A Hypermethylated Phenotype Is a Better Predictor of Survival than MGMT Methylation in Anaplastic Oligodendroglial Brain Tumors: A Report from EORTC Study 26951

Abstract

**Purpose:** The MGMT promoter methylation status has been suggested to be predictive for outcome to temozolomide chemotherapy in patients with glioblastoma (GBM). Subsequent studies indicated that MGMT promoter methylation is a prognostic marker even in patients treated with radiotherapy alone, both in GBMs and in grade III gliomas.

**Experimental Design:** To help determine the molecular mechanism behind this prognostic effect, we have conducted genome-wide methylation profiling and determined the MGMT promoter methylation status, 1p19q LOH, IDH1 mutation status, and expression profile on a series of oligodendroglial tumors [anaplastic oligodendrogliomas (AOD) and anaplastic oligoastrocytomas (AOA)] within EORTC study 26951. The series was expanded with tumors of the same histology and treatment from our own archive.

**Results:** Methylation profiling identified two main subgroups of oligodendroglial brain tumors of which survival in the CpG island hypermethylation phenotype (CIMP+) subgroup was markedly better than the survival of the unmethylated (CIMP+/C0) subgroup (5.62 vs. 1.24 years; \(P < 0.0001\)). CIMP status correlated with survival, MGMT promoter methylation, 1p19q LOH, and IDH1 mutation status. CIMP status strongly increases the predictive accuracy of survival in a model including known clinical prognostic factors such as age and performance score. We validated our results on an independent data set from the Cancer Genome Atlas (TCGA).

**Conclusion:** The strong association between CIMP status and MGMT promoter methylation suggests that the MGMT promoter methylation status is part of a more general, prognostically favorable genome-wide methylation profile. Methylation profiling therefore may help identify AODs and AOAs with improved prognosis.

Introduction

Glioblastomas (GBM) are the most common and aggressive type of glial brain tumor (1, 2). GBMs in which the MGMT promoter is methylated show an improved survival when treated with temozolomide, a finding that has resulted in MGMT promoter methylation to serve as a predictive biomarker in GBMs (3, 4). The predictive effect of MGMT promoter methylation can be explained by a reduced ability to respond to DNA damage by alkylating agents for methylated tumors.

Two large randomized controlled trials that focused on patients with grade III glioma unexpectedly also revealed a prognostic effect of MGMT promoter methylation in patients treated with radiotherapy only (5, 6). Similarly, a retrospective survey in GBM suggests that MGMT promoter methylation also has prognostic significance in patients with GBM treated with radiotherapy only (7). The current understanding of the function of the MGMT protein does not explain the observed prognostic effect of MGMT promoter methylation in patients treated with radiotherapy only.

Methylation in cancer often occurs in the promoter regions of tumor suppressor genes (8, 9). Inactivation of
Translational Relevance

Histologic classification, combined with perceived clinical prognostic features, guides treatment decisions for patients with glioma. However, histologic classification of gliomas is difficult and subject to interobserver variation. In this study, we show that methylation profiling can identify prognostically favorable anaplastic oligodendrogliomas and anaplastic-mixed oligoastrocytomas. Prognostic markers identified in this study therefore will help classify gliomas and guide treatment decisions. Our results, obtained from samples treated within a large phase III clinical trial, also help explain the prognostic relevance of MGMT promoter methylation in this tumor type.

gene expression by promoter methylation thus contributes to tumor formation as the second "hit" in tumor suppressor genes [Knudson 2-hit hypothesis (10)]. Several groups have therefore conducted genome-wide methylation profiling in GBMs (11) and astrocytomas (12). Importantly, whole-genome methylation profiling on GBMs can identify a subset of tumors that have a more favorable prognosis (11, 13). These tumors show an overall increase in DNA methylation at CpG sites (CIMP: CpG island methylator phenotype; ref. 13). Later studies showed that histologic subtype was associated with methylation class and IDH1 mutation, and that GBMs generally show less overall CpG methylation than lower grade gliomas (14, 15). Identification of CpG methylation sites in gliomas may therefore identify genes involved in the initiation and/or progression of gliomas [see e.g., (16, 17)]. Moreover, an association between CIMP and MGMT promoter methylation may also provide an explanation for the prognostic effect of MGMT promoter methylation.

