Established and emerging variants of glioblastoma multiforme: review of morphological and molecular features

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Abstract
Since the recent publication of the World Health Organization brain tumour classification guidelines in 2007, a significant expansion in the molecular understanding of glioblastoma multiforme (GBM) and its pathological as well as genomic variants has been evident. The purpose of this review article is to evaluate the histopathological, molecular and clinical features surrounding emerging and currently established GBM variants. The tumours discussed include classic glioblastoma multiforme and its four genomic variants, proneural, neural, mesenchymal, classical, as well as gliosarcoma (GS), and giant cell GBM (gcGBM). Furthermore, the emerging variants include fibrillary/epithelial GBM, small cell astrocytoma (SCA), GBM with oligodendroglial component (GBMO), GBM with primitive neuroectodermal features (GBM-PNET), gemistocytic astrocytoma (GA), granular cell astrocytoma (GCA), and paediatric high-grade glioma (HGG) as well as diffuse intrinsic pontine glioma (DIPG). Better understanding of the heterogeneous nature of GBM may provide improved treatment paradigms, prognostic classification, and approaches towards molecularly targeted treatments.

Key words: glioblastoma multiforme, GBM, brain tumours, glioma, astrocytoma, variant, WHO.

Introduction
Glioblastoma multiforme (GBM), a grade IV astrocytoma as currently defined by the World Health Organization (WHO) classification, is the most common primary brain tumour with a median survival of approximately 1 year following current multi-modal treatments [89,114,162]. Recent data suggest that approximately 30% of all primary and 80% of all malignant brain tumours are accounted for by the broad category of gliomas, while 54% of all malignant brain tumours are GBM and occur at a rate of 3.20 per 100 000 person-years [25]. Marked diversity exists in the clinicopathological characteristics of GBM and recent studies have suggested the presence of a cancer stem cell (CSC) population may account for this heterogeneity as well as provide a mechanism of tumour recurrence and therapeutic resistance (Fig. 1A, B) [15,38,71]. CSCs have shown the ability to undergo continual self-rene-
Fig. 1. Mechanisms of gliomagenesis and glioblastoma multiforme variants

Schematics are shown of gliomagenesis, factors affecting GBM growth and dissemination, as well as defined and emerging GBM variants. A) The stochastic and B) hierarchical/cancer stem cell (CSC) models for gliomagenesis are demonstrated. The stochastic model implies that tumour cells clonally arise from a single cell where each subsequent daughter cell has an equal potential to form a tumour. The CSC model suggests that a specific and small population of cells undergoes self-renewal and differentiation to form additional cells of a tumour. C) Regardless of the model involved in gliomagenesis, a variety of factors affect growth and dissemination. D) Various underlying, complex mechanisms govern GBM heterogeneity. The World Health Organization established GBM variants include classic GBM, gliosarcoma (GS), and giant cell GBM (gcGBM). Recent genomic data has supported the presence of four genomic GBM subtypes in primary, classic GBM, namely proneural, mesenchymal, classical and neural. Emerging GBM variants include fibrillary/epithelial GBM, small cell astrocytoma (SCA), GBM with oligodendroglial component (GBMO), GBM with primitive neuroectodermal tumour (GBM-PNET), gemistocytic astrocytoma (GA), granular cell astrocytoma (GCA), and paediatric high-grade glioma (HGG), and diffuse intrinsic pontine glioma (DIPG).
Presenting age, co-express markers of distinct neural/glial lineages, confer tumorigenicity, and demonstrate chemoresistance. The diagnosis, prognosis, treatment, and investigation of GBM are further complicated by its heterogeneity (Fig. 1C). This article will review the most recent data regarding understanding of genetic features of emerging GBM variants as well as their impact on patient prognosis.

Multiple classification schemes have been designed to organize the heterogeneity of gliomas. These systems have been refined over the past 100 years and include those of Bailey and Cushing [6], Kernohan [74], Ringertz [142], Nelson [31,89], St. Anne-Mayo [31,89], and the most recent being the WHO classification [89]. The WHO system, developed from the St. Anne-Mayo grading scheme, includes grades based on four key histomorphological features, including nuclear atypia, mitotic figures, microvascular proliferation and necrosis [89]. Simplistically, lesions with three to four variables are grade 4 tumours (GBM), those with two are grade 3 tumours (anaplastic/malignant astrocytoma), and those with one parameter are grade 2 tumours (diffuse astrocytoma). Grade 1 tumours (pilocytic astrocytomas) are related but distinct lesions. The current WHO classification system recognizes three distinct GBM variants, namely classic GBM, gliosarcoma (GS), and giant cell GBM (GC-GBM) (Table I, Fig. 1D) [89]. Moreover, recently suggested WHO variants warranting investigation have emerged (Table II, Fig. 1D). Previous studies evaluated the morphological and genetic diversity of GBM and its potential variants within the WHO 2000 guidelines [101]. And since the publication of the guidelines, multiple recent studies have shed new light on GBM heterogeneity.

The criteria designating a unique GBM variant in comparison to patterns of differentiation remain to be explored. Distinct histopathological features as well as the percentage of such features in total tumour may present an initial discussion regarding the definition of a new GBM variant. Moreover, evidence of distinct molecular features and prognostic classification will ultimately solidify the designation of a true tumour variant.

Table I. Characteristics of established GBM tumour variants

<table>
<thead>
<tr>
<th>GBM variant and morphological features</th>
<th>Molecular alterations</th>
<th>Prognostic markers</th>
<th>Survival</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Classic GBM</strong></td>
<td><strong>Molecular alterations:</strong> EGFR, EGFRvIII, p16INK4A, PTEN, p53, MGMT, PI3K/AKT, DH1; Loss chromosome: 1p, 10, 19q. <strong>Genomic subtypes:</strong> Proneural: PDGF, IDH1/IDH2, p53, PI3KCA, PI3KR1, Mesenchymal: NF1, p53, PTEN. <strong>Proliferative/classical:</strong> EGFR, EGFRvIII, PTEN, p16INK4A. <strong>Neural:</strong> nonspecific</td>
<td>EGFRvIII, MGMT, IDH1, PTEN, p53, CD133, proneural subtype</td>
<td>5 year survival: 10.8%</td>
<td>20, 26, 30, 59, 114, 117, 158, 162, 166</td>
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<tr>
<td><strong>Gliosarcoma (GS), ICD-O 9442/3</strong></td>
<td><strong>Features of GBM along with heterogeneous sarcomatous/mesenchymal: differentiation staining for reticulin, laminin, collagen type IV, procollagen type III, fibronectin, vimentin, α1-antitrypsin, and chymotrypsin A.</strong></td>
<td><strong>Meningioma-like features:</strong></td>
<td>Mean OS: 4-11.6 months</td>
<td>48, 52, 58, 63, 107, 108</td>
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<tr>
<td><strong>Giant cell GBM (gcGBM), ICD-O 9441/3</strong></td>
<td><strong>Features of GBM along with prominent multinucleated giant cells and lymphocytic infiltration:</strong></td>
<td></td>
<td>Mean survival: 57 weeks</td>
<td>18, 33, 79, 93, 105, 121, 156</td>
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<td></td>
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<td><strong>Loss chromosome:</strong> 10; Chromosomal polyplody, microsatellite instability</td>
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### Table 2. Characteristics of emerging GBM tumour variants

