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Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge

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Preface

We have extended our understanding of the molecular biology underlying adult glioblastoma over many years. In contrast, high-grade gliomas in children and adolescents have remained a relatively under-investigated disease. The latest large-scale genomic and epigenomic profiling studies have yielded an unprecedented abundance of novel data and revealed deeper insights into gliomagenesis across all age groups, highlighting key distinctions, but also some commonalities. As we are on the verge of dissecting glioblastomas into meaningful biological subgroups, this Review summarizes the hallmark genetic alterations associated with distinct epigenetic features and patient characteristics in both paediatric and adult disease, and examines the complex interplay between the glioblastoma genome and epigenome.

Introduction

Glioblastoma (GBM; synonymous with the formerly used term ‘glioblastoma multiforme’) is the most frequent and most aggressive malignant primary brain tumour[1, 2], classified as grade IV in the World Health Organization (WHO) classification of tumours of the central nervous system (CNS)[1]. GBM can develop from lower-grade diffuse astrocytoma (WHO grade II) and anaplastic astrocytoma (WHO grade III), and is then termed ‘secondary’ GBM. ‘Primary’ (*de novo*) GBMs are more common, and typically manifest rapidly after a short clinical history and without recognizable signs of a preceding precursor lesion[3]. Diagnostic criteria for GBM, including high mitotic activity, microvascular proliferation and/or necrotic areas, are fulfilled by both primary and secondary GBM, making them indistinguishable by histology alone[1].

Overall, primary GBM accounts for 16% of primary brain tumours[2]. The incidence increases with age, affecting an average of 7.2 per 100,000 adults (> 19 years of age) every year, with a peak age at diagnosis between 75 and 84 years (annual incidence rate 14.6 per 100,000)[2]. Due to the lower incidence in children, studies on paediatric high-grade gliomas (HGG) have traditionally combined GBM, anaplastic astrocytomas and diffuse intrinsic pontine gliomas (DIPG). Although most DIPG histologically present as high-grade tumours and share a universally fatal outcome, they comprise tumours with varying histological grade and divergent differentiation[4–7]. Together, up to 0.8 per 100,000 children (age < 19) are estimated to develop high-grade gliomas each year, making them the most common group of malignant CNS neoplasms in this age group alongside embryonal tumours[2]. The current treatment strategy for GBM patients consists of surgery, radiotherapy and chemotherapy. Complete surgical resection of these infiltrative tumours is virtually impossible and surgery is typically not attempted for DIPG patients due to their location. Concurrent adjuvant radiotherapy in combination with temozolomide (TMZ) represents the standard of care for newly diagnosed GBM, but still less than 5% of patients survive longer than five years post diagnosis[2, 8–10]. Since no chemotherapeutic drugs have proven to be effective in the treatment of DIPG, radiation therapy alone represents the current standard

regimen[11, 12]. Prognosis here is even worse, with less than 10% of DIPG patients surviving more than two years after diagnosis[2, 13].

Over recent years, large-scale research efforts spearheaded by The Cancer Genome Atlas (TCGA) network and the paediatric neurooncology community have been directed at studying the molecular biology underlying GBM, and have generated detailed catalogues of genomic and epigenomic alterations. The emerging insights into gliomagenesis have triggered an avalanche of novel perceptions on the epigenomic and genomic landscape, biological subgroups and putative cells of origin of GBM, fuelling hopes for more effective treatment strategies in the near future. In this Review, we discuss the major recent advances in GBM molecular research, and we aim to convey important details, as well as a general overview of GBM biology across all ages.

Molecular subtypes of glioblastoma

Gene expression profiling of glioblastoma—Genome-wide profiling studies using gene expression microarrays were successfully used to compare gene expression patterns in primary *vs.* secondary GBM[14–17] or adult *vs.* paediatric GBM[18], and to identify differentially expressed genes that could be used to distinguish between different groups. In parallel, early reports showed the utility of expression arrays in diagnostic assignment and prognostication within larger glioma cohorts of different grades[19–21] and within GBM cohorts only[22]. Phillips and colleagues[23] described three subclasses of high-grade glioma (termed proneural, mesenchymal and proliferative, based on the functional annotation of signature genes) as being associated with different outcomes, namely prolonged survival of the proneural subclass. Similar subclasses of GBM were also detected in a large cohort of mixed gliomas and data from this cohort also revealed a distinct gene expression cluster enriched for secondary GBM[24]. Partially in line with these previous findings, unsupervised clustering of gene expression data from 200 adult GBM samples from TCGA network[25, 26] identified four different molecular subtypes: proneural, neural, classical and mesenchymal. The proneural subtype was largely characterized by abnormalities in platelet derived growth factor receptor α (*PDGFRA*) or isocitrate dehydrogenase 1 (*IDH1*), whereas mutation of the epidermal growth factor receptor (*EGFR*) was found in the classical subgroup and mutations in neurofibromin (*NF1*) were common in mesenchymal tumours. This classification system has been refined by subdividing proneural GBM into glioma-CpG island methylator phenotype (G-CIMP)-positive and G-CIMP-negative GBM subsets based on characteristic DNA methylation patterns that strongly correspond with *IDH1* mutation status[27] (Figure 1; discussed below). Comparing the transcriptional classification schemes of Phillips *et al.*[23] with those of Verhaak *et al.*[25] suggests that the proneural and mesenchymal signatures are the most robust and account for the greatest transcriptional variance, while the exact number and composition of other signatures may depend on the sample cohort compositions and experimental designs[28, 29]. The transcription factors signal transducer and activator of transcription 3 (STAT3), C/EBPβ and TAZ have been identified as epigenetic master regulators of the mesenchymal signature and might predict poor clinical outcome[30, 31]. Moreover, *CTNND2* (encoding cyclin D2) and *RHPN2* have recently emerged as negative and positive genetic drivers of mesenchymal transformation, respectively[32, 33]. There is also evidence of plasticity between the proneural and mesenchymal subtypes in GBM[23] that

might be driven by the tumour microenvironment, perhaps through microglia and the NF-κB pathway[34]. The role that the proneural and mesenchymal signatures and other signatures might have as predictors of clinical outcome remains to be clarified. The initially reported better prognosis of the proneural subclass[23, 25] was recently shown to only prove true for the G-CIMP subset, while non-G-CIMP proneural and mesenchymal GBM tend to show less favourable outcomes in the first twelve months post diagnosis compared with other GBM subtypes[26].

