Review paper

Folia Neuropathologica

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

Michael Karsy[1,2], Marshall Gelbman[2], Paarth Shah[3], Odessa Balumbu[1,2], Fred Moy[1,2], Erol Arslan[4,5]

[1]Department of Pathology, New York Medical College, Valhalla, NY, USA, [2]Department of Neurosurgery, New York Medical College, Valhalla, NY, USA, [3]Department of Medicine, New York Medical College, Valhalla, NY, USA, [4]Institute of Fertility Preservation, New York Medical College, Valhalla, NY, USA, [5]Department of Obstetrics & Gynecology, Derik State Hospital, Mardin, Turkey

Folia Neuropathol 2012; 50 (4): 301-321 DOI: 10.5114/fn.2012.32361

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-

Communicating author:

Michael Karsy, Department of Pathology, Department of Neurosurgery, New York Medical College, Basic Sciences Building, Room 413, Valhalla, NY 10595, USA, phone: 914-594-4146, fax: 914-594-4163, e-mail: Michael_Karsy@nymc.edu

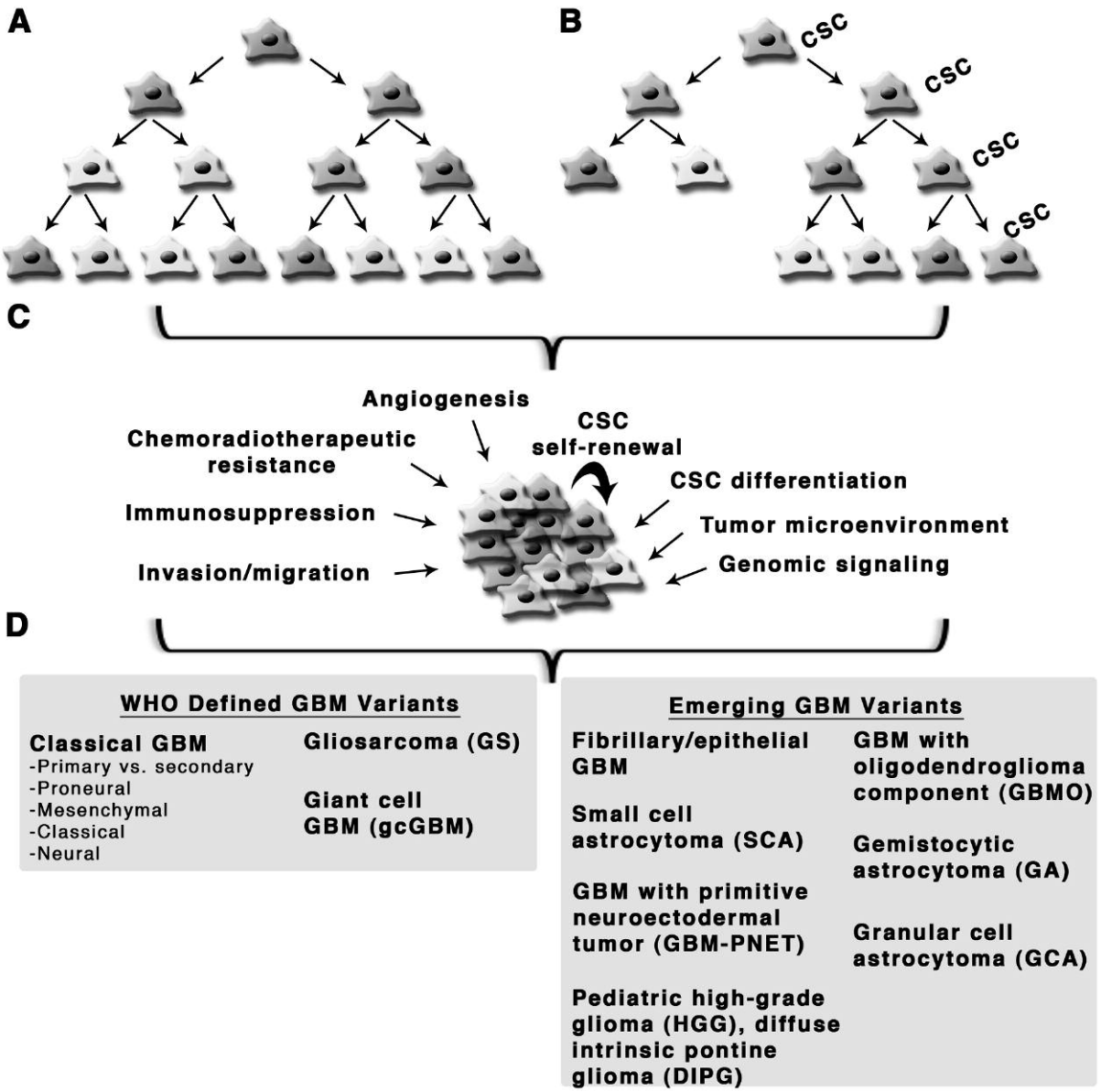

Fig. 1. Mechanisms of gliomagenesis and glioblastoma multiforme variants
Schematics are shown of gliomagenesis, factors affecting GBM growth and dissemination, as well as defined and emerging GBM variants. **A)** The stochastic and **B)** hierarchical/cancer stem cell (CSC) models for gliomagenesis are 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 oligodendroglioma 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).

wal, co-express markers of distinct neuroglial 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

GBM variant and morphological features	Molecular alterations	Prognostic markers	Survival	References
Classic GBM Infiltrating, pleomorphic, hyperchromatic cells with glassy, astrocytic cytoplasm. Frequent presence of pseudopalisading necrosis, neoepithelialization, mitotic figures, and hypercellularity	EGFR, EGFRvIII, $p16^{INK4A}$, PTEN, p53, MGMT, PI3K/AKT, DH1; Loss chromosome: 1p, 10, 19q **Genomic subtypes:** **Proneural:** PDGF, IDH1/IDH2, p53, PI3KCA, PI3KR1 **Mesenchymal:** NF1, p53, PTEN **Proliferative/classical:** EGFR, EGFRvIII, PTEN, $p16^{INK4A}$ **Neural:** nonspecific	EGFRvIII, MGMT, IDH1, PTEN, p53, CD133, proneural subtype	5 year survival: 9.8% Median PFS: 5.3-10.3 months Median OS: 12.7-21.7 months	20, 26, 30, 59, 114, 117, 158, 162, 166
Gliosarcoma (GS), ICD-O 9442/3 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	MGMT, IDH1, p53, PTEN, Rb, STOML3, LHFP, Slug, Twist, MMP-2, MMP-9; PDGFAα, c-kit and B-RAF signalling; Gain chromosome: 7, 9q, 20q, and X; Loss chromosome: 9p, 10, 13q	Meningioma-like features	Mean OS: 4-11.6 months	48, 52, 58, 63, 107, 108
Giant cell GBM (gcGBM), ICD-0 9441/3 Features of GBM along with prominent multinucleated giant cells and lymphocytic infiltration	P53, PTEN, MDM2; Loss chromosome: 10; Chromosomal polyploidy, microsatellite instability		Mean survival: 57 weeks Median survival: ~1 year	18, 33, 79, 93, 105, 121, 156