<table>
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<tbody>
<tr>
<td><strong>Fibrillary/epithelial GBM</strong>&lt;br&gt;Features of GBM along with fibrillary/epithelial differentiation showing the formation of squamous nests and glands staining for EMA, cytokeratin CAM 5.2, E-cadherin, cytokeratin AE1/AE3, cytokeratin 7, pCEA, cytokeratin 5/6 and cytokeratin 20</td>
<td>PS3, p21, EGFR; Chromosome loss 10q22-26, 17p13</td>
<td>E-cadherin</td>
<td>Mean OS: 7 months</td>
<td>73, 88, 104, 106, 143</td>
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<td><strong>Small cell astrocytoma (SCA)</strong>&lt;br&gt;Features of GBM along with mono-morphic proliferation of cells with small nuclei, limited cytoplasm, mild hyperchromasia, limited interlaced stroma, and scant mitotic index</td>
<td>EGFR, EGFRvIII, PTEN</td>
<td></td>
<td>Mean OS: 6-14.3 months</td>
<td>19, 99, 101, 136</td>
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<tr>
<td><strong>GBM with oligodendroglial component (GBMO)</strong>&lt;br&gt;Features of GBM along with oligodendrogial (e.g. fried egg) features</td>
<td>EGFR, p53, IDH1, MGMT; Honeycomb-like features, pseudopalisading necrosis</td>
<td></td>
<td>Mean OS: 19.0-26 months&lt;br&gt;Median PFS: 10.3 months&lt;br&gt;Mean 2-year survival: 60%</td>
<td>17, 56, 103, 137, 146, 160, 167, 168</td>
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<td><strong>GBM with primitive neuroectodermal tumour (GBM-PNET)</strong>&lt;br&gt;Features of GBM along with PNET-like areas showing hypercellularity, minimal fibrillary background, small undifferentiated cells with scant cytoplasm, oval-round hyperchromatic nuclei, and Homer Wright neuroblastic rosettes staining for S-100, synaptophysin, NeuN, and NFP</td>
<td>N-myc, C-myc, IDH1; Loss chromosome 10q</td>
<td>IDH1</td>
<td>Mean survival: 44 months</td>
<td>66, 68, 123</td>
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<tr>
<td><strong>Gemistocytic astrocytoma (GA)</strong>&lt;br&gt;Features of GBM along gemistocytes characterized by glassy, non-fibrillary cytoplasm and peripherally displaced nuclei</td>
<td>PS3, Bcl-2, MIB-1, chromosome 7, 10</td>
<td>Small cell features</td>
<td>Mean OS: 64 months</td>
<td>5, 84, 85, 138, 164, 169</td>
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<td><strong>Granular cell astrocytoma (GCA)</strong>&lt;br&gt;Features of GBMs along with abundant granular cells with large distinct cell borders, round to oval shapes, and abundant eosinophilic granular cytoplasm staining for GFAP, CD68, EMA, and S100</td>
<td>EGFR, p16INK4A, IDH1, MGMT; Gains chromosome 7; Loss chromosome 1p, 8p, 9p, 10, 13q, 22</td>
<td></td>
<td>Mean survival: 7.6 months&lt;br&gt;One-year survival: high-grade (12%)&lt;br&gt;low-grade (40%)</td>
<td>24, 64</td>
</tr>
<tr>
<td><strong>Paediatric high-grade glioma (HGG), diffuse intrinsic pontine glioma (DIPG)</strong>&lt;br&gt;Resembles GBM except for presence in paediatric patients</td>
<td>ADAM3A, AKT, BRAFV600E, CDKN2A/2B, EGFR, PTEN, MGMT, IDH1/2, PDGFRα, p53, Ras/Pi3K, Rb, MET, H3F3A, ATRX, DAXX; Gain chromosome 1q</td>
<td>PS3, PTEN, MIB-1, MGMT, AKT</td>
<td>HGG 2-year survival: 10-30%&lt;br&gt;DIPG 2-year survival: &lt; 10%</td>
<td>34, 51, 91, 118, 119, 129-131, 135, 147, 149, 152, 153, 173</td>
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</table>
The mechanisms of how genomic and molecular abnormalities support the dramatic heterogeneity of GBM as well as its known and potential variants remains to be understood. Moreover, understanding this underlying nature as well as correlation of genetic and pathological features will be important in predicting disease progression and in designing future personalizing therapies.

**Classic glioblastoma multiforme**

Histopathological features of GBM (ICD-O9440/3) are diverse and often nonspecific. GBM shows infiltrating, pleomorphic, hyperchromatic cells with glassy, astrocytic cytoplasm suggestive of an aggressive lesion of glioneuronal origin [20]. Variation in features can range from monotonous, small cell features to large giant cells where determining the difference between differentiation patterns and a distinct, bona fide variant can be difficult. Areas of focal pseudopalisading necrosis and microvascular proliferation, including glomeruloid formation are characteristic. Stains with glial fibrillary acidic protein (GFAP) can be used to identify the astrocytic nature of the tumour while staining with Ki-67/MIB-1 can reflect its rapid proliferation. Secondary structures of Scherer have been described as features of tumour invading normal brain tissue along white matter tracts and blood vessels, where surrounding normal brain generates a gliotic response.

Historically, the underlying genetic basis of GBM has supported the distinction of primary and secondary GBM, each with characteristic clinical and pathological features [114]. Primary GBM typically occurs de novo and with a mean age of 62 years at presentation, while secondary GBMs arise from lower grade gliomas with a mean age of 45 years. While epidural growth factor receptor (EGFR) amplification, EGFR variant III deletion (EGFRvIII), p16INK4A deletion and phosphatase and tensin homolog (PTEN) mutations have been predominant features of primary GBM, these mutations can also be seen in secondary GBM [37,86,114]. Likewise, mutations in tumour suppressor p53, often seen in secondary GBM, can also be observed in primary GBM. Various markers of prognosis in GBM have been investigated, including loss of chromosome 10 with activation of the PI3K/AKT pathway [26], EGFRvIII amplification [158], CD133 [99], O-6-methylguanine-DNA methyltransferase (MGMT) [55,162], and isocitrate dehydrogenase 1 (IDH1) [13,117,166]. However, the role of other cytogenic abnormalities such as deletions in 1p/19q [30], have not shown a consistent effect on prognosis in GBM [67]. Recent studies have also elucidated the molecular features of grade 2 and grade 3 gliomas, which include many of the same driving mutations in GBM, such as p53, IDH1/2, and 1p/19q codeletion [36].