Initial gene expression studies of paediatric HGG highlighted significant differences compared to tumours from adult patients, and suggested the existence of molecularly diverse subsets within paediatric cohorts[18, 35–37]. Activation of a *PDGFRA*-driven gene expression signature has been described in a large proportion of paediatric HGG[4, 36], correlating with the higher frequency of *PDGFRA* amplifications observed in this age group[36–40]. Interestingly, one of these studies further revealed shared gene expression patterns between DIPG and a proportion of midline/thalamic GBM, pointing towards the closely related pathogenesis of these tumours, which was later confirmed by genome sequencing studies[40–42]. Indeed, comparing midline GBM and DIPG that harboured a K27M amino acid change owing to a mutation in *H3F3A*, one of the histone H3.3 genes, with hemispheric GBM with *H3F3A* mutations that result in amino acid changes at G34 revealed specific gene expression profiles that are distinct from each other and from tumours with wild-type *H3F3A* status[40, 41, 43, 44].

DNA methylation profiling of glioblastoma—A number of studies have reported promoter-associated hypermethylation of specific loci in GBM, frequently affecting the expression of genes with known tumour suppressor function such as cyclin dependent kinase inhibitor 2A (*CDKN2A*), *RB1*, *PTEN* and *TP53*[45–48], as well as previously unrecognised regulatory genes involved in cell proliferation (epithelial membrane protein 3 (*EMP3*) and *PDGFB*)[49, 50], invasion (protocadherin γ subfamily A, member 11 (*PCDHGA11*))[51], or radiation sensitivity (suppressor of cytokine signalling 1 (*SOCS1*))[52, 53]. Most prominently, promoter methylation of *MGMT* (encoding O^6-methylguanine methyltransferase) occurs in ~ 45% of GBM in adult patients[26, 54–57], leading to gene silencing and consequently to a reduced proficiency for repairing DNA damage induced by alkylating agent chemotherapy[58–61]. Thus, methylation at this locus has been established as a biomarker for predicting the benefit of TMZ chemotherapy, particularly in elderly GBM patients[56, 57, 61, 62], while both the frequency (16–50%)[63–66] and prognostic significance of *MGMT* silencing in childhood HGG remain controversial[63, 66].

DNA microarray techniques have successfully been applied to study the GBM methylome in a genome-wide manner, and have led to the discovery of clearly defined GBM subgroups based on their global DNA methylation patterns (Figure 1). The first large study, with a purely adult patient cohort, reported three DNA methylation subgroups. One group was tightly associated with *IDH1* mutations and displayed concerted hypermethylation at a large number of loci, and was therefore termed G-CIMP-positive[27]. A later study comparing DNA methylation patterns across both paediatric and adult GBM patients found a similar clustering in tumours from adult patients, and further identified three more distinct clusters composed predominantly of children and adolescents[40]. Two of these corresponded strictly

with recurrent age-specific (K27 or G34) mutations in *H3F3A*, and another group of tumours was enriched for *PDGFRA* alterations (and termed receptor tyrosine kinase 1 (RTK I) and consisted of patients from a more widespread age range[40]. Aberrations of *PDGFRA* are present in approximately half of G-CIMP-negative GBM of the proneural subtype in children and adults[25, 40, 67]. Epigenetic silencing by promoter hypermethylation of the neural lineage markers *OLIG1* and *OLIG2* was observed as a novel and exclusive characteristic in GBM from the G34 cluster and points towards a different pathogenesis of this subgroup. These tumours also showed a considerable decrease in overall DNA methylation on a global scale[40]. This has been previously reported as a common epigenetic feature in primary GBM and was linked to genomic instability of affected regions, deficiency of the methyl group metabolism gene *MTHFR* and increased proliferative activity[68]. A distinct G-CIMP-positive IDH cluster was also identified and, in line with previous reports, tumours from this group were found among younger adult patients, showed proneural gene expression patterns, lacked typical copy-number aberrations (gain of chromosome 7, loss of chromosome 10, *EGFR* amplification, *CDKN2A* and *CDKN2B* deletion), and were associated with a favourable prognosis compared to all other GBM subgroups[27, 40], explaining the increased survival observed in the proneural GBM subtype[23, 25]. The remaining G-CIMP-negative DNA methylation clusters comprised GBM from elderly patients, displaying predominantly classical gene expression, combined gain of chromosome 7 and loss of chromosome 10, and *EGFR*, *CDKN2A* and *CDKN2B* alterations (termed cluster #2 and RTK II)[27, 40], and GBM enriched for mesenchymal gene expression patterns and mutations in *NF1* and *PTEN* (termed cluster #3 and mesenchymal subtype)[27, 40].

Although mutations in *H3F3A* and *IDH1* seem to map one-to-one with three distinct DNA methylation subclasses, recent in-depth analysis of genome-wide DNA methylation and sequencing data by TCGA has found no similarly striking pathognomonic mutations for the additional non-G-CIMP subclasses of adult GBM[26]. This study further confirmed the notion that, despite considerable overlap, established gene expression subclasses do not directly correspond to those defined by DNA methylation profiling[26], unlike DNA methylation profiling in medulloblastoma[69]. Whether DNA methylation profiling provides a more robust and clinically useful platform for GBM subgrouping remains to be tested, but the logistics involved are probably less error-prone than for any RNA-based analyses.

Structural variations in glioblastoma

DNA copy-number aberrations (CNAs) are commonly observed in GBM, and can affect a significant fraction of the tumour genome[67, 70]. Assessing DNA copy-number using high-resolution techniques in large collections of tumour samples has allowed the precise characterization of focal CNA target regions, and genome-wide sequencing studies are beginning to unravel more complex structural rearrangements and hitherto unknown fusion events.

Chromosomal aberrations and genomic rearrangements—Genomic gains of chromosome 7 and losses of chromosome 10 (most often occurring concomitantly; $7^{+}/10^{-}$) represent by far the most common gross chromosomal abnormalities in GBM, being

detected in 83–85% of GBM in adults[26, 67] (Table 1). Consistently throughout reported studies, $7^{+}/10^{-}$ is more frequent in older (age ≥ 70 years) compared to younger (age ≤ 40 years) patients[71], and is therefore highly enriched (> 95 %) in GBM from the classical (or RTK II) subclass, but less common in GBM of the proneural gene expression subtype[23, 25], and virtually absent in *H3F3A*- or *IDH*-mutant G-CIMP-positive tumours[27, 40] (Figure 1). Further broad genomic copy-number changes seen at high frequency (> 20%) in adult GBM patients include gains of chromosomes 19 and 20 (35–40%; enriched in the classical (or RTK II) subtype), and losses affecting chromosomes 9p (38%), 22q (33%), 13q (33%), 14q (27%) and 6q (22%)[26, 67] (Table 1). The number of chromosomal imbalances is generally lower in childhood HGG and a proportion of these tumours (~ 15%) lack any detectable copy-number abnormalities[36, 38, 40, 72]. Paediatric tumours also differ from their adult counterparts in displaying frequent gain of chromosome 1q (20%; enriched in *H3F3A* G34-mutated GBM), while only rarely harbouring aberrations of chromosomes 7 and 10[36, 38].

