Anaplastic transformation in adult diffuse glioma: morphological features and genetic alterations

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**Abstract**

Diffuse gliomas are the most frequent primary central nervous system (CNS) tumors, accounting for about 33% of those neoplasms. Glioblastoma (GB) is the most common and the deadliest form of diffuse gliomas with an overall survival shorter than 18 months in most patients. The World Health Organization (WHO) classifies diffuse gliomas into two main categories according to the presumptive cell of origin (astrocytes vs oligodendrocytes): astrocytomas and oligodendrogliomas. Depending on the presence or absence of signs of anaplasia (high cell density, nuclear atypia, mitoses, microvascular proliferation and necrosis), the WHO classification distinguishes grade II (low grade), grade III (high grade or anaplastic), and grade IV (GB) tumors. Nowadays, molecular data are part of an “integrated diagnosis”, taking into account the histopathological features and the genetic alterations detected in the tumor (histomolecular classification). Key genetic alterations in diffuse gliomas may be diagnostic, prognostic, and/or predictive markers. IDH1/2 mutations characterize grade II-III diffuse gliomas and secondary GB. Diffuse astrocytomas display TP53 mutations whereas oligodendrogliomas harbor 1p/19q codeletion. IDH mutation and 1p/19q codeletion are associated with better prognosis. Some genetic alterations play a key role in anaplastic transformation such as p16 loss, chromosome 9p and 10 loss, chromosome 7 gain and EGFR amplification. The latter represents the genetic signature of primary GB. Such specific genetic alterations in diffuse gliomas are potential candidates to targeted therapy which may offer a cure to this deadly disease.
I. Introduction

1. Definition

Gliomas are the most frequent primary brain tumors. They account for one third of all intracranial tumors (1). Gliomas arise from the constituent glial cells of the brain (i.e. astrocytes, oligodendrocytes, and ependymocytes) or their precursors, hence the names "astrocytomas", "oligodendrogliomas", and "ependymomas" (2). Diffuse gliomas are characterized by their infiltrative pattern of growth; they **diffusely invade the surrounding brain parenchyma**. We therefore distinguish **diffuse gliomas** (i.e. astrocytomas and oligodendrogliomas) from **circumscribed gliomas** (e.g. ependymomas, pilocytic astrocytomas) (3). The latter will not be discussed here. Because of their infiltrative nature, diffuse gliomas cannot be completely surgically resected and because of their inherent tendency to malignant transformation, there is no cure for the disease.

![Figure 1: Axial T1-weighted MRI after Gadolinium injection showing a heterogeneous enhancing tumor (glioblastoma) of the right frontal lobe. Note the mass effect on the midline. Image courtesy of George Jallo, MD (http://emedicine.medscape.com/article/340870-overview).](image-url)
2. Classification

Diffuse gliomas are classified based on the 2007 World Health Organization (WHO) classification of nervous system tumors (2). They are classified based upon the morphological appearance of the tumor cells and their similarity to normal astrocytic or oligodendroglial cells.

These tumors are graded on a scale of II to IV, increasing grade meaning increasing malignancy.

**WHO grade II** refers to lesions that are generally infiltrating and have a low mitotic activity but that may recur after local therapy. Some tumor types tend to progress to higher grades of malignancy.

**WHO grade III** refers to lesions with histopathological features of malignancy, including nuclear atypia and increased mitotic activity. These lesions are anaplastic and most often recur after surgery. They are usually treated with aggressive adjuvant therapy.

**WHO grade IV** refers to lesions (glioblastomas) that are mitotically active, necrosis-prone, and generally associated with a rapid disease course and a fatal outcome. The lesions are treated with aggressive adjuvant therapy.
WHO grade I refers to lesions with **low proliferative potential**, a frequently **discrete nature** (e.g. pilocytic astrocytoma), and the **possibility of cure** following surgical resection alone.

According to the putative glial cells of origin, we distinguish **diffuse astrocytomas**, **oligoastrocytomas** and **oligodendrogliomas**. Due to accumulation of genetic alterations in the tumor cells, diffuse gliomas will progress to astrocytomas grade IV (GB) or GB with oligodendroglial component (GB-O).

