Précis

Definition of terms

- Heterozygous mutation: genetic alteration affecting a single allele
- Homozygous mutation: genetic alteration affecting both alleles
- Somatic mutation: genetic alteration that is acquired, not inherited
- Germline mutation: genetic alteration that is inherited
- Gene amplification: increase in the number of copies of a gene sequence
- Gene deletion: elimination of one or more copies of a gene sequence
- Loss of heterozygosity: loss of a genomic region on one allele, resulting in a single remaining copy
- Oncogene: originates as a proto-oncogene, which normally enables cell growth, but when altered becomes an oncogene, which promotes uncontrolled cell growth
- Tumor suppressor: An element that normally regulates cell cycle progression, the inactivation of which can lead to uncontrolled cell growth
- Genome: the complete set of DNA in an organism, organized into genes
- Epigenome: the complete set of chemical alterations to an organism's DNA and histones
- Transcriptome: the complete set of all RNA molecules transcribed in an organism
- Apoptosis: programmed cell death
- Telomerase: a ribonucleoprotein enzymatic complex that adds DNA repeats to the ends of telomeres
- Alternative lengthening of telomeres: a telomerase-independent mechanism of lengthening telomeres that utilizes homologous recombination
- Oncometabolite: metabolite that can promote malignant transformation when accumulated
- Neomorphic: novel activity not present in the original enzyme, usually conferred by genetic alteration
- Translocation: chromosomal rearrangement between non-homologous chromosomes, common to cancer.

Key points to be covered

- Malignant glioma currently is an incurable disease and is the most common primary malignant brain tumor in adults.
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- According to guidelines set by the World Health Organization (WHO), gliomas are classified into different subtypes based on histology and grade (I–IV).
- Pilocytic astrocytomas are benign gliomas driven by activation of the MAPK pathway, usually accomplished by BRAF alterations.
- Adult ependymomas occur most commonly in the spinal cord, usually with alterations in NF2.
- Astrocytomas can progress to higher grades, including secondary GBM, and have frequent co-occurring alterations in IDH1/2, ATRX, and TERT.
- Oligodendrogliomas are generally less aggressive than astrocytomas, and have co-deletion of 1p/19q and mutations in IDH1/2, the TERT promoter, CIC, and FUBP1.
- Mixed oligoastrocytomas pose challenges for diagnosis and have genetic alterations common to either astrocytomas or oligodendrogliomas.
- Glioblastomas, the most aggressive glioma subtype, arise either as primary or secondary lesions, which are genetically and clinically distinct diseases.
- Primary GBM is the most common glioma, usually presenting in older patients, and is characterized by TERT promoter mutations, EGFR amplification and PTEN loss.
- Secondary GBM occurs in younger patients and arises from lower-grade astrocytomas, bearing similar co-occurring alterations in IDH1/2, ATRX, and TERT.
- Genetic alterations drive gliomagenesis, give insight into glioma biology, assist in diagnosis, and are essential for developing personalized clinical care.
- Signaling pathway alterations in GBM primarily affect the Rb, p53, and RTK/PI3K/AKT pathways.
- GBM can be classified into transcriptional subtypes, known as classical, proneural, neural, and mesenchymal.
- IDH1/2 mutations, most commonly IDH1 R132H, are frequent in astrocytomas, oligodendrogliomas, and secondary GBMs and occur early in gliomagenesis.
- The mutations in IDH1/2 occur in the enzymatic active site, enabling neomorphic production of the oncometabolite D-2HG.
- D-2HG inhibits α-KG-dependent enzymes, resulting in DNA (G-CIMP) and histone hypermethylation.
- MGMT promoter methylation increases sensitivity to alkylating agents and confers a survival benefit in GBM patients treated with temozolomide.
- 1p19q loss of heterozygosity is common in oligodendroglioma and associated with inactivating mutations in the genes CIC and FUBP1.
- Telomere maintenance in glioma is achieved by either TERT promoter mutations, in oligodendroglioma and primary GBM, or ATRX mutations, in astrocytomas and secondary GBM.
- TERT promoter and ATRX mutations are frequent in glioma and are mutually exclusive of each other.
- The mutual exclusivity or co-occurrence of TERT promoter and IDH1/2 mutations effectively stratifies patients into clinically relevant molecular subtypes of glioma.