In this study, we conducted methylation profiling on a set of 68 anaplastic oligodendrogliomas (AOD) and anaplastic oligoastrocytomas (AOA) and correlated the results to molecular (MGMT methylation, IDH1 mutation, LOH on 1p and 19q, and gene expression profiles) and clinical [overall survival (OS)] parameters. Fifty of these tumors were treated as part of the EORTC 26951 clinical trial of which the primary objective was to see if the addition of adjuvant PCV chemotherapy immediately after radiotherapy (59.4 Gy) would improve OS in patients with AOD or AOA with at least 25% oligodendroglial elements according to the 1994 edition of the WHO classification of brain tumors; had at least 3 of 5 anaplastic characteristics (high cellularity, mitoses, nuclear abnormalities, endothelial proliferation, or necrosis); were between 16 and 70 years of age; had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; and had not undergone prior chemotherapy or RT to the skull. Patients provided written informed consent according to national and local regulations for the clinical study and correlative tissue studies. For details of the EORTC study 26951 see reference (18). Additional samples from the Erasmus MC archive were selected on the basis of similar inclusion criteria as the EORTC study 26951: Histologic diagnosis AOA or AOD, age 16 to 70, and patients having undergone similar treatment as the patients in EORTC 26951 (radiotherapy with or without chemotherapy) and with similar clinical characteristics. For research on these samples approval has been obtained from the Erasmus MC institutional review board. Sample collection time for non-EORTC 26951 samples is from 1989 to 2004. Mean follow-up time was defined as the period from date of surgery to date of death. Patients were censored at the date of last follow-up. Patients were eligible for EORTC study 26951 if they had been diagnosed by the local pathologist with AOD or AOA with at least 25% oligodendroglial elements according to the 1994 edition of the WHO classification of brain tumors; had at least 3 of 5 anaplastic characteristics (high cellularity, mitoses, nuclear abnormalities, endothelial proliferation, or necrosis); were between 16 and 70 years of age; had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; and had not undergone prior chemotherapy or RT to the skull. Patients provided written informed consent according to national and local regulations for the clinical study and correlative tissue studies. For details of the EORTC study 26951 see reference (18). Additional samples from the Erasmus MC archive were selected on the basis of similar inclusion criteria as the EORTC study 26951: Histologic diagnosis AOA or AOD, age 16 to 70, and patients having undergone similar treatment as the patients in EORTC 26951 (radiotherapy with or without chemotherapy) and with similar clinical characteristics. For research on these samples approval has been obtained from the Erasmus MC institutional review board. Sample collection time for EORTC 26951 samples is from 1998 to 2002. Sample collection time for non-EORTC 26951 samples is from 1989 to 2004. Mean follow-up time for all samples is 96.4 months. Survival data are collected till death or, for censored patients, either until lost to follow-up or to date.

Nucleic acid isolation, cDNA synthesis, and array hybridization

Genomic DNA was isolated from 5 to 40 cryostat sections of 40 μm thickness using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Quality was assessed on agarose gel electrophoresis, in which a dominant high molecular weight DNA species should be present. For methylation profiling, 1 μg of genomic DNA was subjected to bisulphite modification using the EZ DNA methylation kit (Zymo research company). Bisulphite-converted DNA was then hybridized to Illumina Infinium HumanMethylation27 arrays (Illumina) by

