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LABATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

Evaluation of MGMT promoter methylation status and correlation with temozolomide response in orthotopic glioblastoma xenograft model

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Abstract CpG methylation within the *O*6-methylguanine-DNA-methyltransferase (MGMT) promoter is associated with enhanced survival of glioblastoma multiforme (GBM) patients treated with temozolomide (TMZ). Although MGMT promoter is methylated in ~50% of GBM, several studies have reported a lack of correlation between MGMT methylation and protein expression levels and consequently inaccurate discrimination of TMZ sensitive and resistant patients. To understand the limitations of currently used assays, TMZ responsiveness of 13 GBM xenograft lines was correlated with MGMT protein expression and MGMT promoter methylation determined by (1) standard methylation-specific polymerase chain reaction (MS–PCR), (2) quantitative MS–PCR (qMS–PCR), and (3) bisulfite sequencing. For each xenograft line, mice with established intracranial xenografts were treated with vehicle control or TMZ (66 mg/kg × 5 days), and TMZ response was defined as relative prolongation in median survival for TMZ-treated versus control-treated mice. The relative survival benefit with TMZ was inversely related to MGMT protein expression ($r = -0.75$; $P = 0.003$) and directly correlated with qMS–PCR ($r = 0.72$; $P = 0.006$). There was a direct correlation between MGMT methylation signal by qMS–PCR and the number of methylated CpG sites within the region amplified by MS–PCR ($r = 0.78$, $P = 0.002$). However, bisulfite sequencing revealed heterogeneity in the extent of CpG methylation in those tumors with a robust qMS–PCR signal. Three of the 4 GBM lines with a qMS–PCR signal greater than 10% had at least 1 unmethylated CpG site, while only one line was fully methylated at all 12 CpG sites. These data highlight one potential limitation of the evaluation of MGMT methylation by MS–PCR assay and suggest that more detailed evaluation of methylation at individual CpG sites relative to TMZ response may be worth pursuing.

Keywords MGMT · Methylation · Glioblastoma · Orthotopic xenografts

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Introduction

Temozolomide (TMZ) chemotherapy combined with high-dose radiation therapy improves survival for a subset of patients with glioblastoma multiforme (GBM). Cytotoxic TMZ-induced *O*6-methylguanine lesions are repaired by the *O*6-methylguanine-DNA-methyltransferase (MGMT) protein, and cells lacking MGMT repair activity are significantly more sensitive to the cytotoxic effects of TMZ than cells with normal levels [1]. MGMT expression is