

REVIEW ARTICLE



Grading of adult diffuse gliomas according to the 2021 WHO Classification of Tumors of the Central Nervous System

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The grading of gliomas based on histological features has been a subject of debate for several decades. A consensus has not yet been reached because of technical limitations and inter-observer variations. While the traditional grading system has failed to stratify the risk of IDH-mutant astrocytoma, canonical histological and proliferative markers may be applicable to the risk stratification of IDH-wild-type astrocytoma. Numerous studies have examined molecular markers in order to obtain more clinically relevant information that will improve the risk stratification of gliomas. The *CDKN2A/B* homozygous deletion for IDH-mutant astrocytoma and the following three criteria for IDH-wild-type astrocytoma: the concurrent gain of whole chromosome 7 and loss of whole chromosome 10, *TERT* promoter mutations, and *EGFR* amplification, were identified as independent molecular markers of the worst clinical outcomes. Therefore, the 2021 World Health Organization (WHO) Classification of Tumors of the Central Nervous System adopted these molecular markers into the revised grading criteria of IDH-mutant and -wild-type astrocytoma, respectively, as a grading system within tumor types. Of note, several recent studies have shown that some low-grade IDH-wild-type astrocytoma lacking both the molecular glioblastoma signature and genetic alterations typical of pediatric-type gliomas may demonstrate a relatively indolent clinical course, suggesting the existence of lower-grade adult IDH-wild-type astrocytoma. In terms of oligodendroglioma, IDH-mutant, and 1p/19q codeleted, consistent makers that predict poor outcomes have not yet been identified, and, thus, the current criteria have remained unchanged. Molecular testing to fulfill the revised WHO criteria is, however, not always available worldwide, and in that case, an integrated diagnosis combining all available complementary information is highly recommended. This review discusses controversial issues surrounding legacy grading systems and newly identified potential genetic markers of adult diffuse gliomas and provides perspectives on future grading systems.

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INTRODUCTION

The World Health Organization (WHO) adopted a molecular classification for adult diffuse gliomas in the updated 4th edition in 2016 for the first time^{1–4}. However, the histological grading scale remained unchanged because of the lack of sufficient molecular evidence to revise the traditional histological scale, which was used for many years⁵. The purpose of tumor grading is to provide clinicians with information to predict outcomes, develop a treatment plan, and engage in clinical studies to establish more appropriate treatment regimens. The grading of diffuse gliomas in children and adults has been a subject of discussion for several decades⁶. However, a consensus has yet to be reached because grading has been based on the histological appearance of given tumor samples, which do not always reflect the biological behavior of each tumor, and histological assessments are subjective with inter- and intra-observer variabilities. This short review discusses controversial issues surrounding the grading of adult diffuse gliomas according to the 2021 WHO Classification of Tumors of the Central Nervous System (CNS)⁷ and provides perspectives on future grading systems.

THE WHO GRADING SYSTEM

Historical aspects

One of the pioneers of the modern grading systems of cancers was Albert Broders at the Mayo Clinic, who for the first time coined a numerical grading system that divided tumors into four histological grades of malignancy, which were independent of any clinical history and based on dissimilarities in the given tumors from the normal tissue from which they originated⁸. James Watson Kernohan, a colleague of Broders at the Mayo Clinic, who is regarded as one of the early pioneers of neuropathology, adopted a four-tier system for astrocytic gliomas⁹. This histological grading system of gliomas was broadly accepted and used for the next few decades. However, when the WHO started to publish the classification of tumors series in the 1970s¹⁰, they adopted biology-oriented grading under the leadership of Klaus J Zülch, who was a neurologist/neuropathologist¹¹. Even after the publication of the first edition of the WHO Classification of CNS tumors, several different diagnostic schemes were still used in parallel. However, the second edition of the WHO Classification¹², incorporating the so-called St. Anne-Mayo grading scheme¹³, became universally accepted as the standard for glioma grading.

