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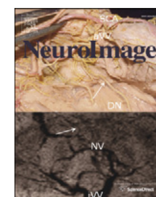

Anatomic localization of O6-methylguanine DNA methyltransferase (MGMT) promoter methylated and unmethylated tumors: A radiographic study in 358 de novo human glioblastomas ☆

Benjamin M. Ellingson [a,*], Timothy F. Cloughesy [b], Whitney B. Pope [a], Taryar M. Zaw [a], Heidi Phillips [c], Shadi Lalezari [b], Phioanh L. Nghiemphu [b], Hassana Ibrahim [a], Kourosh M. Naeini [a], Robert J. Harris [a], Albert Lai [b]

[a] Department of Radiological Sciences, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA
[b] Department of Neurology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA
[c] Department of Tumor Biology and Angiogenesis, Genentech, Inc., San Francisco, CA 94080, USA

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ABSTRACT

Promoter methylation of O6-methylguanine DNA methyltransferase (MGMT) is associated with a favorable prognosis in glioblastoma multiforme (GBM) and has been hypothesized to occur early in tumor transformation of glial cells. Thus, a possible link exists between the site of malignant transformation and MGMT promoter methylation status. Using the Analysis of Differential Involvement (ADIFFI) statistical mapping technique in a total of 358 patients with GBM, we demonstrate that human de novo GBMs occur in a high frequency contiguous with the posterior subventricular zone (SVZ); MGMT promoter methylated GBMs are lateralized to the left hemisphere, while MGMT unmethylated GBMs are lateralized to the right hemisphere; and tumors near the left temporal lobe have a significantly longer overall survival compared with tumors occurring elsewhere, independent of treatment or MGMT methylation status.

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Introduction

Glioblastoma multiforme (GBM) is the most common, and unfortunately also the most lethal primary brain neoplasm. Despite advances in surgery, radiation and drug therapy, the median survival remains relatively unchanged and ranges from 12 to 15 months (Stupp et al., 2005). Although long-term survival is almost universally poor, a handful of prognostic factors have been identified that confer modest difference in survivability. These prognostic factors include age, performance and neurological status, extent of surgical resection, degree of necrosis and enhancement on preoperative magnetic resonance (MR) imaging, adjuvant therapy received, and tumor location (Fontaine and Paquis, 2010; Gorlia et al., 2008; Lacroix et al., 2001).

Although controversial, previous studies support tumor location as playing a role in prognosis (Fontaine and Paquis, 2010; Simpson et al., 1993), likely due to the genetic profile of tumor precursor cells and the stage in the development cycle that these cells transform (i.e. the glioma "cell of origin") (Sanai et al., 2005). For example, an association between brain tumor location, growth pattern and tumor genetic signature has been shown with oligodendroliomas (Zlatescu et al., 2001). As explained in this study, different types of oligodendrogliomas may arise from different precursor cells that are relatively region-specific at inception or during brain development. In support of this hypothesis, germinal regions containing neural stem cells, including the subventricular zone (SVZ), have been proposed as a source for human gliomas (Globus and Kuhlenbeck, 1942). Also consistent with the hypothesis that tumor location reflects the contributions of specific precursor cells is the observation that medulloblastoma arises through abnormalities along a particular developmental pathway in a distinct population of progenitor cells (Marino et al., 2000; Pietsch et al., 1997). Additional evidence of isocitrate dehydrogenase 1 (IDH1) tumors originating from a distinct cell of origin giving rise to their predominant localization within the frontal lobe regions (Lai et al., in press) also supports this theory.

Abbreviations: GBM, glioblastoma multiforme; MGMT, O6-methylguanine DNA methyltransferase; SVZ, subventricular zone; ADIFFI, Analysis of Differential Involvement; MR/MRI, magnetic resonance imaging; IDH1, isocitrate dehydrogenase 1; TMZ, temozolomide; MSP, methylation-specific PCR.

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* Corresponding author at: Department of Radiological Sciences, David Geffen School of Medicine, University of California Los Angeles, 924 Westwood Blvd., Suite 615, Los Angeles, CA 90024. Fax: +1 310 794 8657.
E-mail address: bellingson@mednet.ucla.edu (B.M. Ellingson).

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MGMT promoter methylation and tumor localization

O6-methylguanine DNA methyltransferase (MGMT) promoter methylation is a favorable prognostic factor in patients treated with temozolomide (TMZ), a chemotherapeutic agent shown to prolong survival in patients with GBM (Stupp et al., 2005). The MGMT gene is located on chromosome 10q26 and encodes a DNA-repair protein, which removes alkyl groups from the O6 position of guanine. This is thought to be the same alkylation target of TMZ that triggers cytotoxicity. The MGMT gene is silenced by the methylation of its promoter, resulting in loss of MGMT expression and subsequently decreased DNA-repair activity. In other words, tumors with methylated MGMT promoter are more sensitive to TMZ treatment. These findings underscore the importance of MGMT promoter methylation status in prognosis and therapeutic guidance.