Recent data indicate that the incidence of complex chromosomal rearrangements in the context of a single catastrophic event (*chromothripsis*) is significantly higher in GBM (> 30%) relative to most other tumour types (9%), including breast, colon and lung cancer[70]. Interchromosomal, intrachromosomal (intergenic) and intragenic rearrangements can be detected in the majority (69%) of GBM samples, and these frequently (56%) co-occur with intragenic copy-number differences at the breakpoints[73]. The most prominent intragenic deletions in GBM target parts of the gene encoding either the extracellular domain of EGFR (exons 2–7 to form EGFRvIII) or the carboxy terminus[73–76], and are almost always associated with amplification and co-expression of the wild-type *EGFR* allele[26, 76, 77] (Figure 2 and Table 2). Similarly, a fraction of *PDGFRA*-amplified GBM from both adults and children harbour age-specific intragenic deletion rearrangements of this kinase with constitutively increased activity[78, 79]. Further intragenic deletions disrupt the function of the tumour suppressor candidate gene Fas-associated factor 1 (*FAF1*) as a result of focal deletion of the adjacent gene, *CDKN2C*[73].

A breakpoint-enriched region on chromosome 12q (12q14-15), identified in ~ 5% of GBM, was recently shown to harbour copy-number equal co-amplification of the two known GBM oncogenes *CDK4* and *MDM2*, assembled into double minute chromosomes[73, 80]. Additional fusion transcripts between genes included in the two amplicon segments were also observed[73]. This phenomenon was found to be mutually exclusive with *CDKN2A* and *CDKN2B* deletions and *TP53* mutations, and associated with adverse patient survival[73]. Similarly, a number of gliomas were previously found to harbour double minute chromosomes bearing amplified copies of the *EGFR* gene[81] (Figure 2). More recently, *EGFR* was shown to be further activated by recurrent translocations in 7% of GBM samples, most frequently being fused in-frame to septin 14 (*SEPT14*) or phosphoserine phosphatase (*PSPH*) as the 3′ gene segment, and almost always occurring within amplified regions of the fusion partner genes[26, 32]. In a smaller fraction of GBM (~ 3%), local inversion and in-frame fusion of the tyrosine kinase coding region of fibroblast growth factor receptor 1 (*FGFR1*) to the transforming acidic coiled-coil (TACC) coding domain of *TACC1* (or alternatively fusion of *FGFR3* to *TACC3*) results in constitutive kinase activity[82, 83]. Therefore, recurrent fusion events involving RTK-encoding genes may represent a

promising therapeutic target and provide a strong rationale for the inclusion of these patients in future clinical trials using RTK inhibitors.

Focal copy-number aberrations—The spectrum of high-amplitude focal copy-number alterations in adult GBM highlights a key role of *EGFR* amplifications (43% of cases) and *CDKN2A* and *CDKN2B* deletions (53% of cases)[26], although these events are rarely detected in paediatric HGG (< 5% and ~ 20% of cases, respectively)[38, 40, 72] (Figure 1 and Table 2). Both events are enriched in the classical (or RTK II) and neural molecular subtypes[25] and co-occur with *EGFR* intragenic deletions and/or point mutations[25, 26, 76]. Known genes less frequently targeted by homozygous deletion in adult GBM include *PTEN* (10%), *RB1* (3%), *CDKN2C* and *FAF1* (3%), *QKI* (1.6%), *NF1* (1.3%), *NPAS3* (1.3%) and *TP53* (1%)[26]. High-level amplifications of *CDK4* (13%), *PDGFRA* (11%), *MDM2* (8%), *MDM4* (7%), *MET* (2%) and *CDK6* (1.5%) often co-occur in a variety of combinations[26], with common co-amplification of RTK-encoding genes in a mosaic-like pattern (Box 1). Amplifications of *CCND2*, *CCNE1*, *SOX2*, *MYC* and *MYCN* represent recurrent but less frequent events in adult GBM (< 3%)[26]. Although to some extent most of these gains are also observed in paediatric HGG, focal amplifications of *PDGFRA* (14%) and *MYC* or *MYCN* (8%) are more frequent in the paediatric population[38, 40, 72, 84]. Recurrent focal amplification of *PDGFRA* has been suggested as a key oncogenic event in DIPG in a number of studies[4, 37, 85]. With increasing molecular data on treatment-naïve DIPG, however, the occurrence of this feature seems to be slightly lower than previously reported (< 40%), and enriched in DIPG harbouring histone H3 mutations[40] (C.J., J.G., O.B., C.H. J.M. and N.J., unpublished observations). As *PDGFRA* amplification is somewhat more commonly observed in radiation-induced gliomas[36], one might hypothesize a possible link between *PDGFRA* amplifications and prior radiotherapy, a caveat when studying *post mortem* DIPG (and GBM) tissue obtained from autopsy cases.

Box 1

Tumour heterogeneity in glioblastoma

Intratumoural heterogeneity, referring to the presence of multiple, epigenetically and genetically different cell sub-populations within a single tumour, might contribute to tumour growth, progression and treatment failure[180]. In glioblastoma (GBM), the previously used term ‘multiforme’ describes the histopathologically observed co-existence of morphologically heterogeneous areas[1]. On a molecular level, this phenomenon is well reflected by different area-specific chromosome aberrations[181–183], mutations[184] and gene expression patterns[22, 185, 186]. Multiple receptor tyrosine kinase (RTK) genes (epidermal growth factor receptor (*EGFR*), platelet derived growth factor receptor α (*PDGFRA*) and *MET*) can be amplified and activated in a mutually exclusive manner in adjacent intermingled tumour cells[178, 179, 187], and even multiple *EGFR* mutant alleles can commonly be expressed in a single tumour[26]. Arising from a single precursor and following an early driver event (such as loss of cyclin dependent kinase inhibitor 2A (*CDKN2A*) and *CDKN2B*)[186, 187], activation of different RTKs in equally proliferating sub-populations may have distinct effects on downstream signalling pathways not only within but also between and among heterogeneous sub-populations *via*