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General rules in the current WHO grading system

In the WHO grading system, a biology-oriented grade is generally given to each tumor based on the estimated clinical outcome obtained by epidemiological data. In other words, the grade is fixed to the tumor nomenclature, and variations in the histological appearance of each specimen do not affect the assessment of an individual sample. For example, pilocytic astrocytoma is exclusively assigned to WHO grade I regardless of its histological features. Although a small subset of pilocytic astrocytoma exhibits anaplastic histological features and some patients with such tumors may have a significantly worse prognosis than classic pilocytic astrocytoma, the WHO has not designated a formal grade for these tumors. In addition, the WHO adopted the grading “across tumor types” rather than “within a tumor type”. In this type of grading system, tumors of the same grade roughly have the same clinical outcome regardless of the histological tumor type¹⁴. However, the “grading across tumor types” became irrational because the biology of tumors, primarily defined by genetic alterations, is so heterogeneous that it is more reasonable to grade each tumor individually. For example, many studies reported that IDH-mutant glioblastoma, WHO grade IV, has a markedly better prognosis than IDH-wild-type glioblastoma, WHO grade IV, even though they share identical histology. Another regulation of the WHO grading system is that grading is selected by the natural history of a neoplasm with surgical resection alone and not by the outcome of treatment. This rule has been well accepted in the neuro-oncology community because it reflects the intrinsic biology of each tumor, even though current grading criteria are created based solely on retrospective studies.

Nevertheless, after discovering molecularly defined entities, this rule faces an unresolvable dilemma, namely, nearly all patients with malignant gliomas, including anaplastic astrocytoma and glioblastoma, receive adjuvant therapy after resection; therefore, it became essentially impossible to obtain information on the natural history of molecularly defined high-grade gliomas. Furthermore, a randomized control study to verify the grading criteria for high-grade gliomas was not feasible. The ethics do not allow the existence of patients with a high-grade glioma who does not receive any adjuvant therapy.

Issues in the histological grading system

Four histological markers employed by the WHO grading system to evaluate malignancy are nuclear atypia (A), mitosis (M), microvascular proliferation (previously termed endothelial proliferation: E), and necrosis (N), which are often referred to as the ‘AMEN’ score¹⁵. A significant mitotic count is a requirement for grade III tumors, and microvascular proliferation or necrosis for grade IV tumors, typically diffuse astrocytic tumors. Although the neuropathology community has accepted this system for more than 25 years, inter- and intra-observer variabilities have never been resolved. The system is subjective and has the following technical limitations: the assessment of atypia depends on individual skills and an investigator’s experience. Mitotic counts also rely on the diligence of the examiner. Since gliomas are permeating neoplasms with marked intra-tumoral heterogeneity, mitoses may accumulate focally or be evenly scattered. Therefore, when mitoses are counted in ten consecutive high-power fields (HPF), the mitotic count captured may vary according to the spreading pattern, either evenly or unevenly distributed. This counting method also has a significant pitfall. The area of each HPF depends on the field number (FN) of the ocular lens used. Typical FNs are 20, 22, and 26.5, which correspond to 0.20, 0.24, and 0.34 mm², respectively, as a single HPF area. Differences in ocular lenses significantly affect the number of mitoses captured within a single HPF. In addition, mitoses are often difficult to distinguish from apoptosis. In some institutions, the anti-phosphohistone H3 (pHH3) antibody has been used to

overcome the aforementioned issues (Fig. 1G, I)^{16–19}. This mitosis-specific antibody recognizes the phosphorylation of serine 10 in histone H3 and does not react with apoptosis¹⁶. Therefore, it allows an investigator to quickly identify mitoses, providing increased sensitivity and reducing inter-observer variability. However, one disadvantage of this antibody is non-specific staining, particularly on an automatic immunostainer.

The current grading system was rooted in the 3rd edition of the WHO classification published in 2000²⁰. In that edition, the section on diffuse astrocytoma stated “mitotic activity is absent, but a single mitosis does not yet allow the diagnosis of anaplastic astrocytoma.” This notion is based on a retrospective study conducted by the Mayo Clinic showing that the survival of patients with diffuse astrocytoma with a solitary mitosis did not significantly differ from those without mitosis²¹, which is often referred to as the Modified St. Anne-Mayo scheme. Therefore, a mitotic count greater than 2 in the entire specimen has since been used to designate WHO grade III¹.

The use of Ki-67 antibodies to assess the degree of malignancy has been repeatedly proposed,^{19,22} however, the WHO has never implemented it into the grading system because the immunoreactivity of the Ki-67 antibody is strongly influenced by fixation and the stage duration of formalin-fixed paraffin sections²². Therefore, the Ki-67 index varies with time and location and, thus, is not suitable for assessing biological behaviors across the institution.