Materials and Methods

Samples

Glioma samples were collected from EORTC study 26951 (n = 50) or the Erasmus MC tumor archive (n = 18). One additional control sample (normal adult brain) was obtained from the Dutch Brain Bank. Samples were collected immediately after surgical resection, snap frozen, and stored at −80°C. Clinical data are summarized in Supplementary Table S1. The number of samples chosen was based on the availability of 50 fresh frozen samples from the EORTC study 26951. Additional samples with similar clinical and treatment characteristics were included to increase power of the study whilst maintaining the predominance of EORTC 26951 samples. Part of the molecular data of the EORTC 26951 samples was previously reported (5). Survival time was defined as the period from date of surgery to date of death. Patients were censored at the date of last follow-up. Patients were eligible for EORTC study 26951 if they had been diagnosed by the local pathologist with AOD or AOA with at least 25% oligodendroglial elements according to the 1994 edition of the WHO classification of brain tumors; had at least 3 of 5 anaplastic characteristics (high cellularity, mitoses, nuclear abnormalities, endothelial proliferation, or necrosis); were between 16 and 70 years of age; had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; and had not undergone prior chemotherapy or RT to the skull. Patients provided written informed consent according to national and local regulations for the clinical study and correlative tissue studies. For details of the EORTC study 26951 see reference (18). Additional samples from the Erasmus MC archive were selected on the basis of similar inclusion criteria as the EORTC study 26951: Histologic diagnosis AOA or AOD, age 16 to 70, and patients having undergone similar treatment as the patients in EORTC 26951 (radiotherapy with or without chemotherapy) and with similar clinical characteristics. For research on these samples approval has been obtained from the Erasmus MC institutional review board. Sample collection time for EORTC 26951 samples is from 1998 to 2002. Sample collection time for non-EORTC 26951 samples is from 1989 to 2004. Mean follow-up time for all samples is 96.4 months. Survival data are collected till death or, for censored patients, either until lost to follow-up or to date.

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Service XS (Leiden) according to standard Illumina protocols. Infinium HumanMethylation27 arrays interrogate 27,578 CpG sites across 14,476 genes. Robustness of sample processing was assessed using 2 biological replicates, and resulted in replicates clustering very tightly to each other. These replicates were not included in any analysis. Data are available on request.

Unsupervised clustering analysis

Clustering was conducted using the hierarchical ordered partitioning and collapsing hybrid (HOPACH) algorithm (20). This clustering was used to identify molecular subgroups in gliomas on 2,000 CpG sites with highest variance. Virtually identical clusters were identified using all or the 500, 1,000, 5,000, or 10,000 CpG sites with highest variance. Nonparametric bootstrapping was used to estimate the probability that each sample belongs to a cluster (i.e., fuzzy clustering) and thus determine cluster stability. Samples were assigned to a cluster when at least 50% of bootstrap samples allocated the sample to that specific cluster.

Cluster validation was conducted by representing each of the molecular clusters in our data set by its centroid and classifying external samples to their nearest centroid. Samples belonging to the original data set that were not assigned to one of the 2 large clusters were similarly assigned to the nearest centroid. Robustness of external validation was estimated with the in-group proportion cluster quality measure (21).

Molecular analysis

The IDH1 mutation status was determined by direct sequencing as described (22, 23). 1p19q LOH was determined previously by FISH (19). Probes used were D18S32 (for 1p36), pUC1.77 (for centromere 1), equivalent amounts of bacterial artificial chromosome (BAC) RPCI 11-959O6, 11-95711, and 11-153P24 (for 19p), and BAC 426G3 (for 19q). Expression profiles were determined previously (20). The MGMT methylation status was determined on the snap-frozen DNA used for methylation profiling, using the methylation-specific multiplex ligation–dependent probe amplification (MS-MLPA) assay (ME011, MRC Holland) essentially as described (24). This assay uses a methylation-sensitive restriction enzyme (HhaI) and contains 3 probes within the MGMT promoter/gene. PCR products were separated by capillary gel electrophoresis (ABI Prism 3130 ×; Applied Biosystems) and quantified by use of the GeneMarker Software version 1.7 (SoftGenetics).

The MS-MLPA results were normalized by dividing the peak height for each MGMT probe signal by the mean peak height for 8 control fragments within the same sample. The fraction of methylated MGMT promoter DNA was calculated by normalized values of each probe of HhaI-digested DNA divided by normalized values of corresponding undigested DNAs (24). A sample was considered to have a methylated MGMT promoter when the average fraction of the 3 MGMT probes was more than 0.25.

Statistical analysis

Spearman correlation coefficient was used to assess the strength of the association between parameters. The Fisher exact test was used in the comparisons of patient and disease characteristics between subgroups. The Kaplan–Meier technique was used to assess OS and the log-rank test was computed to compare the survival of subgroups. The Cox regression was used for multivariate OS analysis including age, performance status, IDH1 mutation status, 1p19q LOH, MGMT methylation status, and CIMP. The forward stepwise method was used to select variables with independent prognostic value.

