



Journal List &gt; PLoS One &gt; v.6(1); 2011

PLOS ONE PLoS One. 2011; 6(1): e16146.

PMCID: PMC3017549

Published online 2011 January 7. doi: 10.1371/journal.pone.0016146.

Copyright Shah et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Comprehensive Analysis of MGMT Promoter Methylation: Correlation with MGMT Expression and Clinical Response in GBM

Nameeta Shah,<sup>1</sup> Biaoyang Lin,<sup>1</sup> Zita Sibenaller,<sup>2</sup> Timothy Ryken,<sup>3</sup> Hwahyung Lee,<sup>1</sup> Jae-Geun Yoon,<sup>1</sup> Steven Rostad,<sup>4</sup> and Greg Foltz<sup>1\*</sup>

<sup>1</sup>The Ben and Catherine Ivy Center for Advanced Brain Tumor Treatment, Swedish Neuroscience Institute, Seattle, Washington, United States of America

<sup>2</sup>Department of Radiation and Oncology, University of Iowa Carver College of Medicine, Iowa City, Iowa, United States of America

<sup>3</sup>Iowa Spine and Brain Institute, Waterloo, Iowa, United States of America

<sup>4</sup>Cellnetix Pathology and Laboratories, Seattle, Washington, United States of America

Chris Jones, *Editor*

Institute of Cancer Research, United Kingdom

\* E-mail: [greg.foltz@swedish.org](mailto:greg.foltz@swedish.org)

Conceived and designed the experiments: NS BL GF. Performed the experiments: HL JY. Analyzed the data: NS BL ZS SR GF.

Contributed reagents/materials/analysis tools: NS BL ZS TR SR GF. Wrote the paper: NS BL GF.

Received August 20, 2010; Accepted December 8, 2010.

### Abstract

O<sup>6</sup>-methylguanine DNA-methyltransferase (MGMT) promoter methylation has been identified as a potential prognostic marker for glioblastoma patients. The relationship between the exact site of promoter methylation and its effect on gene silencing, and the patient's subsequent response to therapy, is still being defined. The aim of this study was to comprehensively characterize cytosine-guanine (CpG) dinucleotide methylation across the entire MGMT promoter and to correlate individual CpG site methylation patterns to mRNA expression, protein expression, and progression-free survival. To best identify the specific MGMT promoter region most predictive of gene silencing and response to therapy, we determined the methylation status of all 97 CpG sites in the MGMT promoter in tumor samples from 70 GBM patients using quantitative bisulfite sequencing. We next identified the CpG site specific and regional methylation patterns most predictive of gene silencing and improved progression-free survival. Using this data, we propose a new classification scheme utilizing methylation data from across the entire promoter and show that an analysis based on this approach, which we call 3R classification, is predictive of progression-free survival (HR = 5.23, 95% CI [2.089–13.097], p<0.0001). To adapt this approach to the clinical setting, we used a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) test based on the 3R classification and show that this test is both feasible in the clinical setting and predictive of progression free survival (HR = 3.076, 95% CI [1.301–7.27], p = 0.007). We discuss the potential advantages of a test based on this promoter-wide analysis and compare it to the commonly used methylation-specific PCR test. Further prospective validation of these two methods in a large independent patient cohort will be needed to confirm the added value of promoter wide analysis of MGMT methylation in the clinical setting.

Articles from *PLoS ONE* are provided here courtesy of  
Public Library of Science