THE 5TH EDITION OF THE WHO CLASSIFICATION OF CNS TUMORS

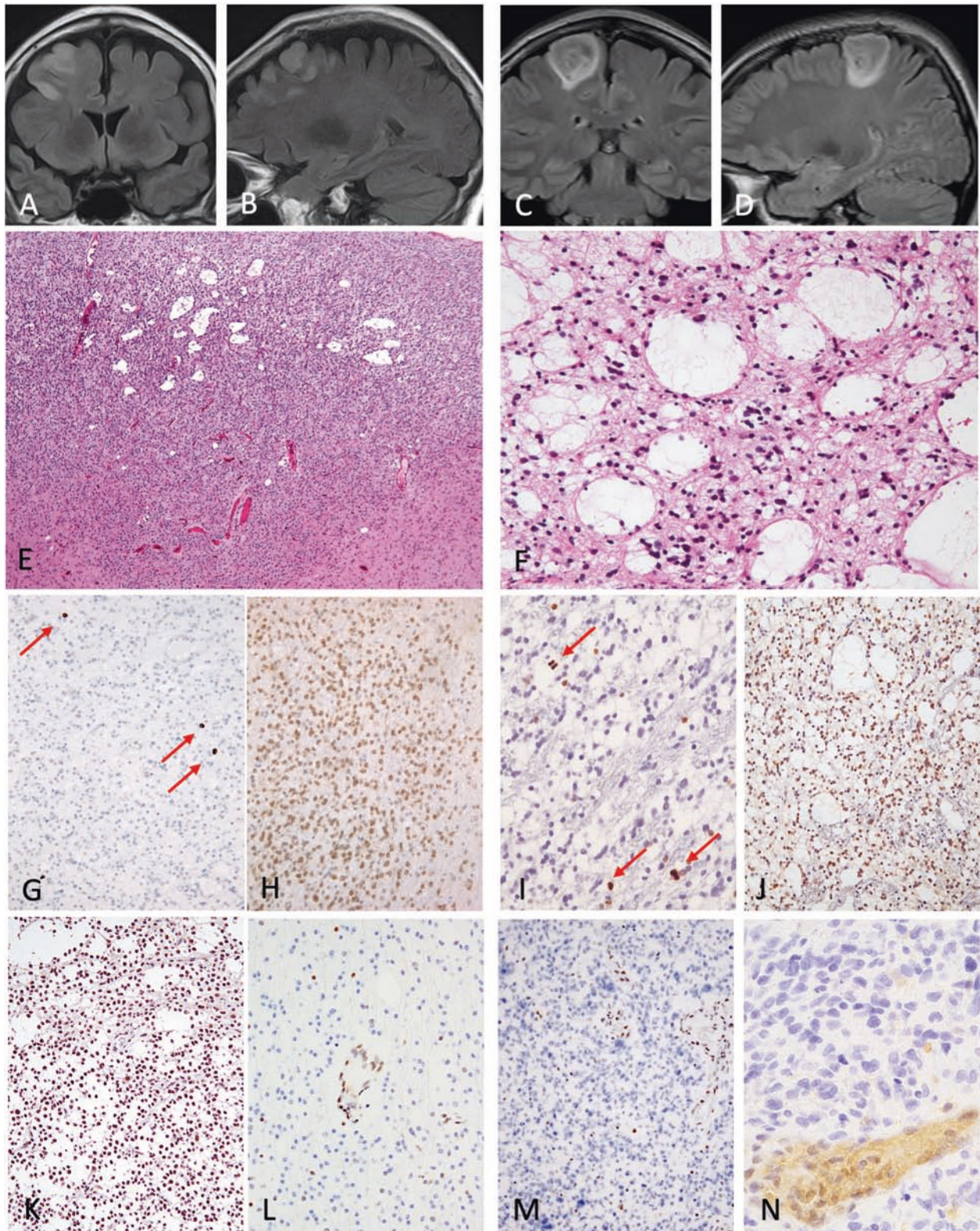
cIMPACT-NOW

After the publication of the 2nd edition of the WHO Classification of CNS Tumors, the WHO has revised it every 7 years, which became too long to incorporate the fruit of the latest research into the classification. To update recent and ongoing advances in research on molecular pathology between WHO revisions, cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy, not officially WHO) was established in 2016²³. cIMPACT-NOW has since convened its separate working committees to address classification and grading issues²⁴.