Introduction

Glia are the most abundant cell type in the human central nervous system (CNS), with functions critical to neural development, plasticity, signal transduction, cell microenvironment, and immunity. Tumors of glial cells account for 80% of all primary CNS malignancies and
are the second most common primary brain tumor in adults.\textsuperscript{1} Gliomas are graded I–IV based on the histopathological and clinical criteria set by the World Health Organization (WHO).\textsuperscript{2} While low-grade (I–II) gliomas are generally slow-growing, high-grade (III–IV) lesions are highly proliferative, invasive malignancies with poor prognosis. The focus of this chapter will be on adult gliomas, in particular pilocytic astrocytoma (grade I), ependymoma (grade I–III), progressive astrocytoma (grade II–III), oligodendroglioma (grade II–III), mixed oligoastrocytoma (grade II–III), and glioblastoma (grade IV, GBM). GBM is the most common malignant primary brain tumor (45.6%) and the most aggressive of the gliomas, with a patient median overall survival of approximately 12 months.\textsuperscript{1,3}

The type and grade of a malignant glioma dictate a great deal regarding the patient's clinical course, including the treatment plan and prognosis. However, accurate histopathologic diagnosis of glioma can be challenging, considering the extent of tumor heterogeneity, overlap of oligodendroglial and astrocytic features in some tumors, and ambiguity in criteria for particular subtypes. Recent studies have clarified a set of distinct genetic alterations that largely characterize each type of glioma, generating tumor-specific molecular signatures. This not only gives insight into the biological mechanisms behind these cancers, but also provides more objective genetics-based markers for diagnosis of glioma subtypes, enabling more accurate prognostic stratification of patients.\textsuperscript{4} This chapter will elaborate further on these key genetic alterations and our understanding of their roles in glioma tumorigenesis and progression.

**The purpose and utility of genomics in glioma**

Cancer at the most basic level is a genetic disease, caused by the accumulation of genetic alterations that enable deregulated cell growth and proliferation, resulting in tumor formation. Investigations into the genetic composition of glioma have revealed frequent mutations and copy number alterations that are characteristic of distinct glioma subtypes. These alterations include recurrent point mutations in the promoter of telomerase reverse transcriptase (\textit{TERT}), seen in over 80% of primary GBMs and oligodendrogliomas, and mutations in the metabolic enzymes isocitrate dehydrogenase 1 and 2 (\textit{IDH1, IDH2}), which are found in over 78% of grade II and III astrocytomas, oligodendrogliomas, and secondary GBMs.\textsuperscript{4,5} Subsequent studies have defined the effects of these mutations on the glioma transcriptome, epigenome, metabolome, and proteome using primary tumor tissues, cell lines, and animal models. These efforts have aided our understanding of the functional purpose of these and other genetic alterations in glioma. Furthermore, identification of these recurrent mutations in tumors alongside histologic examination offers a more precise method of classifying glioma subtypes, providing better prediction of tumor behavior, therapeutic response, and patient outcomes than diagnosis based on histologic features alone.\textsuperscript{4}

Despite advances in surgical management and treatment, malignant glioma remains an incurable disease with a dismal prognosis, accounting for >13,000 deaths per year in the USA.\textsuperscript{1} It is increasingly evident that genomic analysis on a case-by-case basis is a powerful tool in assessing each patient's disease and optimizing management, with the hope that future care will be complemented by effective personalized therapies targeting the genetic aberrations specific to each patient's cancer. With this in mind, the following sections will review the genomic features of each major subtype of glioma in adults and the function of these mutations in tumor biology. We will then discuss the implications of these genetic signatures on the diagnosis and prognosis of patients affected by glioma, illustrating the utility of genomic analysis in both advancing our understanding of glioma and improving management of this disease.
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Classification of adult gliomas