While classification by primary or secondary aetiology has aided in clinical understanding and treatment personalization, recent advances in systems-based analysis of GBM have elucidated a further underlying complexity to GBM. Genomic and proteomic analysis has identified various subtypes of GBM [22,59,117]. These include the proneural subtype mainly distinguished by amplification or mutation of platelet-derived growth factor (PDGF), but also with alterations in IDH1/IDH2, p53, PI3KCA and PI3KR1. Furthermore, the mesenchymal subtype has been described by neurofibromin 1 (NF1) deletions or mutations, along with p53 and PTEN mutation. The proliferative subtype, sometimes termed the classical subtype, has been defined by EGFR mutation or amplification along with EGFRvIII, PTEN, and p16INK4A deletion. Lastly, the neural subtype has been described without a predominant mutation genotype. Moreover, patients with proneural GBM subtypes demonstrate improved survival over the proliferative or mesenchymal groups [125]. Expression profiles of GBM have shown to serve as better markers of prognosis compared to individual genetic or histological features [44].

Molecular heterogeneity may be important in understanding how current therapeutic treatments fail to target cells in the GBM tumour mass thereby selecting for resistant cells that can result in recurrence and poor survival [29]. Microdissection studies of GBM have shown discrete areas of individual tumours to contain distinct chromosomal aberrations [49,65], karyotypes [154], antigenic markers [171], EGFRvIII expression [112], growth factor receptors [57], angiogenic factors [78], and adhesion molecules [11]. This explained heterogeneity has been suggested to derive from a clonal cell type that undergoes evolving mutations or from a CSC that populates the tumour cells and stroma while maintaining a population of undifferentiated cells [15,71]. Despite a lack of an adequate marker of CSCs in GBM, CD133+ has been utilized and studies have supported hierarchical organization of undifferentiated cells [27]. The role of microRNAs in the regulation of CSCs has also been suggested as a mechanism of conferring heterogeneity to GBM [72]. How the GBM cell of origin generates such a large variety of molecular heterogeneity and morphological variants remains unknown.
Gliosarcoma

Gliosarcoma (GS) is a GBM subtype (ICD-O 9442/3) accounting for 1-5% of GBM diagnoses, and presents between ages 50 and 70, with a mean survival of 4-11.5 months [45,89,102]. Initially described by Stroebel and thought to be nondistinct from GBM in terms of age of onset, location, and clinical prognosis, significant evidence now supports GSs as a unique variant [63]. GSs demonstrate a biphasic pattern comprised of glial cells, which express GFAP, and sarcomatous/mesenchymal cells, which express reticulin [89]. Epithelial differentiation with carcinomatous features can also occur in glial portions [115]. The sarcomatous component of this tumour shows atypical, aggressive features and can differentiate along multiple distinct lineages, such as fibroblastic, osteogenic, chondrogenic, adipogenic, and myogenic types, especially upon exposure to radiation treatment [2,7,8,10,52,89,139]. A similar potential variant termed gliofibroma has also been described consisting of biphasic glial and non-sarcomatous fibroblastic components commonly found in paediatric patients [89]. This potential variant has been closely related to GS, with approximately 33 cases currently reported in the literature, and has a mean survival of approximately 17 months [76]. Also reminiscent of GS, lipidized glioblastoma, termed lipoglioblastoma, has also been described as a rare malignant tumour with significant foamy cell presence [62,159]. While early reports of GS suggested the concept of a “collision tumour” with vascular dysplasia resembling sarcomatous features, current models suggest a monoclonal cell of origin or CSC with distinct genetic drivers of GS [35,48].

The clinical course of GS tumours remains poorly understood. GS commonly occurs in the temporal lobes, presents as a circumscribed lesion, can have meningioma-like histological features, and can metastasize extracranially to lungs and liver [45]. GS manifestation in the spinal cord has also been reported [23]. Survival may be greater for GS with meningioma-like features vs. GBM-like features [48]. Also in this meta-analysis, GS tumours showed infrequent EGFR mutations unlike their GBM counterpart and suggested that the role for radiotherapy and chemotherapy treatments continues to be uncertain due to limited data and poor understanding of this entity. A study of 32 cases of GS showed 7 cases of secondary GS after patients underwent irradiation for GBM [124]. However, patients with primary GS that underwent irradiation showed significantly improved survival compared to untreated cases. Interestingly, primary GS showed features of malignant fibrous histiocytoma, fibrosarcoma or osteosarcoma while postirradiated secondary GS commonly showed features of fibrosarcoma, thus suggesting that radiation prompted distinct differentiation patterns. A study of 24 cases of secondary GS suggested that previous treatment with radiation could promote GS development with a mean survival after GS diagnosis of 6.7 months [47]. In a separate study of 30 secondary GS cases developed from primary GBM after treatment with chemotherapy and radiotherapy, a median length of survival of 4.4 months from time of GS diagnosis and median survival of 12.6 months from time of GBM diagnosis was observed [46]. This study also surprisingly showed that concurrent and adjuvant temozolomide treatment yielded significantly worse outcomes. Prediction of GS tumour response to treatment remains a poorly understood area.

Gliosarcoma tumours in paediatric and adult patients show distinct clinicopathological features. A retrospective review of 600 paediatric GBM cases demonstrated GS in 4 patients with approximately 19 cases previously reviewed in the literature [70]. This study showed that paediatric GS tumours had an equivalent male to female ratio, a median age of onset at 11 years, a significant incidence in infants, localization commonly in the cerebral hemispheres, as well as a median overall and event-free survival of 12.1 and 9.8 months, respectively. Interestingly, some studies have suggested that GS cases in paediatric patients show areas of rhabdomyoblastic differentiation suggestive of gliomyosarcoma and osteogenic sarcoma differentiation [148]. However, gliomyosarcoma staining for smooth muscle antigen and factor VIII has also been reported in rare instances of adult GS [75]. Understanding of paediatric GS has been limited as compared to paediatric gliomas due to the rarity of this disease.

Recent studies have greatly elucidated the molecular underpinnings of GS tumours. GS sarcomatous and gliomatous regions show unique expression patterns where regions of sarcoma stain with markers such as laminin, collagen type IV, procollagen type III, fibronectin, vimentin, α1-antitrypsin, and chymotrypsin A while gliomatous regions commonly stain for GFAP and S-100 protein [43,97,144]. However, staining patterns vary widely between tumours and their examined regions. A study of molecular features in 26 cases of GS demonstrated 11.5% had MGMT methylation and 7.7% had IDH1 mutation but these features did not predict overall survival as well as gross total resection and/or
treatment with gamma knife surgery [87]. Analysis of molecular signalling pathways in 6 cases of GS showed that while activating mutations of PDGFRα, c-kit and B-RAF were absent, expression of these signalling pathways was commonly seen in GS [140]. In a previous study of 19 GS tumours, mutations in p53 (26%), PTEN (37%), and the Rb pathway (53%) were commonly seen and also concordant between gliomatous and sarcomatous tumour regions [139]. This study also suggested that GS tumours molecularly resemble primary GBMs. Mutations in p53 have also been seen in both glioma and sarcoma areas of the tumour [12] as well as gains on chromosomes 7, 9q, 20q, X and losses of 9p, 10 and 13q [1,14]. In one study, comparable genotypic patterns of 1p, 9p, 10q, 17p, and 19q loss were seen between glial, sarcomatous and carcinosarcomatous regions [115].