WHO grading of IDH-mutant astrocytoma

After the discovery of isocitrate dehydrogenase (IDH) 1 and 2 in diffuse gliomas²⁵, adult diffuse gliomas are now genetically defined by three major genetic events: the IDH mutation, 1p/19q codeletion, and *TERT* promoter mutations^{26,27}. In astrocytoma, *TP53* and *ATRX* mutations follow IDH mutations. In oligodendroglioma, the 1p/19q codeletion follows IDH mutations²⁶. *TERT* promoter mutations are involved in glioblastoma and oligodendroglioma^{27,28}. The remaining are glioblastoma and so-called triple-negative gliomas, which lack all three major alterations. IDH mutations occur early in gliomagenesis; mutant IDH genes alter IDH enzymes, causing them to produce 2-hydroxyglutarate. This oncometabolite represses DNA demethylation and leads to genome-wide DNA hypermethylation²⁹.

It subsequently became apparent that survival was longer in patients with IDH-mutant astrocytoma than in those with IDH-wild-type astrocytoma^{30,31}, indicating that an independent grade needs to be given to IDH-mutant astrocytoma and IDH-wild-type even though their histological features are identical. Furthermore, retrospective studies found that the mitotic activity threshold did not reflect progression-free or overall survival in IDH-mutant gliomas, suggesting that the histological grading criteria used in the WHO classification do not sufficiently stratify the risk of IDH-mutant astrocytoma^{32,33}. Nevertheless, these studies demonstrated that WHO grading is still valid for IDH-wild-type astrocytoma³². Similarly, proliferative indices (i.e., Ki-67) did not



achieve the sufficient risk stratification of IDH-mutant astrocytoma, but successfully stratified the risk of IDH-wild-type astrocytoma¹⁹. Therefore, the WHO adopted a “within a tumor type” grading scheme to resolve the inconsistencies associated with IDH-mutant and -wild-type astrocytoma in the 5th edition in 2021^{7,34}. To clarify

this revision, the WHO changed all CNS WHO grades to Arabic numerals from Roman numerals in 2021⁷.

In attempts to improve risk stratification, retrospective studies investigated potential molecular markers associated with very poor clinical outcomes that may be incorporated into a more

Fig. 1 Examples of integrated diagnoses for IDH-mutant gliomas in resource-limited settings. Left panel: Oligodendroglioma, IDH-mutant, and 1p/19q codeleted, WHO grade 2 (A, B, E, G, H, K, L). Right panel: Glioblastoma, IDH-mutant, WHO grade 4 (C, D, F, L, J, M, N). **A, B** FLAIR images showing an ill-defined, multi-nodular mass bulging from the cortex in the right frontal lobe. Note FLAIR-high nodules within the mass. **C, D** FLAIR images showing a well-demarcated, heterogenous mass in the right parietal lobe. **E** A nodule protruding from the cortical surface (H&E stain). **F** Elongated and oval tumor cells with fine cytoplasmic processes embedded in the microcystic background (H&E stain). **G** Three mitoses (arrows) positive for the pHH3 antibody in a medium-power field, which does not meet the grade 3 criteria of anaplastic oligodendroglioma. **H** Tumor cells diffusely positive for the IDH1R132H antibody. **I** Three mitoses (arrows) positive for the pHH3 antibody in a high-power field, which meets the histological grade 3 criteria of anaplastic astrocytoma. **J** Tumor cells diffusely positive for the IDH1R132H antibody. **K** Retained ATRX immunoreactivity. **L** Loss of immunoreactivity against H3 K27M me3. **M** Loss of ATRX immunoreactivity in tumor cells and retention in endothelial cells. **N** Loss of methylthioadenosine phosphorylase (MTAP) immunoreactivity in tumor cells, indicating the *CDKN2A* homozygous deletion, which corresponds to WHO grade 4 in the WHO 5th classification.

clinically relevant grading scheme. The *CDKN2A/B* homozygous deletion, amplification of *CDK4*, *RB1* mutations or homozygous deletions, *PIK3CA* or *PIK3R1* mutations, the amplification of *PDGFRA*, amplification of *MYCN*, global DNA methylation, genomic instability, and the loss of chromosome 14 were suggested to be strongly associated with a poor prognosis in patients with IDH-mutant astrocytoma (Table 1).