As stated previously, the WHO has provided guidelines for the classification and grading of CNS tumors into various subtypes based primarily on histopathologic criteria.\(^1\,2\) Low-grade (I–II) gliomas are slow-growing tumors made up of well-differentiated tumor cells. While grade I tumors are largely curable, grade II gliomas often progress to higher grades. High-grade gliomas (III–IV) are rapidly growing, malignant tumors that are made up of anaplastic cells that invade into neighboring tissue and have high rates of recurrence following surgical resection.

The most common adult gliomas fall under the categories of astrocytic, oligodendroglial, oligoastrocytic, and ependymal tumors. The astrocytic tumors, accounting for 75% of gliomas, include: pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III), and glioblastoma (GBM, grade IV) (Figure 1.1).\(^1\,2\) Grade IV GBMs can either arise de novo as primary GBM, or less commonly, from a lower-grade lesion as a secondary GBM. The oligodendrogial tumors account for 5.7% of gliomas, and include oligodendroglioma (also known as well-differentiated oligodendroglioma, grade II), and anaplastic oligodendroglioma (grade III). The mixed-histology oligoastrocytic tumors, which include oligoastrocytomas (grade II) and anaplastic oligoastrocytomas (grade III), account for 3.3% of gliomas. Finally, the

![Glioma Distribution by Histologic Subtype](image)

**Figure 1.1** Distribution of gliomas based on histologic subtype in the USA. Glioblastoma multiforme accounts for the majority of gliomas (54.5%). Astrocytomas of all grades, including glioblastoma multiforme, account for approximately 75% of all gliomas. NOS, not otherwise specified. (Based on statistics from 2007 to 2011 collected by Central Brain Tumor Registry of the United States [CBTRUS].)
ependymomas (grade I–III), account for 6.7% of gliomas (Figure 1.1). Though this list of glioma subtypes is not exhaustive, the most common entities have been included. The following section will detail the key histologic and epidemiologic characteristics of each subtype of glioma, while providing a brief introduction to the most relevant genetic alterations for glioma classification.

Grade I pilocytic astrocytoma

Pilocytic astrocytoma is primarily a pediatric disease, but it also affects young adults. This tumor commonly arises in the cerebellum and is characterized as a slow-growing, well-circumscribed mass that rarely progresses to a higher-grade lesion and responds well to surgical resection.2 Pilocytic astrocytomas are unique among gliomas as they usually possess few genetic alterations. These alterations often lead to activation of a single pathway, the mitogen-activated protein kinase (MAPK) pathway. The most common alteration is KIAA1549-BRAF (73%), a chromosomal tandem duplication that results in the formation of a constitutively active fusion protein. Other alterations found in pilocytic astrocytoma include BRAF point mutations (V600E) and additional BRAF fusions that result in constitutive kinase activation. Inactivating mutations in the gene NF1 are also seen in pilocytic astrocytoma, usually in patients affected by neurofibromatosis type 1 (NF1), who have existing germline mutations and are at increased risk for developing these tumors.6

Grade II–III ependymoma

Ependymomas arise from ependymal cells that line the ventricular system and spinal cord, and account for 7% of all gliomas.1 In children, these tumors most often arise intracranially (90%), while in adults they tend to localize to the spinal cord. Spinal cord ependymomas have a better outcome than intracranial lesions; however, the delicate location of the tumor can make surgical resection challenging and leave patients with neurological deficits.2 Ependymomas are grade II tumors that are slow-growing and generally amenable to surgical excision, while grade III anaplastic ependymomas are proliferative tumors that tend to infiltrate the surrounding tissues or metastasize along the ventricular system.2 Amplification of chromosome 7 and loss of chromosome arm 22q are the most common genetic alterations in adult ependymoma.2 Fifty percent of adults with spinal cord ependymoma have mutations in the neurofibromatosis 2 (NF2) gene, located on 22q, and an increased incidence of ependymoma is seen in patients with the disease neurofibromatosis type 2, who have germline mutations in NF2.2,5