cell-cell interactions[187]. Indeed, a paracrine mechanism has been identified by which cells expressing EGFRvIII recruit wild-type EGFR-expressing cells into accelerated proliferation through upregulation of interleukin 6 (IL-6), leukaemia inhibitor factor (LIF) and GP13, thereby potently driving growth of the entire tumour mass and maintaining tumour cell heterogeneity[188]. These observations have important implications regarding the effects of RTK-targeted therapies, as tumours that have mosaic driver RTK amplifications will probably require the simultaneous use of more than one targeted agent[178, 189]. Furthermore, the impact of sampling bias over space and time is an important diagnostic consideration, particularly in the era of targeted therapy[186]. Tumour heterogeneity in GBM may also arise from clonal evolution, i.e. through the expansion and acquisition of mutations during tumour progression, promoting genetic variability[190]. This concept may be linked with the hierarchical cancer stem cell (CSC) model, which postulates a small sub-population of stem-like cells that give rise to a tumour and are maintained through their capability to self-renew and to produce diverse daughter cells that populate the tumour bulk[191].

Mutational spectrum of glioblastoma

Advances in sequencing technology have led to the identification of increasing numbers of recurrent somatic mutations in the GBM genome of adult patients (Table 2). The number of coding mutations per tumour sample is highly variable (median: ~ 53; range: 3–179)[26]. Frequent mutations in genes such as *PTEN* (29%), *TP53* (29%), *EGFR* (20%), *NF1* (9%), *RB1* (8%), phosphatidylinositol 3 catalytic α (*PIK3CA*; 7%), *PIK3R1* (6%) and *IDH1* (5%) have been reported as significant GBM signature events in earlier studies[67, 86]. The increasing number of samples with genome-wide sequencing data has now facilitated the detection of lower-frequency events in both cancer-related as well as previously un-associated genes such as spectrin α (*SPTA1*; 9%), *ATRX* (6%), *KEL* (5%), gaba-aminobyteric acid α6 (*GABRA6*; 4%), leucine zipper-like transcriptional regulator 1 (*LZTR1*; 3%), *BCOR* (2%), *BRAF* (2%), catenin δ2 (*CTNND2* (2%)) and *QKI* (2%)[26]. Some of those genes (such as *LZTR1*, *CTNND2*, *QKI*, *BRAF* and *BCOR*) are recurrently targeted by both copy-number changes and point mutations, thus underscoring their role as new driver genes[32]. Of note, there is a non-random pattern of mutual exclusivities (such as *PIK3R1* and *PIK3CA*)[26, 67] (C.J., J.G., J.M. and N.J., unpublished observations) and co-occurrences (such as *IDH1*, *TP53* and *ATRX*)[26, 87, 88], and multiple but overall mutually exclusive mutations in genes implicated in regulation of chromatin modification[26], a phenomenon recently observed in various other cancer types[89, 90]. Based on genome-wide sequencing data from 284 GBM samples generated by TCGA[26], 46% of cases were found to have at least one somatic mutation in a gene associated with DNA methylation (*IDH1*), histone modifications (SET domain containing protein 2 (*SETD2*) and lysine-specific demethylase 6A (*KDM6A*), mixed lineage leukaemia 2 (*MLL2*), *MLL3*, *MLL4*, *EZH2* and histone deacetylase 2 (*HDAC2*)) or chromatin remodelling (*ATRX*, CREB binding protein (*CREBBP*), chromodomain helicase DNA binding protein 5 (*CHD5*), *CHD6*, *CHD7*, *CHD8*, *CHD9* and SWI/SNF-related matrix-associated, actin-dependent regulator of chromatin A2 (*SMARCA2*)) (Figure 2).

Importantly, 58–84% of primary GBM in adults have hotspot mutations in the *TERT* gene promoter. These mutations are tightly coupled with *EGFR* amplifications and inversely correlated with *IDH1*, *IDH2*, *TP53* and *ATRX* mutations[26, 91–95], and are associated with increased telomerase expression and activity[26, 92, 94, 96] (Figure 3). *TERT* promoter mutations are found at a much lower rate (3–11%) in childhood GBM[91, 95], which instead frequently display loss of ATRX and an alternative lengthening of telomeres (ALT) phenotype to maintain or increase telomere length[41, 97].

Moreover, recent work has identified novel oncogenic mutations in *PDGFRA* in a proportion of paediatric HGG, often in combination with amplification of the *PDGFRA* locus[4, 79]. Of further note and clinical relevance, inactivating mutations in DNA mismatch repair (MMR) genes (such as *MSH6*) can be induced during TMZ treatment and evoke a GBM hypermutator phenotype, which is causally related to TMZ resistance[67, 98–100].

Histone mutations in childhood high-grade gliomas—The first recurrent histone mutations in human cancer were uncovered by sequencing studies on a large number of paediatric GBM and DIPG patient samples[41, 101]. Heterozygous mutations in *H3F3A* result in amino acid substitutions at positions K27 or G34, and K27 was also found to be mutated in the H3.1 histone genes *HIST1H3B* and *HIST1H3C*[41, 101] (C.J., J.G., O.B., C.H., J.M. and N.J., unpublished observations). These mutations directly or indirectly target important sites on the histone tail for posttranslational modifications (Figure 2). Occurring exclusively in ~ 38% of childhood and young adult (≤ 30 years) HGG[41, 102] (Table 2) and in a mutually exclusive fashion with each other and with mutations in *IDH1*, each histone mutation shows a strong association with distinct molecular features as well as clinical patient characteristics[5, 40, 42] (Figure 1). Mutations in *H3F3A* that result in amino acid changes at G34 occur in adolescents around the age of 20 years that have disease exclusively located in hemispheric regions. A significant number of these tumours have mutations in *TP53*, *ATRX* and (less frequently) *DAXX*[40, 41], and commonly display a global pattern of DNA hypomethylation and an ALT phenotype[40]. Mutations in *H3F3A* that result in amino acid changes at K27 occur in 70–80% of midline GBM and DIPG in younger children[5, 40, 101]. The same substitutions in *HIST1H3B* or *HIST1H3C* seem to be less common and specific to DIPG (11–31%) and to coincide with novel activating mutations in *ACVR1* (also known as *ALK2*) identified in ~ 20% of DIPG cases (C.J., J.G., O.B., C.H., J.M. and N.J., unpublished observations; Suzanne J. Baker, personal communication), providing a potentially druggable target in this subset. Interestingly, recent studies also detected *H3F3A* K27M mutations in a small fraction (< 2%) of midline pilocytic astrocytomas (WHO grade I)[103, 104], conceivably indicating some overlap between the genetics of paediatric low-grade and high-grade gliomas or, alternatively, a remaining degree of diagnostic uncertainty. GBM harbouring mutations in histone H3 genes are mostly devoid of hallmark cytogenetic aberrations, such as *EGFR* alterations and *CDKN2A* and *CDKN2B* deletions observed in adult GBM patients[40] (Figure 1). Interestingly, mutations at K27 seem to confer a dismal prognosis[5, 40], while G34 mutations might be associated with slightly prolonged overall survival[40]. However, these initial observations will have to be tested in larger, prospective patient cohorts.

