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# Integrated molecular analysis suggests a three-class model for low-grade gliomas: A proof-of-concept study

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ABSTRACT

Introduction: We used an integrated molecular analysis strategy to perform class discovery on a population of low-grade gliomas (astrocytomas, oligodendrogliomas, and mixed gliomas) to improve our understanding of the molecular relationships among these tumors and to reconcile genotypic relationships with current histologic and molecular strategies for tumor classification.
Methods: Gene expression profiling was performed on a cross-section of World Health Organization (WHO) grades I–II gliomas. Unsupervised class discovery algorithms identified and validated tumor clusters with genotypic similarity, and these data were integrated with chromosomal copy number assays and RT-PCR data to define molecular tumor subclasses. Machine learning models allowed accurate, prospective classification of unknown tumors into these molecular subgroups. This molecular classification model was compared to current histologic (WHO) and molecular pathologic (chromosome 1p and 19q deletions, p53 alterations, and Ki-67 expression) methods for glioma classification.
Results: Molecular class discovery suggested a three-class model for low-grade gliomas. One discrete cluster of gliomas identified the pilocytic astrocytomas, a second grouped the 1p/19q codeleted oligodendrogliomas, and the mixture of remaining 1p/19q intact gliomas, including astrocytomas, oligodendrogliomas, and oligoastrocytomas, formed a third cluster with a discrete pattern of expression.
Conclusions: Integration of genomic, transcriptomic, and morphologic data for class discovery suggests a three-class model for low-grade gliomas. Class I represents tumors with molecular similarity to pilocytic astrocytomas, class II tumors are similar to 1p/19q codeleted oligodendrogliomas, and class III represents infiltrative low-grade gliomas. This classification is similar to current clinical paradigms for low-grade gliomas; our work suggests a molecular basis for such models. This classification may supplement or may serve as the basis for a molecular pathologic alternative to current grading schemes for low-grade gliomas and may highlight potential targets for future biologically based treatments or strategies for future clinical trials.



## Introduction

Each year, approximately 30,000 patients are diagnosed with central nervous system (CNS) gliomas. The majority of these tumors are classified as high-grade (malignant) gliomas, a collective term that encompasses anaplastic astrocytoma, anaplastic oligoastrocytoma and anaplastic oligodendroglioma (WHO grade III tumors), as well as gliosarcoma and glioblastoma (WHO grade IV tumors) [1]. Regardless of the histologic subtype, treatment of these patients is similar (surgical resection + radiation ± chemotherapy) and survival is generally short (1–5 years) [2–4]. Gliomas with less malignant histologic appearance are classified into WHO grades I–II. The histologic classification schema for these "low-grade gliomas" is complex, using morphologic features, which may be inconsistent or subject to variable interpretation, to assign tumors to several categories, and include astrocytoma (pilocytic, pilomixoid, fibrillary, gemistocytic, protoplasmic), oligodendroglioma, mixed glioma (oligoastrocytoma), and subependymal giant cell astrocytoma (SEGA) [1]. While assignment to select WHO classes (i.e., pilocytic astrocytoma, SEGA) has specific implications for treatment and prognosis, the WHO class for most grades I–II gliomas does not reflect major phenotypic differences. From a biological perspective, it has been difficult to determine to what degree the WHO classification accurately reflects underlying tumor cell biology.

The relative rarity of these tumors and the prolonged survival of patients diagnosed with these lesions (5–20$^+$ years after diagnosis)

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complicate subgroup identification and evidence-based research for low-grade gliomas, which limits large-scale, prospective trials of treatment strategies [1]. Given sparse data, nearly all combinations of observation, biopsy, surgical resection, and adjuvant therapy have been advocated for management of patients with such tumors [5], resulting in variable outcomes. A cycle develops, as multiple treatment strategies are inconsistently applied to small subsets of patients with low-grade gliomas, further limiting the ability to conduct meaningful outcomes research. Accordingly, consensus regarding optimal, biologically based management of most patients with WHO grades I–II gliomas is lacking [5].

This problem highlights the need for a classification system for low-grade gliomas that reflects tumor biology and that can be used prospectively to guide clinical trial design and patient management. A molecular classification system based upon the tumor transcriptome is an attractive option [6,7], because phenotype is associated with genotype. Molecular classification using microarray expression profiles has been investigated in pilocytic astrocytomas [8] and in high-grade gliomas by ourselves [9] and others [10–16], and these studies have identified molecular subgroups with divergent phenotypes (including patient survival), often within a single WHO grade [5,9]. Applying a similar approach to low-grade gliomas may identify molecular subclasses of these tumors with prognostic and therapeutic significance.