New markers may help to better differentiate these GS tumours from GBM and support novel target therapies. A recent study using a comparative genomic hybridization array of glial and mesenchymal areas of 13 GS tumours showed similar gain/loss patterns except for a significant gain at chromosome segment 13q13.3-q14.1 [107]. This area was further shown to contain the gene stomatin (EPB72)-like 3 (STOML3), which is of unknown function but expressed in neuronal cells. This area also contained FRAS1-related extra-cellular matrix protein 2 (FREM2) involved in regulating epidermal-dermal interactions during morphogenesis, as well as lipoma HMGIC fusion partner (LHFP), involved in lipoma formation and hearing. These genes were expressed in 11-20% of mesenchymal areas but not glial areas. This study suggested that these and other yet uncharacterized mechanisms of mesenchymal differentiation in GS exist and may support novel targeted therapies. A separate study of epithelial-mesenchymal transition (EMT) in GS tumours demonstrated expression of Slug, Twist, matrix metalloproteinase-2 (MMP-2) and MMP-9, involved in tumour dissemination, in a majority of GS mesenchymal areas [108]. These results suggest that mechanisms important in EMT may be involved in GS tumours however further examination of how these proteins are involved remains to be seen.

In vitro models of CSC in GS have also been investigated. Tumour-derived tissue expanded in growth factor media was used in an in vitro neurosphere assay, which was used to amplify a subpopulation of cells with gliosarcoma-like properties [32]. This study also showed that GS neurospheres expressed neural stem cell markers Sox2, Msi1 and nestin similarly to GBM and were negative for CD133 expression. Gliosarcoma neurospheres were capable of self-renewal as well as differentiation into astrocytes and mesenchymal cells. When GS neurospheres underwent serial xenograft transplantation, they formed high-grade, invasive tumours reminiscent of parent tumour with biphasic glial and mesenchymal components as well as retained nestin expression. An endogenous rodent model of GS has also been reported from the induction of Fisher 344 rats with 5 mg/kg of MNU for 26 weeks [9]. Tumours in this model show spindle-shaped cells with a sarcomatoid appearance, mutations in p53, and normal expression of p16INK4A and p19ARF [4,151]. Furthermore, tumours derived from this model show an increased expression of TGFα and EGFR along with a decreased expression of FGF-2, FGF-9, FGF-R1, and PDGFRβ [157]. CSCs capable of neurosphere formation, self-renewal, nestin and Sox2 expression, and differentiation into neuronal and glial cells have also been reported from this model [41]. These tumour models support the distinctions between GBM and GS seen clinically.

Giant cell glioblastoma multiforme

Giant cell GBM (gcGBM) is a rare variant of GBM (ICD-O 9441/3) thought to encompass 2-5% of GBM diagnoses [89]. These tumours feature characteristics of GBM including necrosis and atypia along with prominent multinucleated giant cells greater than 500 µm in diameter and lymphocytic infiltration. GcGBMs have also been poignantly termed monstrocellular sarcomas and can variably stain for S-100, vimentin, class III β-tubulin, p53, EGFR and GFAP [89,115,116]. The presence of multinucleated giant cells and lymphocytic infiltration has been reported in multiple studies as favourable features in gcGBM [18,33,105]. However, there may be multiple reasons for this improved survival in gcGBM.

Various clinical features define gcGBM presentation and survival. In a study of 184 pretreatment biopsies of GBM, 12 patients with gcGBM showed significantly improved survival [21]. One study reported a mean age of 46.2 years for 19 patients with gcGBM and an equal prevalence of males to females [100]. In another study of 113 supratentorial GBMs diagnosed between 1987 and 1998, 5.3% survived longer than 5 years with 3 of these being gcGBM [156]. GcGBMs often show distinct surgical borders and present in younger patient populations than GBM [105]. These support the impetus to perform more aggressive surgical resections which may help in part to explain the improve survival.
from this GBM subtype. In a study of 42 cases of gcGBM treated over 34 years at a single institution, gcGBMs were found to be more frequent in younger subjects, showed superficial localization and sharp borders, as well as improved survival compared to reported prognosis in GBM [115]. Furthermore, this study showed that mean survival was improved with combined surgery and radiotherapy (57 vs. 32 weeks), age did not alter survival, and lymphocytic infiltration showed a benefit towards survival. In a study of 16,430 patients from the Surveillance, Epidemiology and End Results (SEER) database diagnosed with GBM, 1% showed gcGBM and demonstrated improved prognosis compared to GBM [79]. In this study, patients with GBM and gcGBM showed similar gender and racial distributions as well as insignificant tumour size and location differences. However, age at diagnosis was significantly younger in gcGBM vs. GBM (51 vs. 62 years) and gcGBMs were more likely to undergo complete resection. And after controlling for multiple factors, a multivariate analysis showed a hazard ratio of 0.76 (95% CI: 0.59-0.97) for patients diagnosed with gcGBM compared to GBM, however median survival for gcGBM continued to be about 1 year. Multiple features are favourable towards prognosis of this entity.

Molecular investigation of gcGBMs has been pursued in a variety of recent studies. Mutations in p53 have been seen in 90% of gcGBMs mostly in locations of the gene unique from usual hot-spot p53 mutations of classic GBM [100]. Furthermore, infrequent EGFR amplification and p16INK4A deletion were seen in this study. Another study of 16 gcGBM tumours showed p53 mutation in 72-84% of giant cells and 4-14% of nongiant cells, in 72-84% of giant cells and 4-14% of nongiant cells, compared to 11-49% polyploidy in classic GBM [93]. GcGBM has also been suggested to be involved in some cases of patients with Turcot syndrome, a rare GBM-forming genetic disorder with biallelic mutation of the DNA mismatch repair genes MLH1, MSH2, MSH6 or PMS2, along with favourable prognosis despite anaplasia and high proliferation [90]. Microsatellite instability has been seen with increased frequency compared to GBM [93]. How these molecular features support a more favourable prognosis for gcGBM continues to be an active area of investigation.

Comparison of paediatric gcGBM and GBM has been recently shown in several investigations. A study of paediatric GBM, aged 3 to 18 years at time of diagnosis, compared 18 cases of paediatric gcGBM and 178 cases of paediatric GBM from the HIT-GBM trial [69]. In this study, patients underwent the best possible surgical resection, standardized fractionated radiotherapy and randomized into one of four types of chemotherapy regimens. Results from this evaluation showed no difference in median age, male : female ratio (~2 : 1), and clinical history between paediatric gcGBM and GBM. Surprisingly, no difference in median overall survival (1.18 vs. 1.08 years) or event-free survival (0.54 vs. 0.53 years) was also observed. While a greater percentage of gcGBM tumours underwent gross-total resection compared to GBM (44 vs. 25%) these results were not significantly different or reported to alter survival when matched with gross-total resected GBM tumours. Thus, while gcGBM portends an improved prognosis in adults, this disease in paediatric patients showed no difference from classic GBM and suggests an alternative mechanism of formation.