The *CDKN2A/B* homozygous deletion and *CDK4* amplification may both be independent predictors of shorter survival in lower-grade astrocytoma³⁵ and glioblastoma³⁶. They have been associated with a lower level of global DNA methylation^{37–40}. WHO grade III astrocytoma with the *CDKN2A/B* homozygous deletion was previously shown to behave almost identically to grade IV astrocytoma; Shirahata and others proposed a novel molecular grading system using the *CDKN2A/B* homozygous deletion to segregate grade IV lesions from grade II and III lesions regardless of histological findings^{33,41–44}. Two studies failed to identify the *CDKN2A/B* homozygous deletion in WHO grade II astrocytoma^{44,45}, while one detected it in 12% of cases³⁵. Previous studies indicated that the *CDK4* amplification combined with the loss of chromosome 14 was associated with a poor prognosis^{41,42}, whereas others did not^{44,45}. Similarly, the homozygous deletion of *RB1* was strongly associated with inferior overall survival in one study⁴⁶, but not in other studies^{44,45}. Aoki and others revealed that altered RB pathway genes, including the *CDKN2A/B* homozygous deletion, amplification of *CDK4*, or *RB1* mutations, were collectively a strong predictor of a poor outcome, but were not when considered independently⁴⁶. Therefore, further studies are warranted to confirm the significance of the amplification of *CDK4* or *RB1* mutations. Although the amplification of *PDGFRA* has repeatedly been associated with a poor prognosis in patients with IDH-mutant astrocytoma, particularly grade II and III tumors^{35,44,47}, one study did not detect this relationship⁴⁶. The significance of other rare genetic alterations, such as *PIK3R* or *PIK3CA* mutations⁴⁶ and the amplification of *MYC*⁴⁴, in IDH-mutant astrocytoma remains unclear and, thus, warrants further study. Based on these findings, the WHO adopted the combination of the *CDKN2A/B* homozygous deletion and traditional histological criteria (anaplasia, mitoses, microvascular proliferation, and necrosis) into a histomolecular grading system of IDH-mutant astrocytoma (Table 2). In this grading system, when the *CDKN2A/B* homozygous deletion is present, grade 4 (IV) is automatically given regardless of the histology of IDH-mutant astrocytoma.

WHO grading of IDH-wild-type astrocytoma

In the WHO 2016 classification, diffuse and anaplastic astrocytoma, IDH-wild-type, were listed as a provisional entity¹. However, recent studies demonstrated that the vast majority of IDH-wild-type diffuse grade II and anaplastic grade III astrocytoma exhibited an aggressive clinical course equivalent to glioblastoma grade IV^{48–51}. More precise molecular analyses, including DNA methylation profiling, revealed that the remaining diffuse and anaplastic astrocytoma, IDH-wild-type, which followed an indolent clinical course, were low-grade gliomas, including pilocytic astrocytoma,

glioneuronal tumors, and pediatric-type diffuse gliomas^{52,53}. The characteristic molecular features of histologically lower grade, but genetically malignant astrocytoma include the amplification of *EGFR*, the concurrent gain of whole chromosome 7 and loss of whole chromosome 10, or *TERT* promoter mutations. *TERT* promoter mutations are the most prevalent among these three alterations and may overlap with each other^{48–50}. Based on these findings, WHO adopted a histomolecular grading scheme for IDH-wild-type astrocytoma that combined traditional histological criteria with three glioblastoma molecular signatures (Table 3)³⁴.

Does adult-type low-grade IDH-wild-type astrocytoma exist?

It currently remains unclear whether true adult-type low-grade IDH-wild-type astrocytoma without pediatric molecular alterations exists. Richardson and others showed that low-grade IDH-wild-type astrocytoma lacking the molecular glioblastoma signature had fewer total copy-number variations (CNV) and less frequent *CDKN2A* homozygous deletions and *PTEN/PIK3CA* alterations, but more frequent *NF1* alterations, indicating the existence of true low-grade IDH-wild-type astrocytoma⁵⁴. Berzero et al. examined 517 grade II gliomas with strict radiological and pathological criteria and identified 29 cases of histologically grade II IDH-wild-type diffuse astrocytoma with the molecular features of glioblastoma⁵⁵. The median overall survival of patients with this group of tumors was 88 months, which was longer than that of glioblastoma. They also demonstrated that grade II IDH-wild-type diffuse astrocytoma is less aggressive than grade III IDH-wild-type diffuse astrocytoma. These findings suggested that the histological grade is still helpful and that strictly defined grade II astrocytoma with *TERT* promoter mutations alone will not behave as glioblastoma, IDH-wild-type⁵⁶. Fujimoto et al. also reported that low-grade IDH-wild-type astrocytoma with either *TERT* promoter mutations or the amplification of *PDGFRA* mostly clustered with glioblastoma in a DNA methylation analysis. They also found that low-grade IDH-wild-type astrocytoma without the molecular features of glioblastoma were a heterogeneous group of tumors using DNA methylation profiling⁵⁷.