Grade II and III astrocytoma: diffuse and anaplastic astrocytoma

Diffuse and anaplastic astrocytomas together account for 12% of all primary CNS malignancies.1 These tumors commonly localize to the cerebral hemispheres and are characterized by indistinct borders with microscopic extensions that spread diffusely into the surrounding tissue. The invasive nature of these tumors makes them difficult to resect completely, predisposing patients to tumor recurrence and progression. Histologically, grade II diffuse astrocytomas are composed of well-differentiated astroglial tumor cells with mildly atypical nuclei and increased cellularity. Grade III anaplastic astrocytomas are characterized by the presence of mitotic figures, as well as increased nuclear atypia and cellularity relative to
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Grade II and III astrocytomas can present de novo and both tend to progress to higher grades (II→III or II/III→IV). However, not all patients survive to the point of developing a grade IV secondary GBM, reflected in part by the incidence rate of grade II and III astrocytomas being two to three times greater than that of secondary GBM. The progression from low-grade (II) astrocytoma to secondary GBM takes an average of 5 years; therefore, efforts are made to treat and closely monitor lower-grade gliomas for progression (Figure 1.2).

Compared to diffuse astrocytomas, anaplastic lesions present in older patients (median age of diagnosis 53 vs. 48 years) and have a poorer prognosis (5-year survival 27.3% vs. 47.4%) (Figure 1.3). Both grade II and III astrocytomas are characterized by a particular genetic signature, consisting of frequent (>75%) co-occurring mutations in the genes IDH1/2, ATRX, and TP53 (Figure 1.4).

Grade II and III oligodendroglioma: well-differentiated and anaplastic oligodendroglioma

Oligodendrogliomas account for 4.9% of all primary malignant brain tumors. The population affected by oligodendroglioma is generally younger than that of astrocytoma, with
the median age of diagnosis for grade II and III oligodendroglioma being 43 and 49 years, respectively (Figure 1.3). In addition, oligodendrogliomas are generally less aggressive tumors than astrocytomas, with 79.5% of grade II and 52.2% of grade III oligodendroglioma patients surviving past 5 years. Oligodendrogliomas frequently arise in the frontal lobes of the cerebral hemispheres and form microcalcifications around neurons and blood vessels that can occasionally be seen on radiographic imaging. Histologically, oligodendrogliomas are characterized by hypercellular masses of uniform and infiltrating cells with rounded nuclei and perinuclear halos, an artifact of formalin fixation and paraffin embedding that gives a characteristic “fried egg” appearance. Well-differentiated oligodendrogliomas (grade II) can evolve into high-grade (grade III) anaplastic oligodendrogliomas, which have increased mitotic activity, vascular proliferation, and necrosis. Oligodendrogliomas most frequently harbor co-occurring IDH1/2 mutations, TERT promoter mutations, 1p/19q chromosomal loss, and alterations in the genes encoding the transcriptional regulators CIC and FUBP1 (Figure 1.4).

Grade II and III oligoastrocytoma: oligoastrocytoma and anaplastic oligoastrocytoma

Oligoastrocytomas have histologic features of both astrocytomas and oligodendrogliomas: either diffusely intermixed or, less commonly, as biphasic tumors with these elements separated into distinct areas. Historically, the diagnosis of oligoastrocytoma has posed a challenge for neuropathologists due to poorly defined criteria for classification and subjectivity in interpretation of histologic features. Although collectively oligoastrocytoma patients have a 5-year survival of 61.1%, there is substantial clinical variability. Recent work has helped ameliorate this histologic and clinical ambiguity by revealing a genetic delineation of oligoastrocytomas into two distinct groups: oligoastrocytomas with co-occurring...
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Figure 1.4 Common genetic alterations in glioma occur in distinct patterns unique to each histology, defining molecular subtypes of glioma. Primary GBM is largely characterized by TERT promoter mutations, while secondary GBM has a mutational spectrum similar to those seen in lower-grade astrocytomas, including co-occurring mutations in IDH1/2, ATRX, and TP53. Oligodendrogiomas have co-occurring mutations in IDH1/2, the TERT promoter, and concurrent loss of 1p19q. While oligoastrocytomas have mixed histologic patterns, genetically they resemble the patterns of either astrocytoma or oligodendroglioma.