**GB** (WHO grade IV) is the most frequent and most aggressive primary brain tumor. Most GB (~90%) develop rapidly in patients over 50 years old without past medical history of diffuse glioma (**de novo** or **primary GB**). **Secondary GB** progress from WHO grade II or III astrocytoma or oligoastrocytoma. They present in younger adult patients (mean age at diagnosis 45 years old). These GB subtypes constitute **distinct disease** entities that evolve through **different genetic pathways**, affect patients at **different ages**, and are likely to differ in prognosis and response to therapy. Histologically, primary and secondary GB are largely **indistinguishable**, but they differ in their **genetic** and **epigenetic profiles**.

![Diagram of glioma classification](image)

**Figure 3**: Classification of adult diffuse gliomas according to the WHO classification (2).
Anaplastic transformation or progression, from low-grade diffuse glioma to highly aggressive tumor (GB), is the consequence of accumulating genetic alterations. The two main types of genetic abnormalities are activation of oncogenes and inactivation of tumor suppressor genes. Oncogenes promote cell survival, cell growth and invasion as well as inhibit apoptosis. Inactivation of tumor suppressor genes leads to impaired regulation of cell growth (5).

II. Morphological features and genetic alterations

As stated earlier, we distinguish primary or de novo GB from secondary GB. Secondary GB results from the accumulation of genetic alterations in a recurrent pattern or order (see below). Primary GB follows different gliomagenesis pathways.

1. Diffuse astrocytomases

A. Morphological features

a. Diffuse astrocytoma (WHO grade II)

Diffuse astrocytomas may arise anywhere in the central nervous system (CNS) but they frequently develop within the cerebral hemispheres. The tumor can measure a few centimeters or involve an entire hemisphere. The most extreme form of the disease, involving the whole CNS (both cerebral hemispheres, the brainstem and the spinal cord) is called “gliomatosis cerebri”. The affected CNS area demonstrates a loss of the normal gray-white junction, with the central region being ivory white and generally firmer than normal brain and occasionally associated with small cysts in the white matter. The tumor may appear well demarcated from the surrounding brain tissue but infiltration beyond the outer margin is always present.

Diffuse astrocytomas show mild to moderate increase in cellularity (2-3 times), moderate nuclear pleomorphism, and low mitotic activity. Intercellular edema and cystic degeneration (bubbly collection of fluid) may be seen. Tumor cells in diffuse astrocytomases feed on preexisting blood vessels (“vascular co-option”). There is no neoangiogenesis. Finally, there is no necrosis (6).
**Figure 4:** Diffuse astrocytoma characterized by discrete hypercellularity, mild nuclear pleomorphism, and a microcystic background. ([http://emedicine.medscape.com/article/1780914-overview#aw2aab6b](http://emedicine.medscape.com/article/1780914-overview#aw2aab6b))

**b. Anaplastic astrocytomas (WHO grade III)**

Anaplastic astrocytomas share the main histopathological features with diffuse astrocytomas but also display **signs of focal or diffuse anaplasia**. Anaplastic astrocytomas show **high cell density** and **nuclear pleomorphism** (figure 5). **Mitoses** are easily found. **Neither microvascular proliferation** (neoangiogenesis) **nor necrosis is present** (2).

**Figure 5:** Anaplastic astrocytomas. Note marked cellular pleomorphism. ([http://www.pubcan.org/cancer/4967/anaplastic-astrocytoma/histopathology](http://www.pubcan.org/cancer/4967/anaplastic-astrocytoma/histopathology)).
c. Glioblastoma (WHO grade IV)

Glioblastoma is composed of poorly differentiated neoplastic astrocytes. The morphology of GB may be extremely heterogeneous, hence the name “glioblastoma multiform” (2). Cellular composition varies from small round tumor cells to multinucleated giant cells. Both cellular and nuclear pleomorphisms are prominent features (figure 6). Mitoses, especially atypical ones, are frequent. The key features distinguishing GB from lower-grade astrocytomas are necrosis and microvascular proliferation (2). Typically, GB have large central necrotic areas surrounded by viable tumor tissue as seen on magnetic resonance imaging (MRI). The histologically characteristic form of necrosis consists of irregular necrotic foci surrounded by radially oriented tumor cells (i.e. pseudopalisading necrosis). Microvascular proliferation appears as glomeruloid vascular structures, which consist of hyperplastic endothelial and smooth muscle cells (2).