Molecular prognostic markers in oligodendroglioma, IDH-mutant, and 1p/19q codeleted

The WHO has never officially disclosed the definite threshold between grades II and III for this tumor. However, the criteria of Giannini^{58–60} have frequently been used: increased cellularity, nuclear pleomorphisms, mitotic activity, microvascular proliferation, and necrosis have been associated with a poor prognosis. A mitotic count greater than 6 per 10 HPF or microvascular proliferation showed the strongest correlations with a poor outcome. Previous studies attempted to identify molecular markers to predict the malignant transformation and outcomes of oligodendroglioma. Their findings revealed that *NOTCH1* mutations or the NOTCH and PI3K pathways were associated with a poor prognosis in patients with oligodendroglioma^{39,46,61}. Furthermore, *TCF12* transcriptional activity was related to a more aggressive tumor type⁶². However, the *CDKN2A* homozygous

Table 1. Genetic alterations that may stratify risk among patients with adult diffuse gliomas.

Genotype		IDH-mutant	IDH-mutant & 1p/19q codeleted	IDH-wildtype
Tumor types & grade	Grade 2	Astrocytoma	Oligodendroglioma	
	Grade 3	Astrocytoma	Anaplastic oligodendroglioma	
	Grade 4	Astrocytoma		Glioblastoma
Cell cycle		CDKN2A/B hd ⁴⁴	CDKN2A hd (aOG) ⁴⁵	
		CDK4 amp ³⁵		
		RB1 mt/hd ⁴⁶		
RTK/PI3K		PIK3A/PIK3R1 mt ⁴⁶		EGFR amp ⁴⁹
		PDGFRA mt ⁴⁷		
Epigenetic		Global DNA methylation ⁷⁵		
Genomic instability				+7/−10 ⁴⁸
Telomere		ATRX mt ¹⁰¹	pTERT mt ³⁰	pTERT mt ⁴⁹
Others		MYCN amp ⁴²	CIC mt ³⁹	
			NOTCH1 mt ⁶¹	

amp amplification, aOG anaplastic oligodendroglioma, hd homozygous deletion, mt Mutation, pTERT TERT promoter.

Table 2. The definition of astrocytoma, IDH-mutant.

• Astrocytoma, IDH-mutant, grade 2
A diffusely infiltrative astrocytic glioma that is well differentiated and lacks histologic features of anaplasia. Mitotic activity is not detected or low. Microvascular proliferation, necrosis, and CDKN2A/B homozygous deletions are absent
• Astrocytoma, IDH-mutant, grade 3
A diffusely infiltrative astrocytic glioma that exhibits focal or dispersed anaplasia and displays significant mitotic activity. Microvascular proliferation, necrosis and CDKN2A/B homozygous deletions are absent
• Astrocytoma, IDH-mutant, grade 4
A diffusely infiltrative astrocytic glioma that exhibits microvascular proliferation or necrosis or CDKN2A/B homozygous deletion or any combination of these features

Table 3. The definition of glioblastoma, IDH-wild-type, grade 4.

An IDH-wild-type, H3-wild-type, diffuse astrocytic glioma and
One or more of the following:
• Microvascular proliferation
• Necrosis
• TERT promoter mutation
• EGFR gene amplification
• +7/−10 chromosome copy-number alterations

deletion was also linked to shorter survival⁴⁵. Therefore, further studies are needed to identify molecular markers associated with a poor prognosis in patients with oligodendroglioma. A transcription factor activity signature has also been related to a poor prognosis in patients with molecular oligodendroglioma treated with adjuvant radiotherapy, suggesting that this signature is a predictive biomarker in oligodendrogliomas⁶³.