Mutations in ATRX, IDH1/2, and TP53, and oligoastrocytomas with co-occurring mutations in the TERT promoter, IDH1/2, and 1p/19q loss of heterozygosity, reflecting the genetic patterns of astrocytomas and oligodendrogiomas, respectively (Figure 1.4). These genetic associations are evident clinically, as oligoastrocytoma patients with 1p/19q co-deletion have a longer progression-free survival than those with intact 1p/19q (60 vs. 30 months), paralleling the generally better prognosis of patients with oligodendrogiomas when compared to those with astrocytomas.
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Grade IV glioblastoma: primary and secondary glioblastoma

GBM is the most common malignant brain tumor, representing almost half (45.6%) of all primary CNS malignancies. GBM is also one of the deadliest cancers, with an average survival of 12 months after diagnosis and a 5-year survival of <5.0%. Histologically, they are characterized by highly anaplastic cells with frequent mitoses, vascular proliferation, and necrosis. As mentioned previously, GBMs can develop in two different ways: either de novo as primary GBMs without evidence of a lower-grade precursor glioma, or as secondary GBMs, which develop from lower-grade gliomas (II–III). While histologically these two types of GBM are largely indistinguishable, there are key differences between primary and secondary GBM in terms of the populations they affect, their clinical behavior, and the underlying genetic alterations that drive their development. Primary GBM, accounting for >90% of GBMs, typically arises in older patients with a mean age of 62 years, while secondary GBM, which accounts for the remaining 5–10% of cases, has a mean age at diagnosis of 45 years. While it usually takes several years for secondary GBM to develop from a lower-grade astrocytoma, most patients with primary GBM experience their first symptoms only 3 months before diagnosis. Secondary GBM is also inherently less aggressive, with patients exhibiting a better overall survival from the time of diagnosis than patients with primary GBM.

Until recently, clinical history was the primary method of differentiating between primary and secondary GBM, due to their histologic similarity. However, recent genomic analyses have revealed that these two subtypes of GBM are not only clinically, but also genetically, distinct diseases. Primary GBM is most clearly distinguished by TERT promoter mutations in the absence of IDH1/2 mutations, while secondary GBM is characterized by co-occurring mutations in IDH1/2, ATRX, and TP53, without TERT promoter mutations (Figure 1.4). Other alterations found in primary GBM, such as EGFR amplification, 10q loss of heterozygosity (LOH), and CDKN2A deletion, will be discussed in the following sections.

Common genetic alterations in glioma

There have been a number of studies aimed at understanding the alterations that drive gliomagenesis from a genetic, epigenetic, and transcriptional viewpoint. While many of these investigations have focused on GBM, the findings have also provided insight into lower-grade gliomas, highlighting the importance of these alterations to glioma initiation and progression. This section outlines many of the significant discoveries resulting from these efforts, starting with the major signaling pathway alterations and transcriptional subtypes of GBM, followed by an in-depth discussion of the recurrent genetic mutations and their functions in glioma, including alterations in IDH1/2, the MGMT promoter, 1p/19q, CIC/FUBP1, ATRX, and the TERT promoter.