A gain-of-function K27M mutation in H3.3 or H3.1 leads to global downregulation of the repressive histone mark H3K27me3 through inhibition of the Polycomb repressive complex 2 (PRC2)[44, 105–107]. K27M-mutant H3.3 was shown to aberrantly bind PRC2 and interfere with the enzymatic activity of EZH2 – the catalytic subunit of PRC2 that establishes the H3K27me3 mark – possibly due to methionine mimicking the structure of monomethyl lysine[106]. Consequent loss of repressive PRC2 activity resulting from a combination of reduced H3K27me3 and DNA hypomethylation has therefore been proposed as the main driver of gliomagenesis in K27M-mutant HGG[44, 106] (Figure 2).

In a similar vein, G34R and G34V-mutant H3.3 can interfere with the regulatory H3K36me3 modification[43, 106]. Genome-wide mapping of H3K36me3 in a G34V-mutant GBM cell line identified *MYCN* among the genes most strongly enriched for H3K36me3 marks, with increased RNA polymerase II binding and transcriptional upregulation of this locus in G34V-mutant cells[43]. Therefore, in addition to *MYCN* focal amplification, H3.3 G34 mutations may represent an alternative mechanism to overexpress MYCN, which was recently shown to drive glioma formation in neural stem cells (NSCs) *in vivo*[108], and could potentially be targeted by bromodomain inhibition[109]. H3K36me3 is further disrupted by mutations in the H3K36 trimethyltransferase SETD2 in children and young adults, which are mutually exclusive with *H3F3A* G34 mutations[110] (Figure 2). Besides its role in transcriptional elongation[106], H3K36me3 was found to be important as a recruitment platform for MMR proteins[111]. Depletion of SETD2 and therefore H3K36me3 levels in HeLa cells leads to an increased spontaneous mutation frequency and chromosomal instability[111]. In line with this, altered histone modifications in subtelomeric regions have been suggested to contribute to deregulated telomere length, also resulting in chromosomal instability. This is reflected by a particularly high number of paediatric GBM displaying ALT[41] (Figure 3). Although genetic hits in *ATRX* and *DAXX* (both essential components for H3.3 incorporation at pericentromeric heterochromatin and at telomeres) are known to promote the ALT phenotype, the contribution of mutant p53[112], histone modifications and subtelomeric DNA hypomethylation to GBM development needs further investigation.

IDH mutations in glioblastomas of young adults—In the early stages of the next-generation sequencing era, the first genome-wide exon sequencing effort in glioma identified heterozygous hotspot mutations at codon 132 (most commonly R132H) in *IDH1* in 12% of GBM[86]. Today, mutations in *IDH1* (and to a lesser extent in *IDH2*) are commonly established as a hallmark molecular feature of secondary GBM in young adults (age 25–45 years; being present in ~ 70% compared to < 5% in primary GBM)[113–116] with predominant localisation in the frontal and temporal lobes[117]. The small number of *IDH*-mutant primary GBM may therefore have rapidly progressed from clinically undiagnosed lower-grade astrocytomas. Mutations in the *IDH* genes are thought to be causative of G-CIMP within the proneural GBM subgroup[23, 25, 27, 40, 118] (Figure 1). The role of mutations in *IDH* genes in gliomas with respect to molecular pathogenesis as well as clinical implications has recently been reviewed in detail[119, 120]. *IDH* mutations seem to require cooperating mutations in *TP53* and *ATRX*[87, 88], and possibly *SETD2*[110], while being less frequently detected in GBM with classical RTK pathway alterations[40, 67, 121]. Besides being a powerful diagnostic marker[122], IDH-mutant GBM have a favourable clinical outcome compared to their non-

mutated counterparts[26, 123]. The main mechanism by which mutant IDH contributes to the pathogenesis of GBM is ascribed to the neomorphic enzymatic activity of mutant IDH proteins, which produce high amounts of the R enantiomer of 2-hydroxyglutarate (2-HG) (a putative oncometabolite) from 2-oxoglutarate (2-OG; also known as α-ketoglutarate)[124, 125]. 2-HG has been shown to inhibit a variety of 2-oxoglutarate-dependent dioxygenases, including key epigenetic regulators such as the DNA demethylating enzymes of the TET[126] or Jumonji domain families[127, 128] (Figure 2). Hence, the methylome of IDH-mutant GBM is dominated by widespread DNA hypermethylation at gene promoters (G-CIMP)[118], irrespective of patient age[40]. The interaction of TET2 with particular transcription factors, such as EBF1, might result in specific rather than random gene promoter hypermethylation[129]. Although there is a strong association of IDH mutation with G-CIMP, early passage neurosphere cultures from IDH wild-type GBM can show a G-CIMP-like phenotype, suggesting intratumoural heterogeneity and/or the involvement of other, as yet unknown, factors[34].

The cells of origin of glioblastoma

Although multiple genetic and epigenetic alterations are known to initiate or promote gliomagenesis, answers as to the cell of origin that acquires the first tumour-promoting mutation remain elusive. Several cell types including NSCs[130, 131], more differentiated progeny such as oligodendrocyte precursors[132, 133], or cells of the astrocytic lineage are discussed as putative glioma-initiating cells. Indeed, all these cell types can give rise to glioma if genetically manipulated (Box 2). Interestingly, malignant transformation seems not to be limited to undifferentiated cell types – transformation of mature neurons or astrocytes by depletion of *Nf1* and *Trp53* has also been shown to generate malignant gliomas *in vivo*[134]. Various pieces of evidence, including differences in tumour location, patient age, mutational spectrum or expression of neuronal lineage markers have led to growing speculation that in addition to acquiring distinct genetic events, at least some of the GBM subgroups described above also have unique cellular origins. This hypothesis might especially prove true for the tight correlations between molecular and clinical characteristics observed in DIPG and midline GBM, which possibly originate from recently identified pontine precursor-like cells[135].