The models’ predictive accuracy was assessed by the Harrel c-index. The c-index is the probability that for 2 patients chosen at random, the patient who had the event first had a higher probability of having an event according to the model. C-index equal to 0.50 represents agreement by chance; c-index = 1.0 represents perfect discrimination. In the absence of an independent data set, the bootstrap technique was used to obtain a c-index corrected for optimism, that is, the model overfitting the data. No adjustment for multiple testing was conducted in this exploratory analysis. The power of multivariate analyses was limited and P values were presented to point out what the main effects or differences are, but not to provide definitive conclusions. The descriptive comparisons of the c-index among different multivariate models allow assessing the relative contribution of CIMP to the classification of patients for their survival. SAS version 9.2 (SAS Institute Inc.) was used for all statistical analyses except the computation of the c-index which was conducted with R software.

Results

Samples

Methylation profiling was conducted on a total of 68 gliomas and 1 control brain. Two biological replicates (i.e., experiments carried out in parallel on independently isolated DNA) were also included. Patients and tumor characteristics are summarized in Table 1, and detailed in Supplementary Table S1. All glioma samples were diagnosed either with AOD or AOA by an expert neuropathologist (J.M. Kros). Fifty of the glioma samples were treated as part of the EORTC study 26951 (18), the remaining 18 samples were treated within the Erasmus Medical Centre. Sixteen of these patients received treatment paradigms similar to that used in the EORTC study 26951 (14 radiation therapy and 2 chemoradiation). One patient received PCV chemotherapy only. One patient refused further treatment after surgical resection; this sample was omitted from any analysis involving clinical parameters.

Our cohort of samples both from EORTC 26951 and Erasmus Medical Centre did not differ from the entire EORTC 26951 cohort (368 patients) with respect to age, performance status, sex, diagnosis (AOD or AOA), IDH1 mutation, 1p19q LOH, and OS (Supplementary Table S2).
MGMT promoter methylation was slightly less frequently observed (63.5% vs. 78.7%) in the present series than the entire EORTC data set.

Methylation profiling

A frequency distribution of the percentage of CpG methylation across our sample cohort shows an approximately bimodal distribution: predominantly unmethylated CpG sites (by far the most frequently observed) and predominantly methylated CpG sites (Supplementary Fig. S1). Few CpG sites show an intermediate percentage of methylation (between 10% and 75%). Unsupervised hierarchical clustering (HOPACH) using the 2,000 most variably methylated CpG loci identified 14 distinct molecular methylation subtypes (list available on request). Virtually identical subgroups were identified using all CpG loci, or the most variable 500, 1,000, 5,000, or 10,000 CpG loci (data not shown). Nonparametric bootstrapping (Fuzzy clustering) confirmed that samples indeed belonged to a defined methylation cluster.

We then focused our analysis on the 2 largest clusters that contain more than 10 samples and reassigned all samples to one of these 2 clusters. Cluster stability was shown as only 2 samples (samples 29 and 54) were assigned to a cluster that was different from the original cluster. The 2 large subgroups are defined by either predominantly methylated or unmethylated CpG islands (Fig. 1). Tumors with a high frequency of CpG island methylation are further referred to as CIMP⁺ tumors.

CIMP status was strongly correlated to other molecular markers such as IDH1 mutation (rho = 0.75; P < 0.001), 1p19q LOH (rho = 0.54; P < 0.001), and MGMT promoter methylation (rho = 0.61; P < 0.001; see also Supplementary Table S3). CIMP status was not correlated to clinical parameters such as age (rho = −0.06; P = 0.65), sex (rho = 0.23; P = 0.064), performance status (rho = 0.0008; P = 0.99), or histologic diagnosis (rho = −0.01; P = 0.91).