Table II. Characteristics of emerging GBM tumour variants

GBM variant and morphological features	Molecular alterations	Prognostic markers	Survival	References
Fibrillary/epithelial GBM 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	P53, p21, EGFR; Chromosome loss 10q22-26, 17p13	E-cadherin	Mean OS: 7 months	73, 88, 104, 106, 143
Small cell astrocytoma (SCA) Features of GBM along with monomorphic proliferation of cells with small nuclei, limited cytoplasm, mild hyperchromasia, limited interlaced stroma, and scant mitotic index	EGFR, EGFRvIII, PTEN		Mean OS: 6-14.3 months	19, 99, 101, 136
GBM with oligodendroglioma component (GBMO) Features of GBM along with oligodendroglial (e.g. fried egg) features	EGFR, p53, IDH1, MGMT; Gain chromosome 7; Loss chromosome 1p, 9p21, 10, 19q	Honeycomb-like features, pseudopalisading necrosis	Mean OS: 19.0-26 months Median PFS: 10.3 months Mean 2-year survival: 60%	17, 56, 103, 137, 146, 160, 167, 168
GBM with primitive neuroectodermal tumour (GBM-PNET) 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	N-myc, C-myc, IDH1; Loss chromosome 10q	IDH1	Mean survival: 44 months	66, 68, 123
Gemistocytic astrocytoma (GA) Features of GBM along gemistocytes characterized by glassy, non-fibrillary cytoplasm and peripherally displaced nuclei	P53, Bcl-2, MIB-1, chromosome 7, 10	Small cell features	Mean OS: 64 months	5, 84, 85, 138, 164, 169
Granular cell astrocytoma (GCA) Features of GBMs along with abundant granular cells with large distinct cell borders, round to oval shapes, and abundant eosinophil granular cytoplasm staining for GFAP, CD68, EMA, and S100	EGFR, $p16^{INK4A}$, IDH1, MGMT; Gains chromosome 7; Loss chromosome 1p, 8p, 9p, 10, 13q, 22		Mean survival: 7.6 months One-year survival: high-grade (12%) low-grade (40%)	24, 64
Paediatric high-grade glioma (HGG), diffuse intrinsic pontine glioma (DIPG) Resembles GBM except for presence in paediatric patients	ADAM3A, AKT, $BRAF^{V600E}$, CDKN2A/2B, EGFR, PTEN, MGMT, IDH1/2, PDGFRA, p53, Ras/PI3K, Rb, MET, H3F3A, ATRX, DAXX; Gain chromosome 1q	P53, PTEN, MIB-1, MGMT, AKT	HGG 2-year survival: 10-30% DIPG 2-year survival: < 10%	34, 51, 91, 118, 119, 129-131, 135, 147, 149, 152, 153, 173

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 epidermal growth factor receptor (EGFR) amplification, EGFR variant III deletion (EGFRvIII), $p16^{INK4A}$ 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 $p16^{INK4A}$ 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 Stroebe 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 extracellular 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 $p16^{INK4A}$ and $p19^{ARF}$ [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, FGFR-1, 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 compare 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 $p16^{INK4A}$ deletion were seen in this study. Another study of 16 gcGBM tumours showed p53 mutation in 75% of samples and focal EGFR overexpression in 56% of tumours, albeit findings were not uniform within all specimens [120]. Point mutations in PTEN and chromosome 10 deletions, where PTEN resides, along with amplification of MDM2 have also been reported [93, 121]. Furthermore, giant cell and nongiant cell populations have shown distinctions in genomic alterations with polyploidy being reported 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-

rillary 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 dedifferentiated 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 $p16^{INK4A}$ 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 cytogenic 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 oligodendroglial 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 oligodendroglioma 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 oligodendroglioma 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 oligodendroglial 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 GBMO 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-

tive study compared 450 GBM and 36 GBMO samples [146]. In this study, GBMO cases showed a lower median age at onset (52.1 vs. 62.24 years) compared to GBM. Among GBMO cases, loss of heterozygosity (LOH) of 1p and/or 19q (75% of samples), LOH of 10q (58% of samples), EGFR amplification (39% of samples), and TP53 mutation (22.2% of samples) were detected. These molecular alterations are consistent with some but not all previous studies [53,82]. Nevertheless, distinctions between rates of alterations in GBMOs and GBM support this distinct entity. The role of 1p/19q codeletion has also been an important facet due to the loss of these markers in conferring better prognosis in oligodendroglioma and medulloblastoma [61]. A study of 1p/19q deletion in 10 GBMs, 2 GBMOs and 8 AOs showed that 1p/19q was codeleted in all AOs but inconsistently altered in GBMs and GBMOs [109]. However, a small sample size, retrospective evaluation, and lack of standardized treatments were significant confounding factors in this study. Another study of 64 GBMs and 24 GBMOs failed to find significant differences in 1p and 19q as well as overall survival [126]. And a study of 19 cases of GBMO demonstrated that calcification, cystic components, LOH of chromosome 10, EGFR amplifications, 9p21 deletions containing $p16^{INK4A}$ were seen in the majority of cases; however, sample size in this study was low and GBMOs were not compared to GBMs seen at the same institution [110]. During a recent study of GBMO and GBM cases, microdissected astrocytic and oligodendroglial components were evaluated by comparative genomic hybridization [77]. This study showed that oligodendroglial and astrocytic components of GBMOs were concordant and that GBMOs could be classified according to chromosomal gains/losses (shown in parentheses) into astrocytic (+7/–10), oligodendroglial (–1p/–19q), intermediate (–1p/+7), and non-specific subtypes. The results support a monoclonal cell of origin along with distinct pathways of gliomagenesis. GBMOs presented in younger patients (55.4 vs. 63.8 years), showed better overall survival (404 vs. 282 days), and responded better to radiotherapy compared to GBM. Furthermore, honeycomb-like features in GBMO may predict better survival than a round cell appearance.

Recent analyses of large tumour databases have better supported a distinct GBMO variant. One group showed that approximately 18.3% of 219 consecutive GBM samples at a single institution were GBMOs, which showed 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 in a survival model, 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 have suggested GBM with PNET-like features (GBM-PNET) as a potential variant of GBM and present in older adults [68]. These tumours contain two distinct architectures, including that of traditional GBM and that of PNET-like areas with hypercellularity, minimal fibrillary background, small undifferentiated cells with scant cytoplasm, oval-round hyperchromatic nuclei, and Homer Wright neuroblastic rosettes [66,89,123]. Furthermore, PNET-like areas show lower expression of GFAP but readily stain for S-100, synaptophysin, NeuN, and neurofilament protein (NFP). Gliomatous and lipomatous degeneration of PNET tumours have been reported, which may support an alternative origin for this tumour [60,134].

A recent study has demonstrated multiple, unique characteristics for GBM-PNETs compared to GBM. In a study of 53 GBM-PNETs (median age 54) and a male: female ratio of 1.3, PNET-like components were observed to be discrete and hypercellular with nodules

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 PTET 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, radiotherapy, temozolomide and platinum-based chemotherapy where, despite limited data, survival continued to be poor with a median of 9.1 months. The synchrony in mutational characteristics between glial and PNET-like areas further supported the hypothesis for monoclonal origin regarding GBM-PNET where the clinical, cytological and immunoreactive features supported the differentiation of GBM-PNET predominantly from secondary GBM. Alternatively, neuroblastic nodules may have represented clonal expansion of tumour stem cell niches within the parent GBM tumour.