Figure 6: Glioblastoma multiforme characterized by abnormal blood vessels and pseudopalisading necrotic foci (upper right-hand corner) (http://www.pathology outlines.com/topic/cnstumorglioblastoma.html).

Since histopathologic features can be extremely variable from one region to another, a single small biopsy specimen might not be representative of the whole tumor, especially of the most malignant areas. Thus, the grade may be underestimated. In GB, if necrosis and abnormal blood vessels are not sampled, the final histopathological diagnosis will be “WHO
grade III (anaplastic) astrocytoma”. It is crucial to correlate the morphological features with the MRI findings (e.g. ring-like contrast enhancement with central necrosis in case of GB).

B. Genetic alterations

The genetic background is very important in diffuse gliomas. The tumors can be distinguished (astrocytoma versus oligodendroglioma, primary GB versus secondary GB) based on the genetic alterations present. Identification of key genetic abnormalities plays a role in diagnosing diffuse gliomas but also in evaluating the prognosis of the disease and the potential response to treatment, especially to targeted therapy (7).

→ IDH1/2 mutation

IDH1/2 gene mutation is the earliest genetic alteration involved in gliomagenesis. It appears in neural stem cells or glial progenitors, leading to premalignant cells (7).

IDH1 is the cytosolic NADP-dependent isocitrate dehydrogenase 1, which takes part in the citric acid cycle. The gene is located on chromosome 2q33. It is mutated in 82% of secondary GB, 88% of low-grade astrocytomas, and 79% of oligodendrogliomas. The mutation is detected in only 5% of primary GB (8). Mutations affect in most cases the amino acid arginine in position 132 of the amino acid sequence (IDH1-R132H variant, >90% of cases). Currently, there are different hypotheses on the oncogenic role of IDH1 mutant. Physiologically, the IDH1 enzyme catalyzes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate thereby reducing NADP+ to NADPH (9). The mutation leads to a decreased level of NADPH, which protects the cell from oxidative stress. IDH1 mutation also leads to HIF-1α (hypoxia inducible factor-1 alpha) stabilization, which plays a role in neoangiogenesis in diffuse gliomas, hence supporting tumor growth and invasion. Thus, IDH1 appears as a tumor suppressor gene. On the other hand, IDH gene mutation leads to decreased production of alpha-ketoglutarate and to production of an “oncometabolite”, 2-hydroxyglutarate (2-HG). The latter seems to be tumorigenic (10-11).

IDH2 is the mitochondrial NADP-dependent isocitrate dehydrogenase 2. The gene is located on chromosome 15q26. IDH2 mutations are rare (<5% of diffuse gliomas); they are more often detected in oligodendrogliomas rather than astrocytomas (12). In all cases, the mutations involve codon 172.

To conclude, IDH1/2 mutations play a key role in tumor formation (probably lead to premalignant cells) in diffuse astrocytomas and oligodendrogliomas (9). Their role, if any, in tumor progression is probably minor.
TP53 mutation and PDGR overexpression

**TP53 gene mutation** characterizes diffuse astrocytomas. TP53 is a tumor suppressor gene, which plays a role in cell cycle regulation. It delays entry into S phase and allows cells to correct DNA replication errors or enter apoptosis (13).