Grading of pediatric-type diffuse gliomas

Neuroepithelial tumors, particularly low-grade gliomas, are the most common solid tumors in children and adolescents, accounting for ~25% of all tumors⁶⁴. They are often referred to as pediatric-type gliomas. Although pediatric-type low-grade gliomas share a similar histology with their adult counterparts, they lack IDH mutations and the 1p/19q codeletion and harbor distinctive genetic abnormalities^{65,66}. The histology of pediatric low-grade diffuse gliomas is often non-specific and overlaps with those of other low-grade tumor

types, including circumscribed gliomas, preventing confident classification. Nevertheless, the prognosis of pediatric-type diffuse gliomas is generally favorable except for frankly malignant counterparts harboring histone H3 alterations⁶⁷. Therefore, the WHO grading system of adult gliomas based on histological findings is not applicable to pediatric gliomas. In the WHO 5th edition, pediatric-type gliomas were divided into two categories, low and high grades; however, specific grades have not been given to all entities³⁴.

EPIGENETIC MARKERS

Glioma epigenetic molecular signatures

Although the epigenetic silencing of O6-methylguanine-DNA methyltransferase (MGMT) by the methylation of its promoter does not correlate with particular subtypes of gliomas, it is a significant biomarker for predicting sensitivity to alkylating agents, such as temozolomide^{68–70}. In elderly patients with glioblastoma, the lack of MGMT promoter methylation has been identified as a negative predictor of responses to alkylating agent chemotherapy^{71,72}. Another important epigenetic signature is the glioma cytosine-phosphate-guanine island methylation phenotype (G-CIMP)⁷³. Nearly all IDH-mutant gliomas are positive for G-CIMP, and MGMT promoter methylation is associated with IDH mutations⁷⁴. More than 75% of G-CIMP-low tumors were found to have alterations in RB pathway genes, including the CDKN2A/B homozygous deletion and amplification of CDK4^{36,39}. Patients with G-CIMP-low IDH-mutant astrocytoma had shorter overall survival than those in the G-CIMP-high group. Therefore, the identification of IDH mutations is indispensable in glioma diagnostic practice but is not yet a part of the grading scheme.

DNA methylation profiling

The genome-wide DNA methylation pattern represents both the cell of origin and somatically acquired DNA methylation changes in cancer. These patterns may remain relatively stable during cancer development, and, thus, an analysis of the tumor DNA status represents those of the original cells, thereby allowing for the more precise identification of tumor types than a morphological analysis^{53,75}. An unsupervised learning approach using DNA methylation and NGS data also enables rare tumors without known canonical genetic alterations to be subclassified within IDH-wild-type gliomas^{46,76}. For example, many IDH-wild-type gliomas belong to pediatric-type gliomas have a single driver gene, typically that in the MAP-kinase pathway⁷⁷. DNA methylation profiling is a reliable and robust approach for the classification of gliomas into molecularly defined subgroups, giving a reasonably accurate estimate of clinical outcomes⁵³.

Grading and CNV

Previous studies reported that IDH-mutant^{35,36} and -wild-type glioblastomas both have higher total CNV levels and evidence of chromothripsis than their lower-grade counterparts⁷⁸, suggesting that total CNV levels are a prognostic factor in diffuse astrocytoma and also that mutations in the genes responsible for overall genomic instability may be an underlying mechanism for astrocytoma with a poor clinical outcome. However, this has not yet been verified because the thresholds for high CNV and somatic mutations varied⁷⁹.

GRADING OF ADULT DIFFUSE GLIOMAS IN RESOURCE-LIMITED SETTINGS

In the WHO 5th classification, genetic testing, such as Sanger sequencing and multiplex ligation-dependent probe amplification, is mandatory for reaching an adequate diagnosis that meets the definition of each tumor type in the classification^{7,34,80–83}. Unfortunately, such genetic testing is not always available, even in developed countries⁸⁴. Nevertheless, the benefits of scientific advances need to be available to every patient with a brain tumor, and the best possible service in each setting should be given to the patients, even using surrogate diagnostic markers. The WHO updated 4th classification¹ and its prior consensus guideline⁸⁵ have proposed an integrated diagnosis that combines all available complementary information. In the “integrated diagnosis,” diagnosis should be layered to provide a format for displaying multiple types of information⁸⁵. It helps to visualize the diagnostic process and eventually increases the correctness of diagnoses in resource-limiting settings using surrogate markers^{83,85–87}. One type of complementary information is MRI^{88,89}. Diffuse gliomas are morphologically highly heterogeneous, and the pathological findings of a surgically resected specimen may only represent part of the entire tumor. Imaging may depict the whole tumor and compensate for the limitations of a pathological investigation⁹⁰. Based on an international survey within the International Society of Neuropathology framework, immunohistochemistry (IHC) was available in the majority of countries surveyed, and an IHC surrogate is helpful for reaching the WHO diagnosis⁸⁴.