Box 2

In vivo tumour models of glioblastoma

In vivo tumour models provide an indispensable tool to study the contribution of cancer-related genes or develop and evaluate novel treatment options. Strategies to reproduce glioblastoma (GBM) tumours in animals include xenograft transplantation models, germline modification of mouse strains and/or somatic gene transfer[192, 193]. Orthotopic injection of primary human GBM cells into immunocompromised mice gives rise to tumours partially retaining histological features and key molecular characteristics of human GBM, but with the disadvantages of altered tumour immunology and absence of a natural microenvironment[194–197]. To recapitulate the diversity of human GBM subgroups[23, 25, 40], genetically engineered mouse models allowing for spontaneous tumour growth have been generated by adopting key glioma signature mutations in

receptor tyrosine kinase (RTK) genes, *TP53*, *PTEN*, *RB*, *NF1* and in cell-cycle pathways[192, 193, 198, 199]. While overexpression of epidermal growth factor receptor (EGFR) alone or EGFRvIII alone is insufficient in effectively promoting tumour formation from neuroglial precursors[200] or astrocytic cells[200, 201], concomitant overexpression of EGFRvIII (and less efficiently wild-type EGFR) with loss of the cyclin dependent kinase inhibitor 2A (*Cdkn2a*) and/or *Pten* and *Trp53* loci is capable of inducing GBM[202–205] that might resemble human tumours of the classical transcriptome subtype[25, 40]. Similarly, PDGF-driven gliomagenesis recapitulates GBM formation in mice when combined with germline loss of *Cdkn2a*, *Pten*, or *Trp53*[206–208]. This model has been successfully used to study paediatric and adult GBM biology[192, 206, 209, 210], and is significantly enriched in proneural and RTK I GBM subtypes[23, 25, 40]. The mesenchymal subtype of GBM might be best investigated using established models based on loss-of-function of *Nf1*, which cooperates with early *Trp53* mutations (and *Pten* haploinsufficiency) to induce astrocytomas of different grades[131, 211–215]. Although molecular profiling of genetically engineered GBM mouse models suggests significant similarities with the adult human disease[199], they may not reflect intratumoural heterogeneity to the same degree observed in primary tumours, and many novel findings of recent genome-wide studies have not yet been successfully transferred into *in vivo* models, including recurrent mutations in isocitrate dehydrogenase 1 (*IDH1*)[216], *H3F3A*, *HIST1H3B*[106], *ATRX* and others. These are now being assessed in the context of reasonable co-oncogenic alterations in order to complement the currently available repertoire of GBM *in vivo* models.

It is widely thought that *IDH1*-mutant GBM might represent a distinct disease entity that probably arises from a different cellular origin compared with GBM in which IDH is not mutated[136]. Based on a current model, mutations of *IDH1* represent an early event in gliomagenesis, priming quiescent neural progenitor cells for additional tumour-promoting genetic hits such as *TP53* mutations or 1p/19q co-deletions, leading to the manifestation of WHO grade II/III gliomas. Subsequent malignant transformation into grade IV GBM is mediated by additional key genomic alterations (such as RTK pathway activation or *PTEN* mutations)[136]. This stepwise progression of secondary, *IDH1*-mutant GBM, with preservation of a proneural expression pattern, substantially differs from *de novo* GBM, which seem to originate as the result of genetic hits in a distinct cell population more closely resembling a NSC[136].

Lineage tracing experiments have indicated that oligodendrocyte precursor cells (OPCs) can be considered as cells of origin for *Nf1* and *Trp53*-mutant GBM, although identical genetic hits can also give rise to glioma when introduced into NSCs[132]. In a premalignant state, *in vivo* proliferation of *Nf1;Trp53*-mutant cells was found to be dramatically increased only in the OPC lineage and not NSCs or progeny lineages of astrocytes, neurons or oligodendrocytes. Fascinatingly, direct introduction of *Nf1* and *Trp53* mutations into OPCs led to the generation of gliomas that molecularly and histologically resembled tumours initiated from NSCs, with transcriptomes of both models corresponding well with the proneural subtype[132].

Assuming that tumours partially retain the gene expression signature of their cellular origin, transcriptional profiles of *H3F3A* K27- and G34-mutant GBM were found to resemble those of distinct anatomical regions at different stages in the developing human brain[40, 137]. Expression of *OLIG1* and *OLIG2* was originally thought to be a common feature of human gliomas[138–140] and OLIG2 was considered essential for promoting gliomagenesis *in vivo*[141, 142]. However, *H3F3A* G34-mutant GBM presumably arise from NSCs or undifferentiated progenitor cells (not of oligodendroglial lineage) which epigenetically repress *OLIG1* and *OLIG2* expression[40] in order to preserve multipotency, unlike other glioma subtypes initiated from OLIG2-positive progenitor-like cells of the subventricular zone[143].

Novel strategies in glioblastoma therapy

Current postoperative standard treatment for GBM patients is mainly based on unselective induction of DNA damage *via* radiotherapy and alkylating agents such as TMZ[8]. The majority of drugs specifically targeting key signalling pathways and mechanisms of gliomagenesis, such as RTK signalling (erlotinib[144–147], gefitinib[147, 148], cetuximab[149] and imatinib[150–153]) or angiogenesis (bevacizumab[154, 155] and cediranib[156]) do not provide a significant survival benefit when tested alone or in combination with other therapies (reviewed in[157]). Comparable results were obtained when combining these drugs with pharmacological histone deacetylase (HDAC) inhibitors (such as panobinostat[158] and vorinostat[158, 159]), although the use of an HDAC inhibitor such as valproic acid in addition to radiotherapy was associated with a better outcome in patients with GBM[160, 161]. It should be noted, however, that efforts to identify molecularly defined patient subgroups for targeted therapies have generally been lacking, and it is possible that within the context of a negative clinical trial, subgroups of patients could be defined that would benefit from such a specific therapeutic approach. Indeed, the identification of several different molecular subgroups[25, 27, 40] would argue against the use of one standardised therapy for GBM and the increasing knowledge about the genetic and/or epigenetic events driving these subgroups offers the opportunity to design innovative patient-tailored treatment protocols.