**PDGF** (Platelet-Derived Growth Factor), a major mitogen (hence, an oncogene) for glia and connective tissue, is involved in normal development, especially of the CNS (14). PDGF occurs as three isoforms (AA, BB, AB) that each binds with different affinities to two different receptors, PDGFRA and PDGFRB. These receptors share similar structures and belong to the protein-tyrosine kinase superfamily of growth factor receptors (15-16). Astrocytomas overexpress the components of the PDGF-PDGFR pathway. This suggests the involvement of both autocrine and paracrine loops in diffuse gliomas, with activation of PDGFRA in the glioma cells and activation of PDGFRB in the intermingled endothelial cells (17). Some diffuse gliomas display **PDFRA gene amplification**, a potential target to imatinib mesylate (Gleevec®) treatment. Overexpression of PDGFRA is likely an early event in gliomagenesis and is related to tumor progression (18).

**Chromosome 9p loss and 19q loss**

When progressing to malignancy, diffuse astrocytomas most often display loss of chromosome 9p (encompassing p16 locus (see below)) and/or loss of chromosome 19q (2).

**Chromosome 10 loss**

Loss of chromosome 10 is the most frequent genetic alteration in glioblastomas, occurring in 60-93% of these neoplasms. Most cases exhibit loss of an entire chromosome, but distinct patterns of deletion on both the long and the short arms suggest the involvement of multiple tumor suppressor genes, such as PTEN (19).

**Chromosome 7 or 7q gain**

Chromosome 7q or 7 gain is frequent in adult diffuse gliomas, especially in GB. It is associated with shorter survival in anaplastic astrocytomas and low-grade astrocytomas. On chromosome 7q is located the EGFR gene whose amplification (see below) promotes cell proliferation and angiogenesis (20).

**EGFR amplification**

EGFR (Epithelial Growth Factor Receptor) is a transmembrane tyrosine kinase receptor. Receptor dimerization and tyrosine autophosphorylation lead to cell proliferation.
EGFR amplification is observed in approximately 40% of GB. It is the genetic signature of de novo GB and is therefore a diagnostic marker of such tumors. No prognostic role has been shown (21).

P16, Rb and CDK4

The cell cycle is divided into four distinct phases: G1, S, G2, and M. Between the different phases there are control points, it enables the cell to check if all the conditions are respected for the division. One of the most critical checkpoints, the transition from G1 to S phase, involves p16 (CDKN2A), Rb (retinoblastoma), E2F, cdk4, cdk6, and cyclin D proteins. During the G1 phase, Rb binds to transcriptional factors (E2F for example) so the transcription is not possible. But when the cell needs to replicate cyclin D binds to cdk4 and cdk6. That form active kinases that phosphorylate Rb. When Rb is phosphotylated, it is not link to E2F so the transcription is possible and the cell can move to the S phase. The p16 protein is one of the most important regulators of the cdk4-cyclin D complex. p16 blocks the binding of cdk4 to cyclin D, preventing phosphorylation of Rb. Inactivation of p16 or Rb or gain of cyclin D1 or cdk4/6 activity have the same consequence, progression to S phase without checking if the cell is normal and can divide (22).

Approximately half of anaplastic astrocytomas and nearly all GB exhibit inactivation of this checkpoint. In astrocytomas, one of the most frequent mechanisms of cancelling this control is through p16 inactivation. Most of the time p16 inactivation occurs through homozygous deletion. Rb inactivation is the second most common mechanism in the formation of astrocytomas, occurring in approximately 20-30% of high-grade astrocytic tumors. CDK4 gene amplification is detected in approximately 10-15% of high-grade astrocytic tumors (22).

2. Oligodendroglioma

A. Morphological features

a. Oligodendroglioma (WHO grade II)

The main histopathological features of oligodendroglioma are round nuclei with perinuclear halos producing a typical “fried egg” appearance (or “honeycomb” pattern). Oligodendrogliomas typically infiltrate the cerebral cortex with neoplastic cells gathering around neurons (perineuronal satellitosis).

Mucoid microcysts and calcifications are also frequent features of oligodendrogliomas, although non specific.
Mitotic activity is usually sparse and proliferation indices are low. There is neither microvascular proliferation nor necrosis (2).