Table 1. Summary of patient characteristics

<table>
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<th>Current study</th>
<th>EORTC 26951 not included in present study</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>68</td>
<td>318</td>
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<tr>
<td>Age (years)</td>
<td>50 (17–70)</td>
<td>49 (16–68)</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>58.8</td>
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<td>IDH1 mutation</td>
<td>46.1%</td>
<td>48.3%</td>
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<tr>
<td>1p19q LOH</td>
<td>23.5%</td>
<td>26.3%</td>
</tr>
<tr>
<td>MGMT methylated</td>
<td>63.5%</td>
<td>78.7%</td>
</tr>
<tr>
<td>Diagnosis (% AOD)</td>
<td>73.5</td>
<td>71.8</td>
</tr>
<tr>
<td>Performance status (%)</td>
<td>36.8</td>
<td>36.8</td>
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Figure 1. Unsupervised clustering based on the 2,000 most variably methylated CpG loci identifies 2 subgroups of gliomas. Subgroups can be separated on the basis of predominantly unmethylated or methylated CpG loci (rows) methylation is color coded from blue ( unmethylated) to yellow (hypermethylated). Sample (columns) numbers are shown above the graph. CIMP status as determined by HOPACH clustering (red, CIMP⁺; green, CIMP⁻). MGMT methylation status (red, MGMT methylated; green, unmethylated; white, not determined). 1p19qLOH (red, LOH; green, retention of either or both arms), and IDH1 mutation status (red, mutated; green, wild-type) is shown below the figure. A second bar below the figure indicates sex (blue, males; pink, females), diagnosis (red, anaplastic oligoastrocytoma; green, anaplastic oligodendroglioma), and molecular cluster based on gene expression profiling (dark red, cl22; red, cl18; orange, cl23; yellow, cl17; green, cl9). * genes associated with gender (see Supplementary Fig. S4). All of the gender-associated CpG sites (89 probes) are methylated in females and unmethylated in males, are located on the X chromosome, and show approximately 50% methylation per CpG site (Supplementary Fig. S4). Location and percentage methylation of these CpG sites is consistent with X chromosome inactivation by methylation in females (9, 45).
Univariate analysis indicates that CIMP status \( [P < 0.0001; \text{HR} = 0.23 (0.12, 0.43)] \) is a strong prognostic factor for OS (Fig. 2). Other prognostic factors include IDH1 mutation status \([P < 0.0001; \text{HR} = 0.30 (0.16, 0.57)]\), 1p19q LOH \([P = 0.004; \text{HR} = 0.34 (0.16, 0.74)]\), MGMT promoter methylation \([P = 0.016; 0.49 (0.27, 0.89)]\), and performance status \([P = 0.032; \text{HR} = 1.55 (1.04, 2.33)]\). In this series, age \((P = 0.199)\) and histopathologic diagnosis \((P = 0.67)\) are not correlated to OS.

When stratified into the different treatment arms, CIMP+ tumors showed an increase in OS in RT-PCV–treated tumors compared with RT only (7.53 vs. 4.33 years, see also Supplementary Fig. S2). This increase in OS was, however, not significant \((P = 0.45)\). No difference in OS can be observed in CIMP– tumors (1.0 vs. 1.21 years). Numbers are, however, too low to draw firm conclusions.

**Multivariate analyses**

Two models were fit with different variables grouping at entry and models were compared by their c-index. Final models of the predictive accuracy for patient survival are shown in Supplementary Table S5. Details of the full analysis are stated in Supplementary Table S5. Our first model included all biological factors (IDH1 mutation, 1p19q LOH, and MGMT methylation) and histology. In this model, IDH1 mutation status \((P = 0.0011)\) and 1p19q LOH \((P = 0.06)\) were the most influential factors \((c\text{-index} = 0.70)\). When CIMP was included in this model, CIMP status was the only factor selected \((P < 0.0001; c\text{-index} = 0.71)\) with comparable predictive accuracy to the model with IDH1 mutation status and 1p19q LOH \((0.71 \text{ vs. } 0.70)\).

Our second model included all biological factors (IDH1 mutation, 1p19q LOH, and MGMT methylation), clinical factors (age and performance status), and histology. In this model, performance status \((P = 0.006)\), age \((P = 0.002)\), IDH1 mutation status \((P < 0.0001)\), and 1p19q LOH \((P = 0.06)\) were selected \((c\text{-index} = 0.74)\). When CIMP was included in this model, CIMP status was not selected for.