**Emerging variants**

GBM can present with dramatic heterogeneity of histopathological and clinical features. The most recent 2007 WHO guidelines for brain tumours found sufficient evidence to support the presence of classic GBM, GS and gcGBM [89]. Furthermore, the guidelines have suggested the possibility of various emerging GBM variants. Multiple, recent in vitro, in vivo, and clinical studies have raised new evidence elucidating such features.

**Fibrillary/epithelial glioblastoma multiforme**

Distinct from WHO grade II fibrillary astrocytoma (ICD-O 9420/3), fibrillary/epithelial differentiation in GBM shows malignant features along with the formation of squamous nests and glands [73,106]. This pattern must often be distinguished from closely mimicking metastatic carcinomas, through the use of GFAP and CAM 5.2 immunostains among others [113]. Some studies have also suggested that fibrillary/epithelial differentiation in GBM may be due to primitive neuroepithelial cells, mechanical compression, or the histological response of host cells to tumour [73,106]. Fibrillary differentiation is a rare event suggested as a potential characteristic of GBM but not a distinct fib-
Foliad GBM variant. However, recent studies have supported a clonal origin for fibrillary GBM. A study of GS with extensive epithelial and glandular differentiation demonstrated concordant alterations in heterozygosity of various evaluated chromosomes (1p, 9p, 10q, 17p, 19q) with losses of 1p36, 9p21, 10q23, and 17p13 suggesting a potential for fibrillary GBM formation [115]. Others have also shown the epithelial and glial components of GBM contain a concordant loss of markers on chromosome 17p13 and 10q22-26 as well as p53 mutation [128]. Concordant mutations of p53 between microdissected portions of glial and fibrillary GBM have also been observed [104]. These studies support a de-differentiated fibrillary/epithelial component of GBM, which may comprise a distinct GBM variant with unique diagnostic and prognostic characteristics.

The defining features of a distinct fibrillary GBM variant continue to be an area of investigation. A study of 58 GBM tumours out of 3500 screened specimens were evaluated by expert pathologists for epithelial, epithelioid and adenoid features [143]. This study demonstrated predominant epithelial features in 35% of cases, epithelioid features in 17% and adenoid features in 48%. Epithelial differentiation was defined as epithelial morphology with squamous nests, true glandular structures and immunohistochemical expression of specific epithelial markers. Epithelioid GBMs contained fewer specific features of epithelial differentiation along with the absence of epithelial marker expression. And adenoid features required the presence of cohesive cells of intermediate size arranged into cords with pseudoglandular spaces and without staining for epithelial markers. The epithelial components of GBM tumours partially or completely stained for epithelial muscle antigen (EMA), cytokeratin CAM 5.2, E-cadherin, cytokeratin AE1/AE3, cytokeratin 7, polyclonal carcinoembryonic antigen (pCEA), in > 70% of samples while also staining for cytokeratin 5/6 and cytokeratin 20 in 7-36% of samples. The glial components of the epithelial tumours were negative for expression of epithelial markers in > 70% of cases except for cytokeratin AE1/AE3 which was positive in 53% of cases. Furthermore, no significant difference in p16INK4A deletion, chromosome 10 loss, or PTEN deletion was seen among samples. However, epithelial GBM showed the highest occurrence of p21 immunonegativity (93% of samples), strong nuclear p53 staining (41% of samples), and strong staining for EGFR (19% of samples). No differences in survival were seen between epithelial, epithelioid and adenoid GBMs with a median overall survival of approximately 7 months.

Markers of fibrillary GBM and its relationship to EMT may play important roles in understanding this variant. Recent studies have suggested that fibrillary/epithelial differentiation may have a unique genetic underpinning and that EMT, important in the spread of metastatic cancers, may be involved in governing GBM dissemination [88,104,111,128,143]. A recent analysis of E-cadherin, an important regulator of dissemination involved in EMT and metastasis, showed that expression correlated to significantly poorer patient prognosis in 27 GBM tumours with epithelial/pseudoepithelial differentiation [88]. Furthermore, survival in these fibrillary GBMs did correlate to age, tumour location or size, extent of resection, β-catenin immunostaining, molecular cytogenetic abnormalities or proliferative indices. This study also evaluated 19 established GBM cell lines, which showed increasing in vivo invasiveness correlating with E-cadherin expression. One GBM cell line (SF767) demonstrating epithelial features, such as E-cadherin staining and filopodia, E-cadherin knockdown diminished cell growth and migration. Despite the uncertain nature of this GBM type as a distinct variant, evidence has begun to suggest underlying molecular mechanisms supporting this tumour and its similarities to metastatic carcinoma.

Small cell astrocytoma

Small cell astrocytoma (SCA) is characterized by monomorphic proliferation of cells with small, round nuclei, limited cytoplasm, mild hyperchromasia, limited interlaced stroma, and scant mitotic index [89]. These tumours may account for 10% of GBM diagnoses with another 11% showing focal, small cell features [122]. Despite the possibility of being mistaken for high-grade oligodendrogial tumours or lower grade astrocytomas, SCAs are aggressive lesions paralleling grade IV gliomas. Multiple studies have correlated the presence of small cell architecture in primary GBM with EGFR amplification [19,136]. In one study, 88% of SCAs (8/9) were amplified for EGFR compared to 42% of (5/12) samples of a control set not containing small cell features, which was validated in a larger set where 67% of (14/21) SCA neoplasms showed EGFR amplification [19]. In another study, 56 cases of GBM were evaluated by chromogenic in situ hybridization and showed 64% (14/22) of SCAs and 23% (5/22) of GBMs showed EGFR amplification [136]. Interestingly, a study of glial and
epithelial microdissected components in SCA showed evidence of human polyomavirus JCV infection, suggesting a role of infection in the monoclonal origin of this tumour [127].