Because WHO grades I–II gliomas are significantly less common than high-grade gliomas, availability of tissue for histologic and molecular analysis has made such investigations more difficult than similar research for high-grade gliomas. A detailed review by Rorive et al. [17] summarized the genomic literature for low-grade astrocytomas to date, citing only 11 studies [12,18–27] that have collected "sound [expression] data" [17] for these tumors. This literature tends to be descriptive, enumerating genes that are differentially expressed between low-grade gliomas and normal brain [8,12,18,19,21,22,24,26,27] or between low-grade gliomas and their high-grade counterparts [12,19–21,23–25]. While such investigations can identify genes that may be markers of malignant progression in gliomas, these analyses have been limited in their ability to discover novel molecular subclasses or to classify unknown tumor samples into such classes in a prospective fashion. We believe that an unbiased investigation of low-grade gliomas, focused on identification and characterization of molecular subgroups, will further the understanding of the underlying biology of these tumors, will stimulate continued exploration of the WHO classification system, and will help establish a theoretical framework in which future attempts to correlate genotype and phenotype can be performed.

In this study, we examine the molecular relationships among a group of 23 tumors representing a cross-section of WHO grades I–II gliomas. We have constructed an integrative data analysis model that limits prospective biases, incorporates genomic, transcriptomic, and histologic data, and facilitates unbiased class discovery based on this integrated molecular data. Our aim is to improve our understanding of the relationships among these tumors and to reconcile these relationships with current histologic and molecular strategies for the classification of low-grade gliomas.

## Materials and methods

### *Inclusion criteria*

Patients selected for inclusion in this study had a confirmed diagnosis of WHO grades I–II glioma (pilocytic astrocytoma, diffuse astrocytoma, oligodendroglioma, or mixed glioma) [1]. Because of the tendency of these tumors to affect young people, patients of any age with the appropriate diagnosis were included. Tissue samples were obtained during initial tumor resections performed at our institution between August 2001 and August 2007 as part of standard medical management. Patients undergoing prior stereotactic biopsy were eligible for inclusion, but patients with prior tumor resections or prior management with chemotherapy and/or radiotherapy were excluded. Patients were also excluded if the available clinical information was insufficient to verify study eligibility or if the pathologic specimen was inadequate for microarray analysis.

### *Sample selection*

This study was approved by the Cleveland Clinic Institutional Review Board. The Brain Tumor and Neuro-Oncology Center database was queried to generate a list of all patients meeting the inclusion criteria. The electronic medical records of these patients were reviewed to verify study eligibility. Dates of death were verified using the Social Security Death Index. Tissue samples from 23 low-grade gliomas (3 pilocytic astrocytomas, 4 grade II astrocytomas, 10 grade II oligodendrogliomas, and 6 grade II oligoastrocytomas) were collected. All samples used in this study were immediately flash frozen at the time of resection and were subsequently stored at -80°C until RNA extraction was performed.

### *Demographics*

Demographic information for the 23 patients included in this study is summarized in Table 1. The patient population was 65% male and 35% female, and the mean age of patients at the time of surgical resection was 34.3 years (±4.66, 95% CI, range 10–60). Twenty tumors were supratentorial (87%) and three were infratentorial (13%). The surgical resection from which tissue used in this study was collected represented the first surgical intervention for 18 patients (79%), while 5 patients had undergone prior stereotactic biopsy (21%). Twenty patients were alive at the time of analysis (87%), and a censored survival point was entered for these patients. The mean survival (including censored survival) was 3.53 years (±0.59, 95% CI) from the time of diagnosis. The extent of resection in all patients was assessed on postoperative, contrast-enhanced MRI or CT imaging.

### *Extent of tumor resection*

We defined gross total resection (GTR) as absence of residual lesional enhancement or MRI FLAIR abnormalities, near-total resection (NTR) as trace amounts of residual enhancement without obvious residual tumor mass (95–99% resection), and subtotal resection (STR) as obvious residual tumor mass, but with resection of >88% of original

**Table 1**
Demographics.

| | |
|---|---|
| Number of patients (*n*) | 23 |
| Age (years) – mean | 34.3 |
| – 95% CI | 4.7 |
| Sex – M (%) | 15 (65%) |
| – F (%) | 8 (35%) |
| Location – supratentorial *n* (%) | 20 (87%) |
| – Location – infratentorial *n* (%) | 3 (13%) |
| Prior stereotactic biopsy – *n* (%) | 5 (21%) |
| – *n* (%) GTR | 12 (53%) |
| – *n* (%) NTR | 6 (26%) |
| – *n* (%) STR | 5 (21%) |
| Survival (censored, years) – Mean | 3.53 |
| – Survival (censored, years) – 95% CI | 0.59 |
| Alive at the time of analysis – *n* (%) | 20 (87%) |

CI: confidence interval; GTR: gross total resection; NTR: near total resection; STR: subtotal resection.