Grading of IDH-mutant gliomas (Fig. 1)

When CT and MRI were unavailable, oligodendrogliomas were characterized by mushroom-like, hypertrophic bulging from the cerebral cortex on autopsy (Fig. 1E)¹¹. On MRI, ~90% of genetically defined oligodendroglioma arise from the frontoparietal lobe^{7,88,91,92}. Some studies suggested that oligodendroglioma with 1p/19q codeletion is characterized by an indistinct tumor border and heterogeneous signal intensity (Fig. 1A, B)^{91–95}, while IDH-mutant astrocytomas often show a rather discrete border (Fig. 1A, C, D)⁹¹ and the T2-FLAIR mismatch sign^{96,97}. The provability of 1p/19q codeleted oligodendroglioma is significantly high when calcification is detected on CT⁹⁸. Fluorescence in situ hybridization, a

microsatellite analysis, or MLAP is often used to detect the 1p/19q codeletion. When these molecular tests are not available, the combination of ATRX and H3K27me3 IHC may provide relevant information equivalent to molecular testing (Fig. 1K, L)^{99–101}, although if a codeletion study is not available, “oligodendroglioma, NOS” should be allotted to the diagnosis¹⁰². The 1p/19q codeletion is mutually exclusive to the loss of ATRX and is associated with the loss of H3K27me3 immunoreactivity; however, retained or inconclusive H3K27me3 mandates molecular testing. In the WHO 2016 classification, the diagnosis of IDH-mutant astrocytoma requires the presence of IDH-1 or 2 mutations and the absence of the 1p/19q codeletion, whereas the WHO 2021 classification does not require the 1p/19q codeletion once the loss of ATRX and a typical astrocytic histology are confirmed (Fig. 1F, M)¹⁰³. Although the loss of p16 CDKN2A antibody immunoreactivity does not correlate with the CDKN2A homozygous deletion in malignant mesothelioma, a strong correlation was previously reported between methylthioadenosine phosphorylase (MTAP) IHC and this deletion¹⁰⁴. In addition, MTAP IHC facilitated the detection of the CDKN2A homozygous deletion in diffuse astrocytoma (Fig. 1N)¹⁰⁵. As mentioned previously, the CDKN2A/B homozygous deletion is often absent in a grade II lesion. Therefore, the WHO grade II histology could be used to exclude the homozygous deletion, particularly in resource-limited settings^{44,45}.

CONCLUSIONS

Although the WHO 5th edition of CNS tumor classification has attempted to introduce newly recognized entities, phase out old tumor types, and adjust the taxonomic structure³⁴, a number of grading issues have not yet been resolved. An integrated diagnosis combining histopathological findings with molecular information has provided clearer insights into the classification of brain tumors than a diagnosis by a histopathological assessment alone⁸⁵. In addition, the unsupervised learning approach using DNA methylation and NGS data, which is free from inter-observer variability, has identified distinctive subgroups with unique molecular characteristics¹⁰⁶. However, it has not yet been established whether these subgroups are clinically relevant. Moreover, numerous studies clearly demonstrated that a given tumor often reveals many molecular alterations over space and time, which results in difficulties selecting the most promising treatment regimen for each patient. Therefore, prospective controlled studies are required to clarify this issue.

The current grading scheme based on the expected natural history may not be rational because the clinical course and outcome of each patient may be extensively modified by the tumor location, extent of resection, and type of adjuvant therapy. In that case, grades cannot be assigned unless their designation does not disturb clinical practice. Therefore, in this molecular era of glioma pathology, the grading system needs to evolve to a type of patient-oriented precision medicine, allowing us to not grade a tumor type as a whole, but to provide the most promising guidance to each patient. To achieve this, clinical and histomolecular information needs to be considered and computational pathology assisted by artificial intelligence adopted in order to accurately assess this information¹⁰⁷. These advances will ultimately improve the lives of individuals affected by CNS tumors.

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This review did not have any ethical issue.

ADDITIONAL INFORMATION

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