Recently, some authors have supported this type of tumour as a distinct variant of GBM. In a study of 229 GBMs, 71 tumours were retrospectively identified as SCAs [122]. In this study, SCA tumours were defined as containing small cell morphology within > 80% of samples. Furthermore, 11% of tumours showed significant, focal, small cell features but did not meet criteria. Among SCA tumours, 37% of samples showed minimal to no radiological enhancement, and 33% showed no endothelial hyperplasia or necrosis, therefore defined as grade III astrocytomas. However, mortality for SCAs of 6 months was similar to grade IV GBMs (11 months). This study also suggested that SCA tumours often mimicked anaplastic oligodendroglioma, anaplastic oligoastrocytoma or glioblastoma with oligodendroglial features due to the frequent presence of branching, chicken wire-like capillary networks, clear perinuclear haloes, per neuronal satellitosis and microcalcifications. However, SCA tumours uniformly lacked 1p/19q deletion thus being distinguished from oligodendroglial tumours. SCAs showed amplification of EGFR and EGFRvIII in 83% and 50% of samples compared to 35% and 21% of GBM samples, respectively. These molecular differences suggest distinct tumours despite their clinical similarity. Overall features suggesting by the authors to define SCA included ring-enhancement and pseudopalisading necrosis, oval nuclei, brisk proliferative indices, and thin, GFAP-positive cytoplasmic processes. Other researchers have reported a median survival of 14.3 months for SCAs, which was not significantly different from classic GBM after adjusting for patient age and surgery type [13]. Despite similar clinical courses, these results support both molecular and histopathological distinction between SCAs and classic GBM.

Glioblastoma multiforme with oligodendroglial component

An emerging variant in the 2007 WHO classification, glioblastoma multiforme with an oligodendroglial component (GBMO) has been termed as a possible distinct entity from GBM [89]. GBMOs resemble GBMs but also contain areas resembling oligodendroglia with the typical fried-egg appearance. GBMO is distinct from anaplastic oligoastrocytoma, oligodendroglioma, and oligoastrocytoma. Significant interest exists in the delineation of this entity since WHO grade III anaplastic oligodendrogial tumours show greater chemosensitivity than GBM and 1p/19q codeletions portend better prognosis, suggesting that the distinction between GBMOs and GBM may impact survival [114,137,172]. Similarly, histological subclassification of GBM also supports improved prognosis in tumours with oligodendrocytic components [17,103]. However, some authors have cautioned that the classification of GBMO tumours, which would have been previously diagnosed as mixed anaplastic oligoastrocytoma (MOA), may arbitrarily increase the incidence of total GBMs as well as the overall survival [95]. Additional studies in order to determine whether distinct prognostic and histological features exist are warranted.

Early studies suggested a distinction between GBMOs and classic GBMs. In regards to survival, analysis of 98 MOA, anaplastic oligodendroglioma (AO), and GBMO tumours showed a median survival time of 24 months for AO vs. 9 months for GBMOs or MOAs [160]. Furthermore, while GBMOs showed worse survival than lower grade astrocytomas, older age and astrocytic elements were seen to increase mortality while necrosis and microvascular proliferation failed to predict survival suggesting distinct features from classic GBM. Nonetheless, GBMOs demonstrated improved survival from GBM. The presence of necrosis has shown to predict poor survival in MOAs independent of patient age (22.8 vs. 86.9 months) [101]. Other studies have shown an overall survival of 20.9 vs. 13.6 months and progression free survival of 10.3 months vs. 7.6 months between GBMOs and GBM, respectively [146]. The results of this study suggested a significant impact of oligodendroglial components on GBM prognosis and molecular characteristics. A recent study of 10 consecutive cases of GBM treated with chemotherapy (nimustine and teniposide) and radiotherapy had a median survival of 26 months (range from 14 to 26 months) while 2-year survival was 60% (range between 20% and 58%), which suggested that aggressive treatment of these patients showed improved outcomes compared to reported rates for GBM in the literature [167]. While many of these early studies showed impressive distinctions between GBMO and GBM, small sample sizes limited solidifications of GBMOs as a distinct entity.

Investigation into molecular differences between GBM and GBMOs has yielded significant insight into the differences between these tumours. A retrospec-
A study of 1p/19q deletion in 10 GBMs, 2 GBMOs and in oligodendroglioma and medulloblastoma [61]. Distinctions between rates of alterations in GBMOs as compared to GBMs were not observed in all previous studies [53,82]. Nevertheless, some but not all studies showed significant differences in 1p and 19q as well as overall survival [126]. A recent study showed that GBMOs demonstrated significantly higher levels of IDH1 mutation (19% vs. 3%), and EGFR amplifications (71% vs. 48%) while codeletion of 1p/19q and MGMT methylation were similar between GBMOs and GBMs. This study utilized expression profiling to classify GBMOs predominantly into proneural and classic GBM subtypes. Incidentally, while this study did not show any prognostic significance for an oligodendroglial component, the presence of pseudopalisading necrosis was a significant predictor of benefit from chemotherapy.

Glioblastoma multiforme with primitive neuroectodermal features

Primitive neuroectodermal tumours (PNET) are rare, neural crest derived tumours commonly occurring in children and young adults (mean age 5.5 years) with CSF dissemination and uniformly poor prognosis [3]. Recent reports suggested GBM with PNET-like features (GBM-PNET) as a potential variant of GBM. Recent analyses of large tumour databases have shown a significantly greater frequency of tumour-related seizures, greater IDH1 mutation (31% vs. < 5%), reduced MGMT expression, and longer survival (19.0 vs. 13.2 months) [168]. Furthermore, this study showed that as an independent component, the presence of an oligodendroglioma component, predicted longer survival regardless of the extent of this feature. Recent analyses of large tumour databases have shown a significantly greater frequency of tumour-related seizures, greater IDH1 mutation (31% vs. < 5%), reduced MGMT expression, and longer survival (19.0 vs. 13.2 months) [168]. Furthermore, this study showed that as an independent component, the presence of an oligodendroglioma component, predicted longer survival regardless of the extent of this feature. Codeletions of 1p/19q were found in < 5% of GBMOs and GBMs. More aggressive therapy had no impact on GBMOs but showed significant improvement in survival with GBMs. Findings from the EORTC 26981/NCIC CE.3 trial examined oligodendroglioma components in 339 confirmed cases of GBMs and found that 15% could be classified as GBMOs [56]. This study showed that GBMOs showed significantly higher levels of IDH1 mutation (19% vs. 3%), and EGFR amplifications (71% vs. 48%) while codeletion of 1p/19q and MGMT methylation were similar between GBMOs and GBMs. This study utilized expression profiling to classify GBMOs predominantly into proneural and classic GBM subtypes. Incidentally, while this study did not show any prognostic significance for an oligodendroglial component, the presence of pseudopalisading necrosis was a significant predictor of benefit from chemotherapy.
of neuronal differentiation [123]. Moreover, neuronal markers, including synaptophysin and NeuN, were specific to PNET-like areas while neuron specific enolase (NSE) was seen in both glial and PNET areas. Furthermore, Ki-67 indices ranged from 30% to 100% and nuclear p53 expression was seen in 83% of cases. GBM components resembled secondary GBM with strong p53 expression and 25% having a prior diagnosis of low grade glioma. Significant portions of glial components showed foci of lower grade glioma (89% of cases), fibrillary astrocytoma (62%), gemistocytic astrocytoma (40%), giant cell astrocytoma (23%), oligoastrocytoma (19%), and oligodendroglioma (2%), while the neuroblastic components showed Homer-Wright rosettes reminiscent of medulloblastoma (60%). Within the PNET-like areas, amplification of n-myc or c-myc was in 43% of samples, suggesting this mutation to be a late event in tumour development. Chromosome 10q deletion was common (50% of samples) in both glial and PNET components, while PTEN deletion, DMBT1 loss and EGFR amplification were rare. Furthermore, this study evaluated the role of gross-total resection. Survival of GBM-PNET subjects was 4.3 ± 2.9 years (M : F ratio 1 : 1.4) while the mean age of GBM subjects was 8.3 ± 4.8 years (M : F ratio 1 : 2). Mutations of p53 and PTEN were seen in 33% and 17% of GBM tumours, respectively, while being found in 8% of GBM-PNET tumours. Furthermore LOH of 17p was seen in 33% of GBMs and no GBM-PNETs, while LOH of 10q was seen in no GBMs and 8% of GBM-PNETs. Amplifications of EGFR, CDK4 and MDM2 as well as homozygous deletion of CDKN2A were absent in all tumours. These results support distinct mechanisms of pathogenesis for GBM-PNET tumours in adult and paediatric patients.