Figure 7: WHO grade II oligodendroglioma. Note round nuclei with perinuclear halos (« honeycomb » pattern).
http://www.pathologyoutlines.com/topic/cnstumoroligodendroglioma.html

b. **Anaplastic oligodendroglioma (WHO grade III)**

Anaplastic oligodendrogliomas show high cellularity. There are nuclear atypia and mitotic figures. In addition to the characteristic branching capillaries of oligodendrogliomas, microvascular proliferation may be present. Foci of necrosis are present in one third of anaplastic oligodendrogliomas (2).

B. **Genetic alterations**

As in astrocytoma development, gliomagenesis in oligodendroglial tumors starts with IDH1/2 mutation in neural stem cells or glial progenitors. The second step of tumorigenesis involves 1p/19q codeletion.

**1p/19q codeletion**

When IDH1/2-mutated glial cells loose simultaneously chromosome arms 1p and 19q (1p/19q codeletion, which is the result of an unbalanced translocation between chromosomes 1 and 19), it gives rise to an oligodendroglioma. This genetic alteration is observed in about 80% of oligodendrogliomas and in less than 10% of (so-called) diffuse astrocytomas. One candidate (tumor suppressor) gene on chromosome 1p is FUBP1 (Far-
Upstream element Binding Protein). On chromosome 19q, the candidate gene is CIC (Capicua transcriptional repressor). These two genes are mutated in 1p/19q codeleted oligodendrogliomas (7). It is important to note that IDH1/2-mutated 1p/19q codeleted oligodendrogliomas have a good prognosis with an overall survival time longer than 15 years.

**P16 deletion**

Deletion of the cell cycle regulator p16 is an uncommon event in oligodendrogliomas, but it is a prognostic factor in oligodendrogioma patients. In a study by Cairncross et al., approximately 15% of anaplastic oligodendrogliomas had p16 gene deletion and these patients exhibited significantly shorter overall survival when compared to patients without this alteration (22).

**Chromosome 9p and 10 loss** also plays an important role in anaplastic transformation of oligodendrogliomas.

3. **Oligoastrocytoma**

   **A. Morphological features**

   Oligoastrocytomas are defined as tumors with two cell components resembling astrocytoma and oligodendroglioma. The two cellular components are usually intermingled. In rare cases, the tumor is biphasic and displays distinct astrocytic and oligodendroglial components (2).

   WHO grade II oligoastrocytomas show moderate cellularity and low mitotic activity. Microcystic changes and calcifications may be present.

   In anaplastic (WHO grade III) oligoastrocytomas, diagnostic features are high cellularity, obvious mitotic activity, cellular pleomorphism and microvascular proliferation. If there is necrosis, the tumor is considered as GB (WHO grade IV) because of the similarly dismal prognosis (2).

   **B. Genetic alterations**

   Oligoastrocytomas are heterogeneous tumors that have molecular features that overlap with either oligodendrogliomas or astrocytomas.

   On a genetic standpoint, 30 to 70% of oligoastrocytomas show LOH (Loss of Heterozygosity) 1p and LOH 19q thus genetically resembling oligodendrogliomas, whereas ~30% show mutations in the TP53 gene or LOH 17p suggesting a relation to astrocytomas (23).
The next edition of the WHO classification of nervous system tumors will not include mixed gliomas. Such tumors will be (re)classified either in astrocytomas or oligodendrogliomas based on the genetic alterations detected (i.e. TP53 mutation versus 1p/19q codeletion).

**Figure 7**: Summary of the most frequent genetic alterations during the progression of adult diffuse gliomas (23-24).
III. Therapy

The standard of care for GB is maximal safe surgical resection, followed by radiation therapy with concurrent and adjuvant temozolomide (according to the Stupp protocol) (26). However, conventional treatments to fight diffuse gliomas have limited efficacy or cause significant side effects. In lower-grade diffuse gliomas, radiotherapy is deferred as long as possible since it may cause cognitive impairments in long-term survivors. Treatments at best, slow down tumor growth and progression. The disease is uniformly fatal. Median overall survival of GB patients (after surgery, radio- and chemotherapy) is only 15 months. Recent molecular advances have contributed to a better understanding of GB pathophysiology, thus offering the possibility of developing new molecular therapies. GB are highly suitable for targeted molecular therapy because they display a set of defined molecular alterations and signaling pathway disruptions that present clear targets.