Recent studies have suggested favourable molecular and prognostic features for GBM-PNETs. A study of 40 cases of grade III or IV glioma demonstrated coexpression of GFAP and NFP as essential to the diagnosis of GBM-PNET [165]. Furthermore, a lack of recurrence was observed in 36% of cases, which underwent gross total resection resulting in a mean survival of 44 months. In another study of 12 patients with GBM-PNET (median age 51.5 years, M : F 4 : 1), mutations in IDH1 were seen in 25% of cases evaluated (n = 2) with overall survival of 15 and 31 months [161]. Furthermore, this study suggested that restricted diffusion on diffusion-weighted imaging correlated with the PNET-like component, CD56 expression with both glial and neuroblastic components, and vimentin staining with the glial component thereby improving identification of this GBM variant. In a study of 86 patients with GBM, PNET-like features defined as neoplastic cells with high N : C ratios, hyperchromatic oval-carrot-shaped nuclei, and absence of honeycomb appearance were seen in 27% of samples, however these features did not correlate with prognosis [163]. These results suggest a promising outcome for these types of tumours and may support aggressive treatment.

Paediatric GBM-PNET tumours have also been reported. In a study of 12 paediatric GBM-PNET and 6 paediatric GBM tumours, an analysis of various molecular markers was employed to differentiate these tumours [81]. The mean age 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-PTEN 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-

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 eosinophil 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 tumours, 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 tumours, LOH at chromosome 1p, 9p, 10q, 17p, 19q, along with mutations of p53, $p16^{INK4A}$ and $p14^{ARF}$, as well as EGFR amplification were seen [24]. Furthermore, losses of 9p and 10q were uniformly seen while p53, $p14^{ARF}$ and $p16^{INK4A}$ 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 tumour. 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 tumours and 6.8% of pontine tumours, 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 tumours seen predominantly in children unlike the greater prevalence of supratentorial GBM in adults. However, the classification of DIPG tumours 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 pathogenic 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 $BRAF^{V600E}$ 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 PDGFRα 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/PI3K 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.

Acknowledgments

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Actor B, Cobbers JMJL, Büschges R, Wolter M, Knobbe CB, Lichter P, Reifenberger G, Weber RG. Comprehensive analysis of genomic alterations in gliosarcoma and its two tissue components. Genes Chromosom Cancer 2002; 34: 416-427.
2. Alatakis S, Stuckey S, Siu K, McLean C. Gliosarcoma with osteosarcomatous differentiation: review of radiological and pathological features. J Clin Neurosci 2004; 11: 650-656.
3. Albuquerque L, Pimentel J, Costa A, Cristina L. Cerebral granular cell tumors: report of a case and a note on their nature and expected behavior. Acta Neuropathol 1992; 84: 680-685.
4. Asai A, Miyagi Y, Sugiyama A, Gamanuma M, Hong SH, Takamoto S, Nomura K, Matsutani M, Takakura K, Kuchino Y. Negative effects of wild-type p53 and s-Myc on cellular growth and tumorigenicity of glioma cells. Implication of the tumor suppressor genes for gene therapy. J Neurooncol 1994; 19: 259-268.
5. Avninder S, Sharma MC, Deb P, Mehta VS, Karak AK, Mahapatra AK, Sarkar C. Gemistocytic astrocytomas: histomorphology, proliferative potential and genetic alterations – a study of 32 cases. J Neurooncol 2006; 78: 123-127.
6. Bailey P, Cushing H. A Classification of the Tumors of the Glioma Group on a Histogenetic Basis With a Correlated Study of Prognosis. JB Lippincott Co., Philadelphia 1926.
7. Banerjee AK, Sharma BS, Kak VK, Ghatak NR. Gliosarcoma with cartilage formation. Cancer 1989; 63: 518-523.
8. Barresi V, Cerasoli S, Morigi F, Cremonini AM, Volpini M, Tuccari G. Gliosarcoma with features of osteoblastic osteosarcoma: a review. Arch Pathol Lab Med 2006; 130: 1208-1211.
9. Barth RF, Kaur B. Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. J Neurooncol 2009; 94: 299-312.
10. Barut F, Kandemir NO, Ozdamar SO, Gul S, Bektas S, Gun BD, Bahadir B. Gliosarcoma with chondroblastic osteosarcomatous differentation: report of two case with clinicopathologic and immunohistochemical features. Turk Neurosurg 2009; 19: 417-422.

