2 Molecular Classifications

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Summary

The field of glioma classification is currently entering a new era with the introduction of paradigms based on molecular information. Rather than supplanting traditional morphology-based classification schemes, it is anticipated that emerging molecular biologic, genomic, transcriptomic, and proteomic data will complement and augment existing morphologic and immunophenotypic data, providing for a more accurate and refined stratification of glioma patients for directed therapies and for the resolution of several problematic issues inherent in histological classifications. Two different approaches are contributing to the improvement of glioma stratification. The first is the analysis of alterations of a limited number of genes or gene products of recently demonstrated impact on patient survival and response to therapy, such as deletion status of chromosomes 1p and 19q in oligodendroglial tumors, and *O*(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in glioblastoma. The second is a more comprehensive analysis of the tumor genome, transcriptome, or proteome, which may in itself provide refined subclassification, or may identify specific relevant biomolecules for use in the single gene analysis approach. Both paradigms have already exerted a tangible and growing impact on glioma classification, yet it is highly likely that we have only just begun to exploit their potential contributions.

Key Words: Molecular classification; glioma; transcriptomics; genomics; proteomics; tissue microarray; MGMT; PTEN; 1p; 19q.

INTRODUCTION

The histopathologic classification of diffuse gliomas by microscopy has a long and storied history. Many different classification and grading systems of increasing precision and clinical utility have been proposed. Currently applied classifications, such as the modified Ringertz systems of Burger *(1)* and Nelson *(2)*, the St. Anne-Mayo system *(3)*, and the World Health Organization (WHO) system *(4)* are largely based on morphologic pattern recognition and relative weighting of various histologic features, with supportive input provided by immunophenotypic studies using monoclonal (MAbs) and polyclonal antibodies (PAbs) directed against various differentiation and cell proliferation makers. The clinical usefulness of these time-tested glioma classification systems cannot be overemphasized. In addition, the technical simplicity and cost efficiency of rendering a diagnosis based on the examination of a hematoxylin and eosin-stained tissue section by simple light microscopy are unparalleled. Nevertheless, morphology-based tumor stratification exhibits a number of shortcomings (Table 1). Among these are (1) subjectiveness, (2) inability to substratify patients within a given major tumor category, such as anaplastic astrocytoma (AA), and (3) inability to predict

From: *Current Clinical Oncology: High-Grade Gliomas: Diagnosis and Treatment*
Edited by: G. H. Barnett © Humana Press Inc., Totowa, NJ

Table 1
Some Shortcomings of Traditional Morphology-Based Tumor Classification Systems

- Subjective classification criteria open to variable interpretation and relative weighting by individual evaluators.
- Nonpredictive of individual patient survival within a given tumor type and grade.
- Nonpredictive of individual tumor response to particular therapeutic regimens.

Table 2
Two Current Paradigms for Molecular Classification of Gliomas

- Sequential assay of a small number of genes or gene products of known significance (e.g., 1p, 19q, 9p, 10q, *PTEN*, *P16^{INK4A}*, *TP53*, *EGFR, MGMT*).
- Simultaneous assay of a large number (hundreds or thousands) of genes or gene products by any one of a number of high-density, high-throughput techniques, followed by statistical analysis to identify glioma subsets and robust classifiers. Techniques include gene expression profiling, array comparative genomic hybridization and proteome profiling.

patient response or lack thereof to specific therapeutic regimens. Subjectiveness or ambiguity of interpretation is usually not an issue for gliomas that exhibit classical morphology—such as typical glioblastomas that show pleomorphism, fibrillary cytoplasmic processes, florid microvascular proliferation, and zones of tumor necrosis with pseudopalisading—or for oligodendrogliomas with monotonous, uniform cells, bland round nuclei, and prominent perinuclear halos; however, in a significant percentage of diffuse gliomas, there exists either a mixture of cells with either astrocytic or oligodendroglia features, or a large percentage of cells exhibit a combination of astrocytic and oligodendroglial features. At different times throughout the history of glioma classification, the neuropathology Zeitgeist has variously favored including such morphologically ambiguous tumors in the astrocytic camp, the oligodendroglial camp, or in a hybrid category called mixed oligoastrocytoma in which there may be topographically separate areas of astrocyte-featured cells and oligodendrocyte-featured cells, or the two populations may be intimately intermixed, or there may be a *tertium quid* variant in which all of the tumor cells display features that are intermediate between those of classical astrocytoma and classical oligodendroglioma *(5)*.

Therefore, there is a clearly defined need for a more refined, individually tailored, and less subjective glioma classification. Contemporary molecular and genomic techniques provide a wealth of possible avenues for improving current glioma stratification.

MOLECULAR CLASSIFICATION PARADIGMS

There are two paradigms for the molecular classification and substratification of diffuse gliomas that are currently enjoying widespread investigation and application (Table 2). The first is based on the assay of only a very small number of genes or gene products of known importance. The second approach employs a more global analysis of hundreds or thousands of genes using contemporary high-density, high-throughput genomic technologies. Both approaches have proven fruitful in preliminary molecular classification attempts.

Table 3
Contemporary Genomic, Transcriptomic, and Proteomic Techniques Used for Data Acquisition for Molecular Classification Studies

DNA	*RNA*	*Protein*
Genome	*Transcriptome*	*Proteome*
Array CGH	Expression microarrays	MALDI-TOF MS
FISH + TMA	SAGE	Protein arrays
		Antibody arrays
		TMA + Ab

Abbr: Ab, monoclonal and polyclonal antibodies; CGH, comparative genomic hybridization; FISH, fluorescent *in situ* hybridization; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SAGE, serial analysis of gene expression; TMA, tissue microarray.