Given the high prevalence of IDH mutations in GBM and other malignancies such as acute myeloid leukaemia (AML), several studies have sought to therapeutically target the neomorphic enzymatic activity of mutant IDH1 and IDH2 using specific inhibitors[162–164]. Recently, the small molecule AGI-5198 was shown to inhibit R132H-mutant IDH1, leading to reduced levels of 2-HG and substantial growth reduction of glioma cells *in vitro* and human glioma xenografts *in vivo*[162]. Interestingly, pharmacological blockade of mutant IDH1 did not reverse the G-CIMP in glioma xenografts, but induced expression of genes associated with astrocytic and oligodendrocytic differentiation[162]. Although not tested in GBM yet, a recent study has reported on the successful inhibition of mutant IDH2 by a small molecule (AGI-6780) in AML[164]. This encouraging data demonstrates that these new drugs might represent an effective treatment option for patients with an IDH-mutant GBM.

In contrast, tumour growth and disease progression of GBM lacking mutations of *IDH1* or *IDH2* have recently been shown to utilize alternative energy resources such as branched-chain amino acids (BCAA) to compensate for increased metabolic demand. Expression of

branched-chain amino acid transaminase 1 (*BCAT1*) was found to be highly dependent on intracellular levels of 2-OG, which is mainly produced by (wild-type) IDH1[165]. Impaired BCAA metabolism via knockdown of BCAT1 or overexpression of mutant IDH1 resulted in reduced cell proliferation[165], rendering the reprogramming of energy metabolism an attractive target for innovative therapeutic approaches in GBM. Interestingly, the metabolic enzyme pyruvate kinase M2 (PKM2) was shown to have a non-metabolic function as a key regulator of histone phosphorylation and acetylation. Activation of EGFR (and also of PDGFR) results in activation of ERK2, which was found to phosphorylate PKM2 leading to nuclear translocation and promotion of the Warburg effect[166]. Nuclear PKM2 was found to phosphorylate histone H3 at threonine 11, causing dissociation of HDAC3 from the *CCND1* and *MYC* gene promoters[167] (Figure 2). Subsequent expression of cyclin D1 and MYC due to increased acetylation of histone H3 at lysine 9 was found to induce cell proliferation and gliomagenesis[166].

The identification of histone mutations in a large proportion of HGG from paediatric and adolescent patients[41, 101] has raised expectations for targeted treatment approaches that tackle these tumours[40, 43]. Although the precise mechanism of K27M-mediated PRC2 inhibition is not fully understood, pharmacological intervention targeting K27M-mutant H3.3 or downstream consequences of this change might represent the ultimate therapeutic goal. Care must be taken here, however, since the role of the PRC2 complex in oncogenesis seems to be highly context-dependent, and unspecific pharmacological H3K27me3 upregulation in K27M-mutant tumours could in fact promote disease progression, as has been shown for other malignancies harbouring EZH2 activating mutations[168–170]. The existence of ALT as the predominant telomere maintenance mechanism in the majority of H3-mutant HGG (especially those with G34R and G34V alterations)[41] might also represent a promising biological target for the development of more powerful drugs in the future, as we begin to understand the underlying molecular details. In contrast, pharmacological inhibition of telomerase activity might be more effective in GBM in adult patients, given their dependence on high levels of telomerase expression[171] (Figure 3). Despite the manifold differences between GBM subtypes, the evolving overall theme of epigenetic deregulation holds promise for epigenetic modulators to be active across subsets of epigenetically distinct tumours, as has recently been shown for a new class of agents targeting the BET domain proteins[172].

Despite the identification of these multiple new targets in certain patient subgroups, bridging the gap between identification of recurrent genomic drivers, such as *EGFR*, and successful targeted therapies remains a considerable challenge, as indicated by the above-mentioned failures of many of the agents tested to date. The development of novel drugs requires accurate assessment of pharmacodynamic and pharmacokinetic parameters of drug activity in preclinical and clinical trials, and penetrance of the blood brain barrier is an additional challenge (although the integrity of this barrier in GBM is debatable). Although some drugs show detectable activity both *in vitro* and *in vivo*, downstream signal transducers may remain unaffected[173], and even for drugs able to induce a clinical response in GBM patients, treated tumours ultimately progress. One reason for the failure of early clinical trials may be a lack of patient selection for a specific therapy, implying that molecular determinants of the

response to a chosen intervention are needed[174]. Another could be pre-existing[175] or emerging tumour resistance mechanisms in response to treatment, for example through activation of alternative RTKs as a result of EGFR inhibition[176].

Due to the inter- and intratumoural molecular heterogeneity of GBM, it is likely that only a small proportion of patients will benefit from therapeutic interventions aimed at any one target, but synergistic combination approaches may prove more effective[177–179]. This likelihood raises the need for prospective tissue collection in order to maximize information gained from clinical trials. Molecular pathological stratification criteria are needed in this and other cancers to optimize enrolment in the therapeutic arm most adapted to tumour biology, and efficacy of a specific agent should be considered in the context of known and clinically relevant biological subsets of glioma.

Summary

Recent large-scale profiling studies have revealed a number of novel findings that have changed the way we look at the genomic and epigenomic landscape of GBM. The community is slowly gaining insight into the complex interactions between genetic alterations and changes in DNA methylation, histone modifications, chromatin remodelling and gene expression, and how deregulation on different levels might contribute to GBM pathogenesis. IDH and H3-mutated GBM are outstanding examples whereby disrupted epigenetic mechanisms due to genetic mutations result in the establishment of mutation-specific epigenetic and transcriptional programs. These and other recurrent genetic aberrations occur in a specific context of cellular origin, co-oncogenic hits and epigenetic phenomena, and are present in distinct patient populations. The biological discrimination of GBM subgroups should therefore guide the design of future clinical trials. In addition, the complex interplay of ‘hard-wired’ genetic events and potentially reversible alterations of the GBM epigenome offers novel opportunities for the development of molecularly targeted therapies, which might represent a promising strategy to tackle this deadly brain tumour.

