sequencing in clinical settings. In this paper, we investigated the clinical applicability of MGMT methylation analysis using amplicon sequencing of bisulfite-treated DNA. **Methods:** Three surgically resected GBM specimens from Kyorin University Hospital were analyzed, all of which had previously undergone MGMT methylation analysis by pyrosequencing using frozen tissue. Bisulfite-treated DNA was PCR-amplified using the corresponding primer set from the pyrosequencing protocol. Library preparation was performed using the Rapid Sequencing DNA V14-Barcoding Kit [SQK-RBK114. 24] (Oxford Nanopore Technologies). Sequencing was conducted using a Flongle flow cell (ONT), and methylation status was determined based on nucleotide sequences at CpG sites within the amplicons. **Results:** The amplicons contained 16 CpG sites. Sequencing results indicated that cytosine (C) represented methylated sites, while thymine (T) indicated unmethylated sites. The average proportions at CpG sites were as follows: Case 1 - C 0%, T 90%; Case 2 - C 21%, T 60%; Case 3 - C 45%, T 23%. These results suggested unmethylated, methylated, and methylated MGMT promoter status, respectively, consistent with the pyrosequencing findings. **Discussion:** Amplicon sequencing of the MGMT promoter region was successfully performed using bisulfite-treated DNA and a benchtop long-read sequencer. Although read evaluation was performed manually in this study, future implementation of alignment tools capable of accommodating the relatively high error rate of nanopore sequencing will be essential for large-scale analyses.