11. Bello L, Francolini M, Marthyn P, Zhang J, Carroll RS, Nikas DC, Strasser JF, Villani R, Cheresh DA, Black PM. Alpha(v)beta3 and alpha(v)beta5 integrin expression in glioma periphery. Neurosurgery 2001; 49: 380-389, discussion 390.
12. Biernat W, Aguzzi A, Sure U, Grant JW, Kleihues P, Hegi ME. Identical mutations of the p53 tumor suppressor gene in the gliomatous and the sarcomatous components of gliosarcomas suggest a common origin from glial cells. J Neuropathol Exp Neurol 1995; 54: 651-656.
13. Birner P, Toumangelova-Uzeir K, Natchev S, Guentchev M. Expression of mutated isocitrate dehydrogenase-1 in gliomas is associated with p53 and EGFR expression. Folia Neuropathol 2011; 49: 88-93.
14. Boerman RH, Anderl K, Herath J, Borell T, Johnson N, Schaefer-Klein J, Kirchhof A, Raap AK, Scheithauer BW, Jenkins RB. The glial and mesenchymal elements of gliosarcomas share similar genetic alterations. J Neuropathol Exp Neurol 1996; 55: 973-981.
15. Bonavia R, Inda MDM, Cavenee WK, Furnari FB. Heterogeneity maintenance in glioblastoma: a social network. Cancer Res 2011; 71: 4055-4060.
16. Brat DJ, Scheithauer BW, Medina-Flores R, Rosenblum MK, Burger PC. Infiltrative astrocytomas with granular cell features (granular cell astrocytomas): a study of histopathologic features, grading, and outcome. Am J Surg Pathol 2002; 26: 750-757.
17. Brennan C, Momota H, Hambardzumyan D, Ozawa T, Tandon A, Pedraza A, Holland E. Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. PLoS ONE 2009; 4: e7752.
18. Brooks WH, Markesbery WR, Gupta GD, Roszman TL. Relationship of lymphocyte invasion and survival of brain tumor patients. Ann Neurol 1978; 4: 219-224.
19. Burger PC, Pearl DK, Aldape K, Yates AJ, Scheithauer BW, Passe SM, Jenkins RB, James CD. Small cell architecture – a histological equivalent of EGFR amplification in glioblastoma multiforme? J Neuropathol Exp Neurol 2001; 60: 1099-1104.
20. Burger PC, Scheithauer BW, Vogel FS. The Brain: Tumors. In: Burger PC, BW Scheithauer, FS Vogel (eds.). Surgical Pathology of the Nervous System and its Coverings. Churchill Livingstone, New York 2002.
21. Burger PC, Vollmer RT. Histologic factors of prognostic significance in the glioblastoma multiforme. Cancer 1980; 46: 1179-1186.
22. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008; 455: 1061-1068.
23. Carstens PH, Johnson GS, Jelsma LF. Spinal gliosarcoma: a light, immunohistochemical and ultrastructural study. Ann Clin Lab Sci 1995; 25: 241-246.
24. Castellano-Sanchez AA, Ohgaki H, Yokoo H, Scheithauer BW, Burger PC, Hamilton RL, Finkelstein SD, Brat DJ. Granular cell astrocytomas show a high frequency of allelic loss but are not a genetically defined subset. Brain Pathol 2003; 13: 185-194.
25. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004-2008. Source: Central Brain Tumor Registry of the United States, Hinsdale, IL 2012; website: www.cbtrus.org
26. Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A, Loeffler JS. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. J Clin Oncol 2004; 22: 1926-1933.
27. Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL, Rivers CS, Modrusan Z, Nacu S, Guerrero S, Edgar KA, Wallin JJ, Lamszus K, Westphal M, Heim S, James CD, VandenBerg SR, Costello JF, Moorefield S, Cowdrey CJ, Prados M, Phillips HS. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell 2010; 17: 362-375.
28. Chimelli L, Symon L, Scaravilli F. Granular cell tumor of the fifth cranial nerve: further evidence for Schwann cell origin. J Neuropathol Exp Neurol 1984; 43: 634-642.
29. Chorny JA, Evans LC, Kleinschmidt-DeMasters BK. Cerebral granular cell astrocytomas: a Mib-1, bcl-2, and telomerase study. Clin Neuropathol 2000; 19: 170-179.
30. Colman H, Aldape K. Molecular predictors in glioblastoma: toward personalized therapy. Arch Neurol 2008; 65: 877-883.
31. Daumas-Duport C, Scheithauer B, O'Fallon J, Kelly P. Grading of astrocytomas. A simple and reproducible method. Cancer 1988; 62: 2152-2165.
32. deCarvalho AC, Nelson K, Lemke N, Lehman NL, Arbab AS, Kalkanis S, Mikkelsen T. Gliosarcoma stem cells undergo glial and mesenchymal differentiation in vivo. Stem Cells 2010; 28: 181-190.
33. Donev K, Scheithauer BW, Rodriguez FJ, Jenkins S. Expression of diagnostic neuronal markers and outcome in glioblastoma. Neuropathol Appl Neurobiol 2010; 36: 411-421.
34. Faury D, Nantel A, Dunn SE, Guiot M-C, Haque T, Hauser P, Garami M, Bognar L, Hanzely Z, Liberski PP, Lopez-Aguilar E, Valera ET, Tone LG, Carret AS, Del Maestro RF, Gleave M, Montes JL, Pietsch T, Albrecht S, Jabado N. Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. J Clin Oncol 2007; 25: 1196-1208.
35. Feigin I, Allen LB, Lipkin L, Gross SW. The endothelial hyperplasia of the cerebral blood vessels with brain tumors, and its sarcomatous transformation. Cancer 1959; 11: 264-277.
36. Figarella-Branger D, Maues de Paula A, Colin C, Bouvier C. Histomolecular classification of adult diffuse gliomas: the diagnostic value of immunohistochemical markers. Rev Neurol (Paris) 2011; 167: 683-690.
37. Frederick L, Wang XY, Eley G, James CD. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res 2000; 60: 1383-1387.
38. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F, Vescovi A. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 2004; 64: 7011-7021.
39. Geddes JF, Thom M, Robinson SF, Révész T. Granular cell change in astrocytic tumors. Am J Surg Pathol 1996; 20: 55-63.
40. Geranmayeh F, Scheithauer BW, Spitzer C, Meyer FB, Svensson-Engwall A-C, Graeber MB. Microglia in gemistocytic astrocytomas. Neurosurgery 2007; 60: 159-66; discussion 166.
41. Ghods AJ, Irvin D, Liu G, Yuan X, Abdulkadir IR, Tunici P, Konda B, Wachsmann-Hogiu S, Black KL, Yu JS. Spheres isolated from 9L gliosarcoma rat cell line possess chemoresistant and aggressive cancer stem-like cells. Stem Cells 2007; 25: 1645-1653.
42. Giangaspero F, Cenacchi G. Oncocytic and granular cell neoplasms of the central nervous system and pituitary gland. Semin Diagn Pathol 1999; 16: 91-97.