Topographic distribution of MGMT promoter methylated tumors is consistent with the hypothesis of a distinct "cell of origin". Specifically, MGMT promoter methylation is thought to occur as part of a genetic signature that develops from lower-grade gliomas (Eoli et al., 2007), and this transformation is thought to occur early in tumor development within glial cells predestined for specific locations (Drabycz et al., 2010). This appears plausible, especially in light of evidence supporting GBM development from neural stem cells (Nicolis, 2007) and the fact many gliomas are contiguous with the SVZ (Alvarez-Buylla and Garcia-Verdugo, 2002), known to harbor neural stem cells. Two recent studies examined the relationship between MGMT promoter methylation status and tumor location, but arrived at different conclusions. In a 2007 study by Eoli et al. (Eoli et al., 2007), MGMT promoter methylated tumors were found to occur more often in parietal and occipital lobes, whereas tumors without MGMT promoter methylation were more often in the temporal lobes. In a recent publication by Drabycz et al., (2010), no significant difference between the locations of MGMT methylated and unmethylated GBM tumors was found. Both these studies, however, were limited by relatively small sample sizes ($n=86$, 45 methylated, 41 unmethylated; and $n=72$, 36 methylated, 36 unmethylated patients, respectively).

Despite noting a general higher frequency in different regions of the brain, no tools have been developed for voxel-wise statistical comparison for testing tumor localization. The current study involves a new technique called Analysis of Differential Involvement (ADIFFI) maps and applies this technique to MGMT promoter methylated versus non-methylated tumors in order to determine whether these tumors are localized to a particular area of the brain more often than chance. In addition, the current study examined 358 patients, representing the largest and most comprehensive study examining radiological difference between MGMT methylated versus non-methylated GBMs.

Methods

Patients

All patients participating in this study signed institutional review board-approved informed consent to have their data collected and stored in our institution's neuro-oncology database. Data acquisition and storage were performed in compliance with all applicable Health Insurance Portability and Accountability Act (HIPAA) regulations. The study spanned April 2000 through March 2011. A total of $n=358$ patients with de novo GBM were enrolled in this retrospective study who met the following criteria: 1) pathology confirmed GBM with no previous history of primary CNS tumors, 2) pre-surgical T2/FLAIR images and/or post-contrast T1-weighted images, and 3) tissue available for testing MGMT promoter methylation status. Additional patient characteristics are summarized in Table 1.

Table 1
Patient characteristics. T2/FLAIR = number of patients with adequate T2-weighted or fluid-attenuated inversion recovery (FLAIR) images. T1 + C = number of patients with adequate post-contrast T1-weighted images. KPS = Karnofsky Performance Status. * = Standard Deviation.

Total	358
T2/FLAIR	353
Methylated	128
Unmethylated	225
T1 + C	323
Methylated	123
Unmethylated	200
Gender	
Male	222
Female	136
Age	$56.4 \pm 10.2^*$
KPS	$72.4 \pm 10.1^*$

MGMT methylation analysis

MGMT methylation analysis was performed by methylation-specific PCR (MSP) according to a previously published protocol (Hegi et al., 2005) with some slight modifications as described in another publication (Lai et al., 2010). To generate bisulfite modified DNA, genomic DNA isolated from formalin fixed, paraffin-embedded tissue using Recoverall Total Nucleic Acid Isolation Kit (Ambion, Austin, TX) was modified using the EZ DNA Methylation-Gold Kit (ZymoResearch, Orange,CA) following the manufacturer's protocol. Samples were subjected to a two-stage nested PCR strategy using: first-stage primers (5′-GGATATGTTGGGATAGTT-3′ and 5′-CCAAAAACCCCAAACCC-3′) and second-stage primers (unmethylated reaction: 5′-TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3′ and 5′-AACTCCACACTCTTCCAAAAACAAAACA-3′; methylated reaction: 5′-TTTCGACGTTCGTAGGTTTTCGC-3' and 5′-GCACTCTTCCGAAAACGAA-ACG-3′). PCR products were analyzed on 3% agarose gels. Positive and negative control samples for the MSP reaction were U87MG DNA treated with SssI methyltransferase (New England Biolabs, Ipswich, MA) and whole-genome amplification ofU87MGDNAusing the GenomiPhi V2 Amplification Kit (Amersham Biosciences, Piscataway, NJ), respectively.

Magnetic Resonance Imaging

Data was collected on either a 1.5 T (GE LX Echospeed or GE HDx Excite; General Electric Medical Systems, Waukesha, WI; Siemens Avanto TIM Class or Siemens Sonata Maestro Class; Siemens Medical Solutions, Erlangen, Germany) or 3.0 T (Siemens Trio TIM Class or Siemens Allegra TIM Class; Siemens Medical Solutions, Erlangen Germany) using pulse sequences supplied by the scanner manufacturer. Standard anatomical MRI sequences consisted of axial T1 weighted, T2-weighted fast spin-echo, and fluid attenuated inversion recovery (FLAIR). Additionally, gadopentate dimeglumine (Gd-DTPA, Magnevist®; Berlex, Wayne, NJ; 0.1 mmol/kg) or gadobenate dimeglumine (Gd-BOPTA, Multihance®; Bracco S.p.A., Milano, Italy; 0.1 mmol/kg) enhanced axial and coronal T1-weighted images (i.e. post-contrast, contrast-enhanced, T1-weighted images). Axial images were used for ADIFFI analysis, which consisted of slices 3–5 mm thick and 0–1 mm interslice gap. Echo and repetition times (TE and TR) for MR acquisition differed from scanner to scanner according to field strength and our specific clinical protocols.

Image registration

All images for each patient were registered to a high-resolution (1.0 mm isotropic), T1-weighted brain atlas (MNI152; Montreal Neurological Institute) using a mutual information algorithm and a 12-degree of freedom transformation using FSL (FMRIB, Oxford, UK; http://www.fmrib.ox.ac.uk/fsl/). Fine registration (1–2 and 1–2 voxels) was then performed using a Fourier transform-based, 6 degree

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