**Integrated analysis with expression profilling**

We next integrated our methylation data with gene expression profiles to determine the effect of methylation on gene expression. Expression and methylation data were available for 27 samples included in this study and were integrated on the basis of “gene symbol” as indicated by the respective annotation files (20). Results are shown in Fig. 3. In general, few genes \((994 \text{ of } 2,965, 33.3\%)\) that are highly methylated \((>75\% \text{ methylation})\) are expressed \((\text{RMA levels} > 6.5)\). In contrast, genes that are unmethylated \((<10\% \text{ methylation})\) show a much wider range in gene expression levels, though most \((7,476 \text{ of } 9,422, 79.3\%)\) are expressed at RMA levels exceeding 6.5. The absence of methylation of genes therefore appears to be permissive for gene expression. An unmethylated gene is not necessarily expressed at high levels as a multitude of additional molecular mechanisms are involved in gene expression.

We have recently conducted expression profiling on a large cohort of gliomas and identified 7 “intrinsic molecular subtypes” that correlate better with survival than histology (20). We determined whether these “intrinsic molecular subtypes” correlate with the tumors’ CIMP status. In our study, all but one CIMP+ tumors are in the prognostically favorable molecular clusters 9 \((\text{characterized by a high incidence of } 1p19q \text{ LOH and } IDH1 \text{ mutation})\) and 17 \((\text{characterized by a high incidence of } IDH1 \text{ and } TP53 \text{ mutations})\). Conversely, tumors within the prognostically unfavorable molecular clusters 18 \((\text{characterized by a high frequency of } EGFR \text{ amplification})\) and 23 \((\text{no clear molecular marker identified})\) are all CIMP– \((P < 0.0001, \text{the Fisher exact test})\). Only one sample was available of cluster 22 \((\text{secondary tumors and high frequency of } IDH1 \text{ mutations})\), which clustered among the CIMP– tumors. Prognostically favorable tumors are therefore identified both by
methylation profiling (CIMP\(^+\)) and expression profiling (clusters 9 and 17). However, methylation profiling does identify fewer prognostically relevant molecular subgroups than expression profiling (20, 25, 26).

**Cluster validation**

We next aimed to determine whether our CIMP\(^+\) and CIMP\(^-\) methylation clusters can also be identified in an external data set. We therefore validated our methylation clusters on the methylation data from the Cancer Genome Atlas (TCGA) external data set (assessed December 2010) containing GBMs only (13). The “clusterRepro” R-package (see methods) was used to classify each TCGA sample to one of the 2 clusters (CIMP\(^+\) or CIMP\(^-\)) identified in the present study. Of the 269 samples included in this study, 245 were assigned to the CIMP\(^+\) cluster, the remaining 24 samples were assigned to the CIMP\(^-\) cluster. Survival data were available for 190 of these samples of which 176 were assigned to the CIMP\(^+\) cluster and 14 to the CIMP\(^-\) cluster. Similar to our data set, the CIMP\(^+\) tumors had a significantly better survival than the CIMP\(^-\) tumors (Fig. 4; \(P = 0.0001\)).

In summary, our CIMP status was originally defined in gliomas by the TCGA (13). We have therefore also assigned our data set according to the TCGA-defined CIMP status based on 1,503 CpG sites as reported (13). When the 68 samples of current study are assigned to one of these clusters, the CIMP status remained identical in 64 of 68 samples (Supplementary Table S1) with assigned to one of these clusters, the CIMP status remained identical in 64 of 68 samples (Supplementary Table S1) with highly concordant survival (Supplementary Fig. S3).

We next used samples of the TCGA to validate the association between CIMP status and “intrinsic molecular subtype.” For 217 samples of the TCGA data set, paired expression and methylation data are available. Each sample was assigned to a defined gene expression–based intrinsic subtype as described previously (20). When integrating the molecular expression clusters with methylation, all but one (8 of 9) samples within the CIMP\(^+\) subgroup were found in the prognostically favorable cluster 17 (IDH1 and TP53) and all but one 172 of 173 CIMP\(^+\) tumors are within prognostically unfavorable tumors (clusters 18 and 23). Both CIMP\(^-\) (9 of 35) and CIMP\(^-\) (26 of 35) tumors are found in cluster 22, which has unfavorable prognosis but contains secondary GBMs and a high percentage of tumors with IDH1 mutation. This observation largely confirms our hypothesis that prognostically favorable/unfavorable tumors can be identified both by methylation and expression profiling.