43. Grant JW, Steart PV, Aguzzi A, Jones DB, Gallagher PJ. Gliosarcoma: an immunohistochemical study. Acta Neuropathol 1989; 79: 305-309.
44. Gravendeel LAM, Kouwenhoven MCM, Gevaert O, de Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK, De Moor B, Eilers PH, van der Spek PJ, Kros JM, Sillevis Smitt PA, van den Bent MJ, French PJ. Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. Cancer Res 2009; 69: 9065-9072.
45. Han SJ, Yang I, Ahn BJ, Otero JJ, Tihan T, McDermott MW, Berger MS, Prados MD, Parsa AT. Clinical characteristics and outcomes for a modern series of primary gliosarcoma patients. Cancer 2010; 116: 1358-1366.
46. Han SJ, Yang I, Otero JJ, Ahn BJ, Tihan T, McDermott MW, Berger MS, Chang SM, Parsa AT. Secondary gliosarcoma after diagnosis of glioblastoma: clinical experience with 30 consecutive patients. J Neurosurg 2010; 112: 990-996.
47. Han SJ, Yang I, Tihan T, Chang SM, Parsa AT. Secondary gliosarcoma: a review of clinical features and pathological diagnosis. J Neurosurg 2010; 112: 26-32.
48. Han SJ, Yang I, Tihan T, Prados MD, Parsa AT. Primary gliosarcoma: key clinical and pathologic distinctions from glioblastoma with implications as a unique oncologic entity. J Neurooncol 2010; 96: 313-320.
49. Harada K, Nishizaki T, Ozaki S, Kubota H, Ito H, Sasaki K. Intratumoral cytogenetic heterogeneity detected by comparative genomic hybridization and laser scanning cytometry in human gliomas. Cancer Res 1998; 58: 4694-4700.
50. Harris CP, Townsend JJ, Brockmeyer DL, Heilbrun MP. Cerebral granular cell tumor occurring with glioblastoma multiforme: case report. Surg Neurol 1991; 36: 202-206.
51. Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, Weller M, Herold-Mende C, Unterberg A, Jeuken JW, Wesseling P, Reifenberger G, von Deimling A. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. Acta Neuropathol 2009; 118: 469-474.
52. Hayashi K, Ohara N, Jeon HJ, Akagi S, Takahashi K, Akagi T, Namba S. Gliosarcoma with features of chondroblastic osteosarcoma. Cancer 1993; 72: 850-855.
53. He J, Mokhtari K, Sanson M, Marie Y, Kujas M, Huguet S, Leuraud P, Capelle L, Delattre JY, Poirier J, Hoang-Xuan K. Glioblastomas with an oligodendroglial component: a pathological and molecular study. J Neuropathol Exp Neurol 2001; 60: 863-871.
54. Heesters MA, Koudstaal J, Go KG, Molenaar WM. Analysis of proliferation and apoptosis in brain gliomas: prognostic and clinical value. J Neurooncol 1999; 44: 255-266.
55. Hegi ME, Diserens A-C, Gorlia T, Hamou M-F, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005; 352: 997-1003.
56. Hegi ME, Janzer R-C, Lambiv WL, Gorlia T, Kouwenhoven MCM, Hartmann C, Deimling von A, Martinet D, Besuchet Schmutz N, Diserens AC, Hamou MF, Bady P, Weller M, van den Bent MJ, Mason WP, Mirimanoff RO, Stupp R, Mokhtari K, Wesseling P, European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups, and National Cancer Institute of Canada Clinical Trials Group. Presence of an oligodendroglioma-like component in newly diagnosed glioblastoma identifies a pathogenetically heterogeneous subgroup and lacks prognostic value: central pathology review of the EORTC_26981/NCIC_CE.3 trial. Acta Neuropathol 2010; 123: 841-852.
57. Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B, Nistér M. Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. Cancer Res 1992; 52: 3213-3219.
58. Hoshino T, Wilson BC, Ellis WG. Gemistocytic astrocytes in gliomas. An autoradiographic study. J Neuropathol Exp Neurol 1975; 34: 263-281.
59. Huse JT, Holland EC. Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. Nat Rev Cancer 2010; 10: 319-331.
60. Ishizawa K, Kan-nuki S, Kumagai H, Komori T, Hirose T. Lipomatous primitive neuroectodermal tumor with a glioblastoma component: a case report. Acta Neuropathol 2002; 103: 193-198.
61. Jansen M, Yip S, Louis DN. Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers. Lancet Neurol 2010; 9: 717-726.
62. Johnson MW, Lin D, Smir BN, Burger PC. Lipoglioblastoma: a lipidized glioma radiologically and histologically mimicking adipose tissue. World Neurosurg 2010; 73: 108-111.
63. Jones H, Steart PV, Weller RO. Spindle-cell glioblastoma or gliosarcoma? Neuropathol Appl Neurobiol 1991; 17: 177-187.
64. Joo M, Park S-H, Chang SH, Kim H, Choi C-Y, Lee C-H, Lee BH, Hwang YJ. Cytogenetic and molecular genetic study on glioblastoma arising in granular cell astrocytoma: a case report. Hum Pathol 2011; doi: 10.1016/j.humpath.2011.08.015 [Epub ahead of print].
65. Jung V, Romeike BF, Henn W, Feiden W, Moringlane JR, Zang KD, Urbschat S. Evidence of focal genetic microheterogeneity in glioblastoma multiforme by area-specific CGH on microdissected tumor cells. J Neuropathol Exp Neurol 1999; 58: 993-999.
66. Kandemir NO, Bahadir B, Gul S, Karadayi N, Ozdamar SO. Glioblastoma with primitive neuroectodermal tumor-like features: case report. Turk Neurosurg 2009; 19: 260-264.
67. Kaneshiro D, Kobayashi T, Chao ST, Suh J, Prayson RA. Chromosome 1p and 19q deletions in glioblastoma multiforme. Appl Immunohistochem Mol Morphol 2009; 17: 512-516.
68. Karina A, Jonker BP, Morey A, Selinger C, Gupta R, Buckland ME. Glioblastoma with primitive neuroectodermal tumour-like components. Pathology 2012; 44: 270-273.
69. Karremann M, Butenhoff S, Rausche U, Pietsch T, Wolff JEA, Kramm CM. Pediatric giant cell glioblastoma: New insights into a rare tumor entity. Neurooncol 2009; 11: 323-329.
70. Karremann M, Rausche U, Fleischhack G, Nathrath M, Pietsch T, Kramm CM, Wolff JEA. Clinical and epidemiological characteristics of pediatric gliosarcomas. J Neurooncol 2010; 97: 257-265.
71. Karsy M, Albert L, Tobias ME, Murali R, Jhanwar-Uniyal M. All-trans retinoic acid modulates cancer stem cells of glioblastoma multiforme in an MAPK-dependent manner. Anticancer Res 2010; 30: 4915-4920.