**IDH–R132H targeted therapy**

- IDH1–R132H mutant protein may be a potential target for targeted therapy. A selective **IDH1–R132H mutant enzyme inhibitor**, AGI-5198, has been developed and has been found to block the production of 2-hydroxyglutarate and induce histone demethylation in a dose-dependent manner, although without significant CIMP (CpG island methylator phenotype) profile alteration (IDH1/2-mutated gliomas are characterized by a DNA hypermethylation profile called CIMP). Tumor growth was inhibited, independent of dosage, while the drug was well tolerated without signs of toxicity (27). This therapeutic approach, directed at a tumor-specific mutation, shows great promise.

- IDH1-R132H mutant protein may be a potential target for immunotherapy. IDH1-R132H mutant protein is a potential tumor-specific neoantigen expressed in all tumor cells. It could be used for mutation-specific vaccination because it contains a suitable immunogenic epitope. Peptides encompassing the mutated region are presented on major histocompatibility complexes (MHC) class II and induce mutation-specific CD4+ T Helper-1 (TH1) responses. CD4+ TH1 cells and antibodies spontaneously occurring in patients with IDH1-R132H-mutated gliomas specifically recognize IDH1-R132H. Peptide vaccination of mice devoid of mouse MHC and transgenic for human MHC class I and II with IDH1-R132H p123-142 peptide results in an effective MHC class II-restricted mutation-specific antitumor immune response. As IDH1-R132H protein is present in all tumor cells, an anti-IDH1-R132H specific vaccine may represent a novel therapeutic strategy for IDH1-R132H-mutated gliomas (28).
**EGFR targeted therapy**

EGFR maintains tumor heterogeneity and, by targeting EGFR, a better tumor growth control might be achieved. Soeda *et al.* showed on GB cancer stem cell lines that EGF, but not other growth factors, promoted cancer stem cell proliferation (29). To date, the small-molecule inhibitors of EGFR that were introduced in clinical trials include gefitinib, erlotinib, and nimotuzumab. However, the clinical implications of EGFR amplification or EGFR overexpression in GB are not yet clear. Several studies have shown contradictory results, with a positive impact of EGFR inhibitors on survival in some studies, decreased survival and poor prognosis in others or no impact on survival at all.

**TP53 targeted therapy**

Viruses that can specifically target TP53-deficient tumor cells have been developed. The use of E1B gene-defective adenoviruses represents an extremely promising approach. The genetically modified adenoviruses take advantage of dysfunctional p53 in cancer cells to selectively kill them. The virus induces the S phase of the host cell cycle so that viral DNA replication can proceed. The E1B region of the viral genome encodes a protein that binds and inactivates p53 in infected cells. This binding is essential to virus replication, thus an adenovirus deficient for the E1B protein may replicate only in cells that are deficient for p53 function. This should allow selectivity for tumor cells with mutated TP53 and spare normal cells (30). An example of this is ONYX-015, a mutant adenovirus generated by deletion of an 800 bp fragment of the E1B gene. Recently, human glioma xenografts were shown to be susceptible to ONYX-015 replication *in vivo*, an effect that was enhanced by radiation therapy. These findings led to a phase I clinical trial for recurrent gliomas in which ONYX-015 was administered to the tumor bed following surgical resection. The virus was well tolerated with no evidence of toxicity. However, efficacy was not determined. A variety of other oncolytic viruses are currently under development that may lead to greater efficacy and specificity in the future (31).

**Conclusions**

Morphological features in diffuse gliomas are strongly correlated with distinct genetic alterations, which can be used for developing targeted therapies. The genetic make-up of diffuse gliomas is key to accurately classify those neoplasms and choose the most appropriate treatment for a given patient. Many different gene therapy approaches for malignant gliomas have already been developed, each with its own merits and limitations. In the near future, it will be important to study in which way such new molecular approaches could be implemented and combined with conventional treatment strategies of glioma.
therapy and also to understand the pathophysiology of the disease in order to achieve maximum benefit and minimal toxicity.

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