**Discussion**

In this study, we have identified 2 prognostically different molecular subgroups of anaplastic oligodendroglioma based on methylation profiling. These subgroups can be separated on overall CpG island methylation: CIMP\(^+\) tumors generally have a more favorable outcome than CIMP\(^-\) tumors. CIMP was the only factor selected when all biological factors were included. Predictive accuracy was slightly higher in the model with CIMP alone. CIMP status was not selected in a model including all biological factors (IDH1 mutation, 1p19q LOH, and MGMT methylation), clinical factors (age and performance status), and histology. The prognostic value of CIMP status could be validated on an external data set from the TCGA.

Our results indicate that CIMP status is a strong predictor of survival in AODs and AOA. Other prognostic markers in gliomas include 1p19q LOH (27), IDH1 mutation status (6, 28, 29), and MGMT methylation status (3, 6, 28) or whole-genome approaches such as gene expression profiling (20, 25, 26, 30) and, similar to reported in this study, methylation profiling (13–15). These prognostic markers may help classify gliomas as histologic classification is troublesome and subject to interobserver variation (31–34).

CIMP was first identified in colorectal cancer in 1999 (35). In this study, colorectal cancers could be distinguished on the basis of low or high levels of tumor-specific methylation, the latter being referred to as the CpG island methylator phenotype (CIMP). CIMP has been identified in several other cancer types including gastric, lung, liver, ovarian, and leukemias (36). Although CIMP\(^+\) gliomas are associated with a more favorable prognosis, a high methylation index is associated with a reduced progression free or survival in many other cancer subtypes, including bladder cancer, esophageal adenocarcinoma, neuroblastoma, ovarian cancers, and leukemia [see (36) and references therein]. Furthermore, in colorectal cancer, CIMP is associated with KRAS and BRAF mutations and negatively associated with TP53 mutations and chromosomal instability (37–39). In gliomas, CIMP status appears to be positively correlated...
with TP53 mutations: astrocytic grade II and III tumors often are CIMP\textsuperscript{+}, and these histologic subtypes have a high percentage of TP53 mutations (13, 40). Two recent papers have shown a more causal effect of IDH1 mutation on chromatin remodeling and CIMP status (41, 42). In the present study, however, not all IDH1 mutated tumors are CIMP\textsuperscript{+} (Fig. 1), which indicates that IDH1 mutation status is not the only determinant for CIMP status.

CIMP has been associated with increased (protein) expression of DNA methyltransferase [DNMT; e.g., see ref. 43]. As a result, many genes involved in tumor initiation and/or progression become methylated in CIMP\textsuperscript{+} tumors (44). Reversal of CIMP status may thus provide a potential method in the treatment of CIMP\textsuperscript{+} gliomas. Aberrant methylation in tumors may be reversed by treatment of DNMT1 inhibitors such as decitabine (9, 44). However, in our expression profiling data, the prognostically favorable tumors do not show an increase in DNMT1, 3A, or 3B (data not shown). It therefore remains to be determined whether DNMT1 inhibition is an effective treatment for gliomas.

In summary, our data using mainly samples that were included in the large, prospective, randomized phase III EORTC study 26951 show the association of CIMP status and prognosis in anaplastic oligodendrogial tumors, and their close association with 1p19q co-deletion, MGMT promoter association, and IDH1 mutations. The strong association between CIMP status and MGMT promoter methylation suggests that the MGMT promoter methylation status is part of a more general, prognostically favorable genome-wide methylation profile. This finding appears to explain the observations of the improved outcome in patients with MGMT promoter methylated tumors treated with radiotherapy only. Thus, in anaplastic glioma, MGMT promoter methylation may well be an epiphenomenon of genome-wide methylation. Methylation profiling may thus be helpful for identification of patients with AOD or AOAs with improved prognosis.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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A Prognostic Methylation Phenotype in Oligodendrogliomas


A Hypermethylated Phenotype Is a Better Predictor of Survival than MGMT Methylation in Anaplastic Oligodendrogial Brain Tumors: A Report from EORTC Study 26951

Martin J. van den Bent, Lonneke A. Gravendeel, Thierry Gorlia, et al.

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