72. Karsy M, Arslan E, Moy F. Current Progress on Understanding MicroRNAs in Glioblastoma Multiforme. Genes Cancer 2012; 3: 3-15.
73. Kepes JJ, Fulling KH, Garcia JH. The clinical significance of "adenoid" formations of neoplastic astrocytes, imitating metastatic carcinoma, in gliosarcomas. A review of five cases. Clin Neuropathol 1982; 1: 139-150.
74. Kernohan JW, Mabon RF. A simplified classification of the gliomas. Proc Staff Meet Mayo Clin 1949; 24: 71-75.
75. Khanna M, Siraj F, Chopra P, Bhalla S, Roy S. Gliosarcoma with prominent smooth muscle component (gliomyosarcoma): a report of 10 cases. Indian J Pathol Microbiol 2011; 54: 51-54.
76. Kim Y, Suh Y-L, Sung C, Hong SC. Gliofibroma: a case report and review of the literature. J Korean Med Sci 2003; 18: 625-629.
77. Klink B, Schlingelhof B, Klink M, Stout-Weider K, Patt S, Schrock E. Glioblastomas with oligodendroglial component-common origin of the different histological parts and genetic subclassification. Cell Oncol (Dordr) 2011; 34: 261-275.
78. Koga K, Todaka T, Morioka M, Hamada J, Kai Y, Yano S, Okamura A, Takakura N, Suda T, Ushio Y. Expression of angiopoietin-2 in human glioma cells and its role for angiogenesis. Cancer Res 2001; 61: 6248-6254.
79. Kozak KR, Moody JS. Giant cell glioblastoma: a glioblastoma subtype with distinct epidemiology and superior prognosis. Neurooncology 2009; 11: 833-841.
80. Kösel S, Scheithauer BW, Graeber MB. Genotype-phenotype correlation in gemistocytic astrocytomas. Neurosurgery 2001; 48: 187-93; discussion 193-4.
81. Kraus JA, Felsberg J, Tonn JC, Reifenberger G, Pietsch T. Molecular genetic analysis of the TP53, PTEN, CDKN2A, EGFR, CDK4 and MDM2 tumour-associated genes in supratentorial primitive neuroectodermal tumours and glioblastomas of childhood. Neuropathol Appl Neurobiol 2002; 28: 325-333.
82. Kraus JA, Lamszus K, Glesmann N, Beck M, Wolter M, Sabel M, Krex D, Klockgether T, Reifenberger G, Schlegel U. Molecular genetic alterations in glioblastomas with oligodendroglial component. Acta Neuropathol 2001; 101: 311-320.
83. Kros JM, Schouten WC, Janssen PJ, van der Kwast TH. Proliferation of gemistocytic cells and glial fibrillary acidic protein (GFAP)-positive oligodendroglial cells in gliomas: a MIB-1/GFAP double labeling study. Acta Neuropathol 1996; 91: 99-103.
84. Kros JM, Waarsenburg N, Hayes DP, Hop WC, van Dekken H. Cytogenetic analysis of gemistocytic cells in gliomas. J Neuropathol Exp Neurol 2000; 59: 679-686.
85. Krouwer HG, Davis RL, Silver P, Prados M. Gemistocytic astrocytomas: a reappraisal. J Neurosurg 1991; 74: 399-406.
86. Larysz D, Kula D, Kowal M, Rudnik A, Jarząb M, Blamek S, Bierzyńska-Macyszyn G, Kowalska M, Bażowski P, Jarząb B. Epidermal growth factor receptor gene expression in high grade gliomas. Folia Neuropathol 2011; 49: 28-38.
87. Lee D, Kang SY, Suh Y-L, Jeong JY, Lee J-I, Nam D-H. Clinicopathologic and genomic features of gliosarcomas. J Neurooncol 2012; 107: 643-650.
88. Lewis-Tuffin LJ, Rodriguez F, Giannini C, Scheithauer B, Necela BM, Sarkaria JN, Anastasiadis PZ. Misregulated E-cadherin expression associated with an aggressive brain tumor phenotype. PLoS ONE 2010; 5: e13665.
89. Louis DN, Ohgaki HH, Wiestler OD, Cavenee WK. Astrocytic tumors. In: WHO Classification of Tumours of the Central Nervous System. Louis DN, Ohgaki HH, Wiestler OD, Cavenee WK (ed.). WHO Press, Albany 2007.
90. Lusis EA, Travers S, Jost SC, Perry A. Glioblastomas with giant cell and sarcomatous features in patients with Turcot syndrome type 1: a clinicopathological study of 3 cases. Neurosurgery 2010; 67: 811-817; discussion 817.
91. MacDonald TJ, Aguilera D, Kramm CM. Treatment of high-grade glioma in children and adolescents. Neurooncology 2011; 13: 1049-1058.
92. Markesbery WR, Duffy PE, Cowen D. Granular cell tumors of the central nervous system. J Neuropathol Exp Neurol 1973; 32: 92-109.
93. Martinez R, Roggendorf W, Baretton G, Klein R, Toedt G, Lichter P, Schackert G, Joos S. Cytogenetic and molecular genetic analyses of giant cell glioblastoma multiforme reveal distinct profiles in giant cell and non-giant cell subpopulations. Cancer Genet Cytogenet 2007; 175: 26-34.
94. Martins DC, Malheiros SM, Santiago LH, Stávale JN. Gemistocytes in astrocytomas: are they a significant prognostic factor? J Neurooncol 2006; 80: 49-55.
95. Marucci G. The effect of WHO reclassification of necrotic anaplastic oligoastrocytomas on incidence and survival in glioblastoma. J Neurooncol 2011; 104: 621-622.
96. McNab AA, Daniel SE. Granular cell tumours of the orbit. Aust N Z J Ophthalmol 1991; 19: 21-27.
97. Meis JM, Ho KL, Nelson JS. Gliosarcoma: a histologic and immunohistochemical reaffirmation. Mod Pathol 1990; 3: 19-24.
98. Melaragno MJ, Prayson RA, Murphy MA, Hassenbusch SJ, Estes ML. Anaplastic astrocytoma with granular cell differentiation: case report and review of the literature. Human Pathology 1993; 24: 805-808.
99. Metellus P, Nanni-Metellus I, Delfino C, Colin C, Tchogandjian A, Coulibaly B, Fina F, Loundou A, Barrie M, Chinot O, Ouafik L, Figarella-Branger D. Prognostic impact of CD133 mRNA expression in 48 glioblastoma patients treated with concomitant radiochemotherapy: a prospective patient cohort at a single institution. Ann Surg Oncol 2011; 18: 2937-2945.
100. Meyer-Puttlitz B, Hayashi Y, Waha A, Rollbrocker B, Boström J, Wiestler OD, Louis DN, Reifenberger G, Deimling von A. Molecular genetic analysis of giant cell glioblastomas. Am J Pathol 1997; 151: 853-857.
101. Miller CR ,Perry A. Glioblastoma. Arch Pathol Lab Med 2007; 131: 397-406.
102. Morantz RA, Feigin I, Ransohoff J. Clinical and pathological study of 24 cases of gliosarcoma. J Neurosurg 1976; 45: 398-408.
103. Motomura K, Natsume A, Watanabe R, Ito I, Kato Y, Momota H, Nishikawa R, Mishima K, Nakasu Y, Abe T, Namba H, Nakazato Y, Tashiro H, Takeuchi I, Mori T,Wakabayashi T. Immunohistochemical analysis-based proteomic subclassification of newly diagnosed glioblastomas. Cancer Science 2012.
104. Mueller W, Lass U, Herms J, Kuchelmeister K, Bergmann M, Deimling von A. Clonal analysis in glioblastoma with epithelial differentiation. Brain Pathol 2001; 11: 39-43.