**Gemistocytic astrocytoma**

Gemistocytic astrocytoma (GA), characterized by gemistocytes with glassy, non-fibrillary cytoplasm and peripherally displaced nuclei, is delineated as a WHO grade II tumour (ICD-O 9411/3) however these tumours often behave more aggressively than other lower grade astrocytomas [89,164]. While a tumour sample containing 20% of gemistocytes is defined for a diagnosis of diffuse astrocytoma, the amount of tumour containing gemistocytic components suggesting an aggressive tumour is debatable [85,164]. In fact, this threshold of gemistocytic cells in astrocytomas has been shown to significantly impact overall and progression-free survival [85]. However, other studies have suggested that gemistocytic components in astrocytomas do not correlate with age, p53 expression, or MIB-1 staining, or survival [54,94].

The presence of gemistocytic components in GBM has been uncertain in predicting prognosis. A study of 40 low-grade astrocytomas with progression to WHO grade III or WHO grade IV astrocytomas demonstrated that tumours with > 5% gemistocytes showed significantly poorer survival compared to tumours with < 5% gemistocytes (35 vs. 64 months) [170]. In addi-
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...tion, this study showed that GAs had a greater likelihood of p53 mutations, anti-apoptotic protein Bcl-2 expression, and MIB-1 proliferation indices. While this study suggested that most gemistocytes are nonproliferative and may be terminally differentiated, a sizeable fraction can progress to develop neoplasms. One study of 32 GAs with a mean gemistocytic index of 39.6% (range 12.2-80.8%) suggested that gemistocytes lacked proliferative activity and that in fact the presence of small cells and proliferation index defined tumours with the potential for aggressive growth [5]. Other studies have suggested the quiescent nature of gemistocytes in a variety of brain tumours [83]. And early studies using tritiated thymidine showed little uptake within GAs [58]. A study of 23 biopsies at a single institution showed a high fraction of microglia that correlated with gemistocytic tumour components and lower rates of MHC class II molecule immunoreactivity on gemistocytes [40]. These results suggested that T-cell anergy and immunoregulation could affect gemistocyte proliferation.

Common mutations between gemistocyte-containing and non-containing components suggest a clonal origin for GAs. Microdissected gemistocytes and non-gemistocytic astrocyte cellular components have shown identical p53 mutations [138]. An analysis of 28 GAs containing a mean fraction of 35 ± 9.9% gemistocytes demonstrated p53 mutation in exon 5-8 within 82% of cases while PTEN mutation was rare [169]. Furthermore, p53 mutation was synchronous between gemistocytic and fibrillary tumour components in 4 tumours. This study also showed that p53 mutation was characteristic of GAs while PTEN mutations were not commonly found in low-grade and anaplastic GAs. Furthermore, mutations in p53 have been suggested to exist in tumours of younger patients, tumours with a greater portion of atypical gemistocytes, tumours with significant smaller cell components, and subjects with shorter postoperative survival [80]. Similarly, chromosomal analysis of chromosome 7 and 10 alterations has also been found to be concordant in a subset of GAs suggesting some, but not all, gemistocytic cells to be neoplastic [84]. One study suggesting the presence of small cells better predicted poor prognosis, showed that GA tumour had a mean MIB-1 index of 3.7% (range 0.5-10.5%) primarily restricted to small cell components [5]. Furthermore, this study showed that p27 and cyclin D1 immunoreactivity localized to small astrocytic cells, as well as p53 but not EGFR expression in both gemistocytes and small cell areas. These molecular distinctions suggest the presence of GAs apart from classic GBM but additional studies are warranted.

Granular cell astrocytoma

Granular cell astrocytomas (GCA) are rare infiltrative malignant gliomas characterized by abundant granular cells with large distinct cell borders, round to oval shapes, and abundant eosinophilic granular cytoplasm [89]. GCAs commonly stain for periodic acid-Schiff (PAS), GFAP, CD68, EMA, and S100 with granular components encompassing > 30% of tumour cells [155]. In one analysis, intracerebral GCAs were more aggressive than granular cells found at other sites [92]. Delineation of these tumours may be important since their intratumoral and peritumoral lymphocytic infiltration and occasional macrophage presence can mimic demyelinating or infectious histological features [42]. Some reports have suggested that granular cells are unique entities characterized by transformed neoplastic astrocytes [3,50,98]. However, the presence of granular cell features in multiple tumour types such as glioblastoma, meningioma, ganglioglioma, neurinoma and others, along with distinct molecular features suggests that granular features may represent a degenerative process in brain tumours rather than a distinct variant [141]. However, despite often being classified as low grade tumours with low MIB-1 indices, GCAs often display aggressive features, can degenerate, and confer poor patient prognosis similarly to classic GBM.

GCAs demonstrate reduced patient survival despite their poorly understood nature. These tumours have been reported in the meninges, choroid plexus, pituitary gland, trigeminal nerve, optic nerves, cerebellum, and spinal cord though they most are cerebral lesions [28,39,42,96,145,155]. However, limited understanding of this tumour exists due to its rarity and approximately 50 cases have been reported [155]. One-year survival from GCA is reportedly 12% for high-grade GCA and 40% for low-grade GCA with extension to multiple cerebral lobes as seen in 35% of reported cases, may confer a poor prognosis despite a lack of aggressive histological features [150]. A study of 22 cases of GCAs (age range from 29 to 75 years) with a nearly 3 : 1 male : female ratio (17 men, 5 women) showed sheets of monomorphic round cells packed with eosinophilic, PAS-staining granules comprising 30-95% of cells [16]. Furthermore, this study demonstrated lymphocytic infiltration in 63% of cases, transition to
infiltrating astrocytoma in 72% of cases, GFAP staining in 95% of tumors, common staining for S-100, KP-1, ubiquitin, and EMA, as well as MIB-1 index correlating with WHO grade. Furthermore, 83% of followed cases recurred after surgery with a mean survival of 7.6 months.