Signaling pathway alterations in glioblastoma

In 2008, groups at Johns Hopkins University, Duke University, and The Cancer Genome Atlas (TCGA), carried out large-scale investigations using genetic sequencing, copy number analysis, and gene expression profiling to identify the major genetic alterations in GBM. Three dominant signaling pathways were found to be affected by somatic mutation, gene amplification, and/or homozygous deletion: the p53 (TP53, MDM2, MDM4), Rb (RB1, CDK4/6 and CDKN2A/B), and RTK/P13K/PTEN (EGFR, PDGFRA, PIK3CA, PIK3R1, PTEN, AKT) pathways. A subsequent study by the TCGA in 2013 confirmed and expanded on many of
these findings. It is worth noting that these pathways have substantial overlap, such as CDKN2A (Rb pathway) and AKT (RTK/P13K/PTEN pathway) both affecting MDM2 levels (p53 pathway), illustrating that single alterations can have complex and far-reaching effects on multiple cellular processes.

**Genetic alterations in the p53 pathway: TP53 and CDKN2A/MDM2/MDM4**

Alterations to the p53 pathway are found in the vast majority of GBMs (86%), with deletion or mutation of TP53 (27.9%), amplification of MDM1/2/4 (15.1%), and homozygous deletion of CDKN2A/B (61%) occurring in a largely mutually exclusive fashion. p53 is a tumor suppressor encoded by the TP53 gene and that functions as a critical regulator of the mammalian cell cycle, promoting repair pathway activation, growth arrest, or apoptosis in response to stressors such as DNA damage, oxidative stress, and oncogene activation. Though tumor suppressors generally require loss of both alleles in order to lose regulatory control, TP53 mutations can act in a dominant negative fashion, wherein a heterozygous mutation is sufficient to compromise native p53 regulatory function and promote oncogenesis. MDM2/4 and p53 interact through an auto-regulatory negative-feedback loop in which p53 activates transcription of MDM2/4, which in turn targets p53 for ubiquitin-mediated proteasomal degradation, limiting p53’s growth-suppressive activity in the absence of cellular stress. CDKN2A encodes two proteins through alternate reading frames p16ink4a and p14arf. p14arf responds to over-activation of growth signaling pathways by inhibiting MDM2, allowing p53 to accumulate within the cell, inducing cell cycle arrest and/or apoptosis.

**Genetic alterations in the Rb pathway: RB1, CDK4, and CDKN2A/B**

The Rb pathway is altered in 79% of GBMs, with inactivating alterations in RB1 (7.6%), amplifications of CDK4 (14%), and homozygous deletions of CDKN2A/B (61%) occurring with high mutual exclusivity. RB1 loss of heterozygosity is also found in approximately 40% of GBMs. Rb is a tumor suppressor that normally binds and inactivates E2F transcription factors, which are potent drivers of G0→S cell cycle progression. Rb is phosphorylated by cyclin-dependent kinases, CDK4 and CDK6, blocking the ability of Rb to bind E2F and allowing cell cycle progression. In addition to its role in the p53 pathway described above, CDKN2A is also involved in the Rb pathway, where P16ink4a inhibits cyclin-dependent kinases, leaving Rb bound to E2F and halting the cell cycle. The high frequency of CDKN2A deletions and its regulatory role in both the p53 and Rb pathways indicate the importance of CDKN2A loss of function as a mechanism for GBM tumorigenesis.

**Genetic alterations in the RTK/P13K/AKT pathway: EGFR, PDGFRA, PIK3CA, PIK3R1, PTEN, AKT**

Alterations to the RTK/P13K/AKT pathway occur in 90% of GBMs, with 39% of cases bearing two or more alterations in this pathway. Amplification of genes that encode receptor tyrosine kinases (RTKs) occurs in 66% of GBMs (EGFR 57.4%; PDGFRA 13.1%; FGFR 3.2%; MET 1.6%), leading to increased activation of the P13K/AKT signaling pathway. Normally, cells depend on RTKs to respond to external growth factor cues to activate pathways that stimulate cell growth and proliferation. However, cancer cell proliferation is innately uncoupled from such external cues, allowing tumors to grow in the absence of environmental proliferative signals. In GBM, this independence is accomplished through alterations of RTKs, such as gene amplification and/or mutations that lead to receptor auto-activation. EGFR amplification is the most common of these, occurring in more than half of GBMs. Among EGFR-amplified tumors, many also have EGFR mutations, the most common of