105. Müller W, Slowik F, Firsching R, Afra D, Sanker P. Contribution to the problem of giant cell astrocytomas. Neurosurg Rev 1987; 10: 213-219.
106. Mřrk SJ, Rubinstein LJ, Kepes JJ, Perentes E, Uphoff DF. Patterns of epithelial metaplasia in malignant gliomas. II. Squamous differentiation of epithelial-like formations in gliosarcomas and glioblastomas. J Neuropathol Exp Neurol 1988; 47: 101-118.
107. Nagaishi M, Kim Y-H, Mittelbronn M, Giangaspero F, Paulus W, Brokinkel B, Vital A, Tanaka Y, Nakazato Y, Legras-Lachuer C, Lachuer J, Ohgaki H. Amplification of the STOML3, FREM2, and LHFP Genes Is Associated with Mesenchymal Differentiation in Gliosarcoma. Am J Pathol 2012; 180: 1816-1823.
108. Nagaishi M, Paulus W, Brokinkel B, Vital A, Tanaka Y, Nakazato Y, Giangaspero F, Ohgaki H. Transcriptional Factors for Epithelial-Mesenchymal Transition Are Associated with Mesenchymal Differentiation in Gliosarcoma. Brain Pathol 2012; 22: 670-676.
109. Nagasaka T, Gunji M, Hosokai N, Hayashi K, Ikeda H, Ito M, Inao S. FISH 1p/19q deletion/imbalance for molecular subclassification of glioblastoma. Brain Tumor Pathol 2007; 24: 1-5.
110. Nakamura H, Makino K, Kuratsu J-I. Molecular and clinical analysis of glioblastoma with an oligodendroglial component (GBMO). Brain Tumor Pathol 2011; 28: 185-190.
111. Neelima R, Gopalakrishnan CV, Thomas B, Radhakrishnan VV. Glioblastoma multiforme with epithelial differentiation. Neurol India 2011; 59: 918-920.
112. Nishikawa R, Sugiyama T, Narita Y, Furnari F, Cavenee WK, Matsutani M. Immunohistochemical analysis of the mutant epidermal growth factor, deltaEGFR, in glioblastoma. Brain Tumor Pathol 2004; 21: 53-56.
113. Oh D, Prayson RA. Evaluation of epithelial and keratin markers in glioblastoma multiforme: an immunohistochemical study. Arch Pathol Lab Med 1999; 123: 917-920.
114. Ohgaki H, Kleihues P. Genetic profile of astrocytic and oligodendroglial gliomas. Brain Tumor Pathol 2011; 28: 177-183.
115. Ozolek JA, Finkelstein SD, Couce ME. Gliosarcoma with epithelial differentiation: immunohistochemical and molecular characterization. A case report and review of the literature. Modern Pathology 2004; 17: 739-745.
116. Palma L, Celli P, Maleci A, Di Lorenzo N, Cantore G. Malignant monstrocellular brain tumours. A study of 42 surgically treated cases. Acta Neurochir (Wien) 1989; 97: 17-25.
117. Parsons DW, Jones S, Zhang X, Lin JC-H, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. 2008. An integrated genomic analysis of human glioblastoma multiforme. Science 2008; 321: 1807-1812.
118. Paugh BS, Broniscer A, Qu C, Miller CP, Zhang J, Tatevossian RG, Olson JM, Geyer JR, Chi SN, da Silva NS, Onar-Thomas A, Baker JN, Gajjar A, Ellison DW, Baker SJ. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. J Clin Oncol 2011; 29: 3999-4006.
119. Paugh BS, Qu C, Jones C, Liu Z, Adamowicz-Brice M, Zhang J, Bax DA, Coyle B, Barrow J, Hargrave D, Lowe J, Gajjar A, Zhao W, Broniscer A, Ellison DW, Grundy RG, Baker SJ. Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. J Clin Oncol 2010; 28: 3061-3068.
120. Peraud A, Watanabe K, Plate KH, Yonekawa Y, Kleihues P, Ohgaki H. p53 mutations versus EGF receptor expression in giant cell glioblastomas. J Neuropathol Exp Neurol 1997; 56: 1236-1241.
121. Peraud A, Watanabe K, Schwechheimer K, Yonekawa Y, Kleihues P, Ohgaki H. Genetic profile of the giant cell glioblastoma. Lab Invest 1999; 79: 123-129.
122. Perry A, Aldape KD, George DH, Burger PC. Small cell astrocytoma: an aggressive variant that is clinicopathologically and genetically distinct from anaplastic oligodendroglioma. Cancer 2004; 101: 2318-2326.
123. Perry A, Miller CR, Gujrati M, Scheithauer BW, Zambrano SC, Jost SC, Raghavan R, Qian J, Cochran EJ, Huse JT, Holland EC, Burger PC, Rosenblumb MK. Malignant gliomas with primitive neuroectodermal tumor-like components: a clinicopathologic and genetic study of 53 cases. Brain Pathol 2009; 19: 81-90.
124. Perry JR, Ang LC, Bilbao JM, Muller PJ. Clinicopathologic features of primary and postirradiation cerebral gliosarcoma. Cancer 1995; 75: 2910-2918.
125. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006; 9: 157-173.
126. Pinto LW, Araújo MBM, Vettore AL, Wernersbach L, Leite ACC, Chimelli LMC, Soares FA. Glioblastomas: correlation between oligodendroglial components, genetic abnormalities, and prognosis. Virchows Arch 2008; 452: 481-490.
127. Piña-Oviedo S, De León-Bojorge B, Cuesta-Mejías T, White MK, Ortiz-Hidalgo C, Khalili K, Del Valle L. Glioblastoma multiforme with small cell neuronal-like component: association with human neurotropic JC virus. Acta Neuropathol 2006; 111: 388-396.
128. Plessis du DG, Rutherfoord GS, Joyce KA, Walker C. Phenotypic and genotypic characterization of glioblastoma multiforme with epithelial differentiation and adenoid formations. Clin Neuropathol 2004; 23: 141-148.
129. Pollack IF. Multidisciplinary management of childhood brain tumors: a review of outcomes, recent advances, and challenges. J Neurosurg Pediatr 2011; 8: 135-148.
130. Pollack IF, Finkelstein SD, Woods J, Burnham J, Holmes EJ, Hamilton RL, Yates AJ, Boyett JM, Finlay JL, Sposto R, and Children's Cancer Group. Expression of p53 and prognosis in children with malignant gliomas. N Engl J Med 2002; 346: 420-427.
131. Pollack IF, Hamilton RL, Burger PC, Brat DJ, Rosenblum MK, Murdoch GH, Nikiforova MN, Holmes EJ, Zhou T, Cohen KJ, Jakacki RI, Children's Oncology Group. Akt activation is a common event in pediatric malignant gliomas and a potential adverse prognostic marker: a report from the Children's Oncology Group. J Neurooncol 2010; 99: 155-163.
132. Pollack IF, Hamilton RL, James CD, Finkelstein SD, Burnham J, Yates AJ, Holmes EJ, Zhou T, Finlay JL, Children's Oncology Group. Rarity of PTEN deletions and EGFR amplification in malignant