PATIENT STRATIFICATION BASED ON ASSAY OF A SMALL NUMBEROF MOLECULAR MARKERS

The term "molecular classification," in the most basic sense, implies the use of molecular data to effect or facilitate the reliable and reproducible stratification of patients into groups that have differing prognostic or therapeutic implications. Contemporary basic and translational molecular biological research has yielded a number of molecular marker assays with proven prognostic and/or therapeutic significance in high-grade gliomas. The most salient of these is deletion testing for markers on chromosomes 1p and 19q in oligodendrogliomas. Co-deletion of markers on both chromosomal arms is associated with better response to therapy, increased time to recurrence, and increased survival compared with morphologically similar tumors that lack this molecular signature *(6–14)*. In this instance, the assessment of only two chromosomal regions yields a clinically significant dichotomous molecular classification of oligodendroglial tumors. Assay of a few additional molecular markers, such as TP53 mutation can be used to generate an even finer molecular substratification of anaplastic oligodendrogliomas into four prognostically significant groups *(6)*. Another prominent example of molecular stratification using only a single gene assay is the assessment of epigenetic silencing of *O*(6)-methylguanine-DNA methyltransferase (MGMT) by promoter methylation in high-grade astrocytomas. Patients with tumors that exhibit MGMT gene promoter methylation survive longer after treatment with temozolomide and radiation therapy compared with patients with tumors that lack this feature *(15–17)*. Thus, MGMT promoter methylation status permits the molecular subclassification of glioblastoma patients into two different treatment response groups.

MOLECULAR STRATIFICATION BASED ON TRANSCRIPTOME PROFILING, COMPARATIVE GENOMIC HYBRIDIZATION, AND PROTEOME PROFILING

A number of contemporary high-density genomic, transcriptomic, and proteomic techniques are available that could potentially provide data useful for tumor molecular classification and novel class discovery (Table 3). In theory, quantitative information on DNA alterations, mRNA (cDNA) levels, or protein composition and quantities could be used to

Table 4
Technology-Driven Revolutions in the History of Tumor Nosology

- Light microscopy
- Electron microscopy
- Immunocytochemistry
- Genomics & Transcriptomics
- Proteomics Next!

separate gliomas into meaningful subsets for diagnostic, prognostic, and/or therapeutic purposes. Currently, transcriptome profiling *(18–26)* and array comparative genomic hybridization *(27–30)* are the dominant methodologies that have been used to generate molecular classifications of the diffuse gliomas. Initial experiments using proteomic data to subclassify gliomas have also been reported *(31)*.

Several conclusions can be drawn from the collective experience on molecular classification of gliomas using genomic and transcriptomic techniques to date: (1) molecular classification can separate tumor types and grades as well as and often better than histopathologic classification, (2) gene expression profiling can identify subgroups within histologic tumor types that are not identifiable by morphologic or immunophenotypic evaluation, and (3) nosologic groups identified by expression profiling have prognostic significance with respect to patient survival. In addition, it is clear that the analysis of hundreds or thousands of genes or gene products, although a logical starting point, is not necessary once robust classifier genes have been identified. Rather, the paradigm that is evolving is the initial quantitation of thousands of genes, followed by statistical analysis to identify small sets of only a few genes that are strong classifiers. These markers can then be used to stratify tumors for various ends, such as prognosis, susceptibility to specific therapeutic regimens, or resistance to specific therapies. Sets as small as three genes have proven as powerful as the indiscriminant evaluation of hundreds of genes in classifying gliomas *(19)*. This then raises the possibility of design of relatively simple chip sets for diagnosis that would be feasible from both technological and cost efficiency perspectives for widespread routine diagnostic application. Additionally, it may also be possible to select for robust classifier gene sets that code for expressed proteins for which antibodies could then be raised, thereby "translating" genomic classification into protein immunohistochemical classification. In contrast to high-density gene expression platforms and array comparative genomic hybridization (CGH) technology, immunohistochemistry is available in virtually all modern hospital diagnostic clinical laboratories.

CONCLUSIONS

Numerous studies by multiple institutions over the past several years have convincingly demonstrated the power and ability of molecular approaches to classify and substratify diffuse gliomas. In the words of Nutt and colleagues, "Gene expression-based classification of malignant gliomas correlates better with survival than histological classification *(25)*." At the same time, it is equally true that classical histopathological evaluation of gliomas provides much useful information and no cogent arguments for discontinuing morphologic and immunophenotypic diagnosis and classification studies have been advanced. Morphologic evaluation has the advantages of simplicity, cost effectiveness, and a long history of proven

useful application. Although it is hazardous to attempt to predict the future, it seems likely that morphology will continue for some time to provide the foundation for glioma classification, with molecular analysis judiciously applied as warranted to provide more refined stratification as continued research unveils additional applications.

The history of tumor classification is replete with examples of the influence of technological advances (Table 4). The invention of the light microscope was arguably the single greatest advance in tumor nosology, introducing the modern era. Other waves of progress were provided by the transmission electron microscope for ultrastructural analysis and the introduction of immunocytochemistry for differentiation antigen identification. Currently, transcriptomic techniques (gene expression profiling) and genomic techniques (array comparative genomic hybridization and related techniques) are exerting a strong influence on the field. Proteomic technology is likely to have an increasing influence in the future as the field matures.

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http://www.springer.com/978-1-58829-511-8

High-Grade Gliomas
Diagnosis and Treatment
Barnett, G.H. (Ed.)
2007, XIII, 495 p., Hardcover
ISBN: 978-1-58829-511-8
A product of Humana Press