Molecular mechanisms of GCAs have also been investigated. In a study of 11 GCAs (age range from 46 to 75 years) with ~2:1 male:female ratio (7 men, 4 women) and granular cell areas ranging from 30% to 100% of tumors, LOH at chromosome 1p, 9p, 10q, 17p, 19q, along with mutations of p53, p16INK4A and p14ARF, as well as EGFR amplification were seen [24]. Furthermore, losses of 9p and 10q were uniformly seen while p53, p14ARF and p16INK4A mutations as well as EGFR amplifications mutations were uncommon. Interestingly, higher frequencies of chromosomal aberrations were seen as compared to infiltrating astrocytomas at comparable WHO grades. A recent study detailing histological and molecular features of GCAs demonstrated frequent mitosis, pseudopalisading necrosis, and endothelial hyperplasia, which were reminiscent of GBM [64]. Furthermore, gains of chromosome 7, losses of chromosomes 1p, 8p, 9p, 10, 13q and 22 were observed along with EGFR amplification, CDKN2A deletion, IDH1 mutation and MGMT promoter methylation. Gains in chromosome 7 and losses in chromosome 10 have been observed in other studies of GBM with predominant granular cell features [141]. However, these studies have failed to support a unique molecular signature for GCAs suggesting that multiple genotypes can support this type of tumor. Similarly to GAs, GCAs mimic a benign pathological process despite distinct molecular mechanisms [29].

**Paediatric high-grade glioma and diffuse intrinsic pontine glioma**

Paediatric high-grade glioma (HGG), accounting for 2.8% of CNS tumors and 6.8% of pontine tumors, show many distinct clinical and molecular features from adult GBM [89,91]. A subset of malignant glioma that occurs in the brainstem includes diffuse intrinsic pontine gliomas (DIPG), which are aggressive tumors seen predominantly in children unlike the greater prevalence of supratentorial GBM in adults. However, the classification of DIPG tumors as HGGs is controversial [91]. Current treatment involves surgery and multiagent chemotherapy, however for supratentorial HGG, 2-year survival rates range from 10% to 30% while for DIPG survival is < 10% [135]. Despite similar driving mutations to adult GBM, HGGs are very distinct lesions.

Factors conferring a poor prognosis include high tumour grade, smaller tumour resections, p53 overexpression, PTEN mutation, high MIB-1 index, and overexpression of MGMT [91,130,133,135]. Interestingly, a long-term overall survival is significantly greater for infants compared to older children suggesting distinct pathological processes [135,147]. A study of 231 children with HGG showed mutations of p53 in 33% of available samples (n = 40/121), where p53 overexpression but not p53 mutation correlated with reduced 5-year progression-free survival [130]. Furthermore, this study showed that while a significant number of HGGs contained p53 mutations, secondary progression from lower-grade gliomas was an unusual course for this disease unlike in adults. One interesting case of HGG was detected prior to birth at 37 weeks of gestation and demonstrated an absence of p53 and EGFR staining as well as MIB-1 index of 87.5% [153]. Mutations in IDH1/IDH2, PTEN or EGFR are less frequent than in adult GBM [51, 132]. Despite a rarity of PTEN mutations, activated AKT has been frequently observed (79% of samples) in one series of HGG and correlated with a poor prognosis [131]. In addition, combined Ras and AKT activation have been seen in a series of 32 HGGs correlating to poor survival [34]. Mutations in BRAF, namely the missense activating BRAFV600E mutation, in combination with CDKN2A inactivation have also been seen in small series of paediatric astrocytomas [149]. Mutations in MGMT were seen in approximately 11% of one series (n = 12/109), and these cases had a significantly worse 5-year progression-free survival (8.3% vs. 42.1%) [133]. A variety of therapies have been suggested to design rational targeted therapies based on these mutations, however, such treatments in HGGs as well as immunotherapeutic approaches have not successfully improved long-term outcomes [129].

Molecular subtyping of HGG may be a method of improving personalized treatments. A recent genomic study of 78 HGGs, including 7 DIPGs demonstrated significant copy number alterations in PDGFRA and deregulation of its downstream molecular signalling pathways [119]. Gains of chromosome 1q were frequent in HGG (30%) vs. adult GBM (9%) while gains of chromosome 7 were more frequent in adult GBM (13% vs. 74%). Losses of chromosome 10q were more common in adult GBM (35% vs. 80%). Furthermore, mutations...
in IDH1 were not seen in HGG. PDGFRA amplification and chromosome 1q gain were more frequent in HGGs that received radiation. Subtyping of HGG into proneural, proliferative, mesenchymal, and undetermined categories was seen by principle component analysis, however, markers of these subtypes were distinct from adult GBM subtypes. These result suggested PDGFRA signalling to be a key player in HGG formation. In another genomic study of single-nucleotide polymorphisms in DIPG, 11 samples underwent analysis by a 250k SNPs array, which identified gains in PDGFRA in 36% of samples, and poly ADP-ribose polymerase (PARP-1) pathway genes in 27% of samples [173]. Furthermore, analysis of genome copy number abnormalities in 43 DIPGs and 8 low-grade brainstem gliomas demonstrated gene amplifications of the Ras/Pi3 K signalling pathway in 47% of tumours, the Rb cell-cycle regulatory pathway in 21% of tumours, and concurrent amplification in 21% of tumours [118]. PGFRA and MET were commonly upregulated genes. This study also suggested that gene expression patterns of DIPG differed from those of HGG while low-grade brainstem and non-brainstem gliomas showed similar expression patterns. These data highlight the similarities and distinctions between DIPG and HGG.

Key genes involved in HGG formation have been recently identified and highlighted the unique methods by which this tumour forms as compared to adult GBM. A recent landmark study used whole-exome sequencing of 48 HGGs with matched germline tissue identifying 80 somatic mutations in tumours, including two single-nucleotide polymorphisms in H3F3A which encodes the histone H3.3 protein variant involved in DNA organization [152]. H3F3A mutations were seen in 36% (32/90) of HGGs but only 3% (11/318) of young adult GBMs. Interestingly, this study was the first to associate a histone mutation with a human disease. Mutations in ATP-dependent helicase (ATRX) and death-associated protein 6 (DAXX), involved in chromatin remodelling, as well as p53 were also predominant features of HGG, which all significantly overlapped with H3F3A mutations.

Conclusions

Significant progress has been made in the histological, clinical and molecular understanding of GBM and its variants. Recent studies have also provided impressive information regarding potential novel variants and their distinguishing factors. Nevertheless, the need for improved diagnostic and prognostic markers of GBM variants are needed in order to delineate true variants from histopathological differentiation features. Furthermore, the need for large tumour database in order to accumulate sufficient samples for the evaluation of these extremely rare tumour variants is warranted. And finally, the need for uniform diagnostic criteria defining such emerging variants will be necessary for future studies. Understanding these GBM variants may aid in elucidating the mechanisms of this tumour’s marked heterogeneity and resistance to treatment.

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