gliomas of childhood: results from the Children's Cancer Group 945 cohort. J Neurosurg 2006; 105: 418-424.
133. Pollack IF, Hamilton RL, Sobol RW, Burnham J, Yates AJ, Holmes EJ, Zhou T, Finlay JL. O6-methylguanine-DNA methyltransferase expression strongly correlates with outcome in childhood malignant gliomas: results from the CCG-945 Cohort. J Clin Oncol 2006; 24: 3431-3437.
134. Prayson RA. Lipomatous supratentorial primitive neuroectodermal tumor with glioblastomatous differentiation. Ann Diagn Pathol 2009; 13: 36-40.
135. Qaddoumi I, Sultan I, Gajjar A. Outcome and prognostic features in pediatric gliomas: a review of 6212 cases from the Surveillance, Epidemiology, and End Results database. Cancer 2009; 115: 5761-5770.
136. Quezado M, Ronchetti R, Rapkiewicz A, Santi M, Blumenthal DT, Rushing EJ. Chromogenic in situ hybridization accurately identifies EGFR amplification in small cell glioblastoma multiforme, a common subtype of primary GBM. Clin Neuropathol 2005; 24: 163-169.
137. Quon H, Abdulkarim B. Adjuvant treatment of anaplastic oligodendrogliomas and oligoastrocytomas. Cochrane Database Syst Rev 2008; (2): CD007104.
138. Reis RM, Hara A, Kleihues P, Ohgaki H. Genetic evidence of the neoplastic nature of gemistocytes in astrocytomas. Acta Neuropathol 2001; 102: 422-425.
139. Reis RM, Könü-Leblebicioglu D, Lopes JM, Kleihues P, Ohgaki H. Genetic profile of gliosarcomas. Am J Pathol 2000; 156: 425-432.
140. Reis RM, Martins A, Ribeiro SA, Basto D, Longatto-Filho A, Schmitt FC, Lopes JM. Molecular characterization of PDGFR-alpha/PDGF-A and c-KIT/SCF in gliosarcomas. Cell Oncol 2005; 27: 319-326.
141. Rickert CH, Paulus W. Genetic characterisation of granular cell tumours. Acta Neuropathol 2002; 103: 309-312.
142. Ringertz N. Grading of gliomas. Acta Pathol Microbiol Scand 1950; 27: 51-64.
143. Rodriguez FJ, Scheithauer BW, Giannini C, Bryant SC, Jenkins RB. Epithelial and pseudoepithelial differentiation in glioblastoma and gliosarcoma: a comparative morphologic and molecular genetic study. Cancer 2008; 113: 2779-2789.
144. Rutka JT, Giblin JR, Hřifřdt HK, Dougherty DV, Bell CW, McCulloch JR, Davis RL, Wilson CB, Rosenblum ML. Establishment and characterization of a cell line from a human gliosarcoma. Cancer Research 1986; 46: 5893-5902.
145. Saad A, Mo J, Miles L, Witte D. Granular cell astrocytoma of the cerebellum: report of the first case. Am J Clin Pathol 2006; 126: 602-607.
146. Salvati M, Formichella AI, D'Elia A, Brogna C, Frati A, Giangaspero F, Delfini R, Santoro A. Cerebral glioblastoma with oligodendrogliomal component: analysis of 36 cases. J Neurooncol 2009; 94: 129-134.
147. Sanders RP, Kocak M, Burger PC, Merchant TE, Gajjar A, Broniscer A. High-grade astrocytoma in very young children. Pediatr Blood Cancer 2007; 49: 888-893.
148. Sarkar C, Sharma MC, Sudha K, Gaikwad S, Varma A. A clinicopathological study of 29 cases of gliosarcoma with special reference to two unique variants. Indian J Med Res 1997; 106: 229-235.
149. Schiffman JD, Hodgson JG, Vandenberg SR, Flaherty P, Polley M-YC, Yu M, Fisher PG, Rowitch DH, Ford JM, Berger MS, Ji H, Gutmann DH, James CD. Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. Cancer Res 2010; 70: 512-519.
150. Schittenhelm J, Psaras T. Glioblastoma with granular cell astrocytoma features: a case report and literature review. Clin Neuropathol 2010; 29: 323-329.
151. Schlegel J, Piontek G, Kersting M, Schuermann M, Kappler R, Scherthan H, Weghorst C, Buzard G, Mennel H. The p16/Cdkn2a/Ink4a gene is frequently deleted in nitrosourea-induced rat glial tumors. Pathobiology 1999; 67: 202-206.
152. Schwartzentruber J, Korshunov A, Liu X-Y, Jones DTW, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tönjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jäger N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bognar L, Ebinger M, Schuhmann MU, Scheurlen W, Pekrun A, Frühwald MC, Roggendorf W, Kramm C, Dürken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifenberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM, Jabado N. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 2012; 482: 226-231.
153. Seker A, Ozek MM. Congenital glioblastoma multiforme. Case report and review of the literature. J Neurosurg 2006; 105: 473-479.
154. Shapiro JR, Yung WK, Shapiro WR. Isolation, karyotype, and clonal growth of heterogeneous subpopulations of human malignant gliomas. Cancer Res 1981; 41: 2349-2359.
155. Shi Y, Morgenstern N. Granular cell astrocytoma. Arch Pathol Lab Med 2008; 132: 1946-1950.
156. Shinojima N, Kochi M, Hamada J-I, Nakamura H, Yano S, Makino K, Tsuiki H,Tada K, Kuratsu J, Ishimaru Y, Ushio Y. The influence of sex and the presence of giant cells on postoperative long-term survival in adult patients with supratentorial glioblastoma multiforme. J Neurosurg 2004; 101: 219-226.
157. Sibenaller ZA, Etame AB, Ali MM, Barua M, Braun TA, Casavant TL, Ryken TC. Genetic characterization of commonly used glioma cell lines in the rat animal model system. Neurosurg Focus 2005; 19: E1.
158. Simmons ML, Lamborn KR, Takahashi M, Chen P, Israel MA, Berger MS, Godfrey T, Nigro J, Prados M, Chang S, Barker FG, Aldape K. Analysis of complex relationships between age, p53, epidermal growth factor receptor, and survival in glioblastoma patients. Cancer Res 2001; 61: 1122-1128.
159. Singh AD, Iftinca M, Easaw JC. Lipidized glioblastoma: Pathological and molecular characteristics. Neuropathology 2012.
160. Smith SF, Simpson JM, Brewer JA, Sekhon LHS, Biggs MT, Cook RJ, Little NS. The presence of necrosis and/or microvascular proliferation does not influence survival of patients with anaplastic oligodendroglial tumours: review of 98 patients. J Neurooncol 2006; 80: 75-82.
161. Song X, Andrew Allen R, Terence Dunn S, Fung K-M, Farmer P, Gandhi S, Ranjan T, Demopoulos A, Symons M, Schulder M, Li JY.

Glioblastoma with PNET-like components has a higher frequency of isocitrate dehydrogenase 1 (IDH1) mutation and likely a better prognosis than primary glioblastoma. Int J Clin Exp Pathol 2011; 4: 651-660.
162. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJB, Janzer RC, Ludwin SK,Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups, and National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009; 10: 459-466.
163. Takeuchi H, Hosoda T, Kitai R, Kodera T, Arishima H, Tsunetoshi K, Neishi H, Yamauchi T, Sato K, Imamura Y, Itoh H, Kubota T, Kikuta K. Glioblastoma with oligodendroglial components: glioblastoma or anaplastic oligodendroglial tumors. Brain Tumor Pathol 2012; 29: 154-159.
164. Tihan T, Vohra P, Berger MS, Keles GE. Definition and diagnostic implications of gemistocytic astrocytomas: a pathological perspective. J Neurooncol 2006; 76: 175-183.
165. Varlet P, Soni D, Miquel C, Roux F-X, Meder J-F, Chneiweiss H, Daumas-Duport C. New variants of malignant glioneuronal tumors: a clinicopathological study of 40 cases. Neurosurgery 2004; 55: 1377-1391, discussion 1391-1392.
166. Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR,Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN, Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010; 17: 98-110.
167. Vordermark D, Ruprecht K, Rieckmann P, Roggendorf W, Vince GH, Warmuth-Metz M, Kölbl O, Flentje M. Glioblastoma multiforme with oligodendroglial component (GBMO): favorable outcome after post-operative radiotherapy and chemotherapy with nimustine (ACNU) and teniposide (VM26). BMC Cancer 2006; 6: 247.
168. Wang Y, Li S, Chen L, You G, Bao Z, Yan W, Shi Z, Chen Y, Yao K, Zhang W, Kang C, Jiang T. Glioblastoma with an oligodendroglioma component: distinct clinical behavior, genetic alterations, and outcome. Neurooncology 2012; 14: 518-525.
169. Watanabe K, Peraud A, Gratas C, Wakai S, Kleihues P, Ohgaki H. p53 and PTEN gene mutations in gemistocytic astrocytomas. Acta Neuropathol 1998; 95: 559-564.
170. Watanabe K, Tachibana O, Yonekawa Y, Kleihues P, Ohgaki H. Role of gemistocytes in astrocytoma progression. Lab Invest 1997; 76: 277-284.
171. Wikstrand CJ, Bigner SH, Bigner DD. Demonstration of complex antigenic heterogeneity in a human glioma cell line and eight derived clones by specific monoclonal antibodies. Cancer Res 1983; 43: 3327-3334.
172. Wolańczyk M, Hułas-Bigoszewska K, Witusik-Perkowska M, Papierz W, Jaskólski D, Liberski PP, Rieske P. Imperfect oligodendrocytic and neuronal differentiation of glioblastoma cells. Folia Neuropathol 2010; 48: 27-34.
173. Zarghooni M, Bartels U, Lee E, Buczkowicz P, Morrison A, Huang A, Bouffet E, Hawkins C. Whole-genome profiling of pediatric diffuse intrinsic pontine gliomas highlights platelet-derived growth factor receptor alpha and poly (ADP-ribose) polymerase as potential therapeutic targets. J Clin Oncol 2010; 28: 1337-1344.