Glossary

WHO Classification of tumours of the central nervous system	Classification system where histological grading is applied as a means of predicting the biological behaviour of a tumour. It ranges from benign tumours (grade I) to highly aggressive, rapidly progressing tumours with frequently fatal outcome (grade IV)
Glioma	Glioma refers to tumours that have histologic features similar to normal glial cells, i.e. astrocytes (astrocytoma), oligodendrocytes (oligodendroglioma), or ependymal cells (ependymoma), but is often used to imply only astrocytic or oligodendroglial tumours
Diffuse intrinsic pontine glioma (DIPG)	Highly infiltrative glial tumour arising in the pons. Occurs almost exclusively in children, with a peak age at diagnosis of between 5 and 9 years

Temozolomide (TMZ)	Alkylating chemotherapeutic agent used for the treatment of GBM. Triggers tumour cell death through extensive DNA damage
CpG Island Methylator Phenotype (CIMP)	DNA methylation pattern of widespread CpG island promoter methylation. CIMP is frequently reported to be associated with distinct tumour subgroups, patient prognosis and response to treatment
Chromothripsis	Clustered chromosomal rearrangements in one or a few chromosomes during cancer development, thought to occur through a one-step catastrophic genomic event
High amplitude focal copy-number alteration	Small fragment (typically ~ 3 Mb or smaller in size) of amplified or homozygously deleted DNA, often resulting in numerous copies of oncogenes or deletion of both copies of tumour suppressor genes
Double minute chromosomes	Small circular fragment of extrachromosomal DNA frequently harbouring one or more oncogenes
Alternative lengthening of telomeres (ALT)	One or more mechanism(s) by which 5–10% of human cancers maintain or increase the overall length of their telomeres without the need of increased telomerase activity. The exact molecular mechanism(s) of ALT remain elusive, but may rely on recombination-mediated elongation
Warburg effect	Predominant production of energy by a high rate of glycolysis followed by lactic acid fermentation in the cytosol observed in most cancer cells in the presence of oxygen
Polycomb repressive complex 2 (PRC2)	One of two classes of polycomb-group proteins. PRC2 has methyltransferase activity and primarily trimethylates histone H3 on lysine 27 (i.e. H3K27me3), a mark of transcriptionally silent chromatin

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Biographies

Dominik Sturm

Dominik Sturm is a physician scientist in the Division of Pediatric Neurooncology at the German Cancer Research Center (DKFZ), Heidelberg, Germany. His recent research activities focused on elucidating the molecular biology underlying childhood high-grade gliomas and diffuse intrinsic pontine gliomas, and applying novel molecular diagnostic tools to improve childhood brain tumour classification. Having received his MD in 2011, he is currently in clinical training as a paediatrician at the Department of Pediatric Oncology and Hematology at the Heidelberg University Medical Center for Children and Adolescents.

Sebastian Bender

Sebastian Bender is a postdoctoral research fellow in S.M.P.'s laboratory at the German Cancer Research Center (DKFZ), Heidelberg, Germany. He received his PhD from the University of Heidelberg, Germany, in 2013 for studying the molecular consequences of histone H3.3 mutations in paediatric high-grade gliomas. His main research focus is the characterization of molecular mechanisms driving tumourigenesis in childhood brain tumours.

David T.W. Jones

David Jones is a senior postdoctoral scientist at the DKFZ, where he has played a major part in the International Cancer Genome Consortium (ICGC) PedBrain Tumour sequencing project. He received his PhD from the University of Cambridge in 2009, working in the group of V. Peter Collins. In 2008, he was the first to describe a highly recurrent *BRAF* fusion gene occurring in two-thirds of the most common childhood brain tumour (pilocytic astrocytoma). His primary research focus is the application of cutting-edge genomics techniques to identify new diagnostic, prognostic and therapeutic targets in the field of neurooncology.

Peter Lichter

Peter Lichter pioneered the development of technologies to delineate almost any chromosomal region by fluorescence *in situ* hybridization (FISH) and to detect DNA copy-number alterations *via* high resolution comparative genomic hybridization (arrayCGH). Applying these, as well as next-generation DNA sequencing approaches, he made major contributions to decipher the higher order genome organization and to elucidate pathomechanisms of tumour aetiology and progression, including the description of novel prognostic or predictive gene signatures.

Jacques Grill

Jacques Grill is the Head of the Pediatric Brain Tumour Program at Gustave Roussy, Villejuif, France, and Principal Investigator of Several International Trials including HERBY, a randomized phase II study for paediatric non-brainstem high-grade gliomas and BIOMEDE, a biology-driven trial for diffuse intrinsic pontine glioma. He is the group leader of a research team of the CNRS Unit 8203 dedicated to the discovery of new biomarkers and therapeutics targets in glial tumours.

Oren Becher

Oren Becher is an assistant professor in the Departments of Pediatrics and Pathology at Duke University. His laboratory is focused on the development of genetically engineered Diffuse Intrinsic Pontine Glioma (DIPG) mouse models, dissection of the function of novel genetic alterations in DIPG, and the evaluation of the novel therapeutics.

Cynthia Hawkins

Cynthia Hawkins is a paediatric neuropathologist and scientist at The Hospital for Sick Children (SickKids) in Toronto, Canada. Her clinical practice includes diagnosis of paediatric brain tumours and she serves as a central pathology reviewer for several national and international paediatric brain tumour clinical trials. The Hawkins laboratory is focused on the study of paediatric high grade glioma, in particular diffuse intrinsic pontine glioma. Her lab uses cancer genetic techniques in combination with *in vitro* and *in vivo* models to better understand what cellular processes underlie paediatric high grade glioma and how we can use this information to improve our ability to diagnose and treat children with brain tumours.

Jacek Majewski

Dr. Jacek Majewski obtained his BS in Physics and MS in Electrical Engineering from Stanford, and PhD in Biology from Wesleyan University. Having vowed never to live in New York, he nevertheless followed his fiancée to the Rockefeller University as a post-doctoral fellow in statistical genetics. He is currently a Canada Research Chair and Associate Professor at the Department of Human Genetics at McGill University. His research involves genomics and analysis of high throughput data aimed at understanding human genetic disorders. The approaches include RNA sequencing, exome, and whole genome sequencing, with specific applications to monogenic disorders and cancer.

Chris Jones

Chris Jones is a research team leader at in the Divisions of Molecular Pathology and Cancer Therapeutics at The Institute of Cancer Research (ICR) in London, UK. He completed his first degree in toxicology and pharmacology and later a PhD in molecular biology at the School of Pharmacy, University of London, UK. He moved to the ICR in 2001 and took up his post focusing on the genomics of childhood cancer in 2003. He is a Fellow of the Royal College of Pathologists by published works and is active in international paediatric brain tumour collaborative groups and clinical trials.

Joseph F. Costello

Dr. Costello is a Professor of Neurosurgery at UCSF, holds the Karen Osney Brownstein Endowed Chair in Neuro-oncology and serves as the Director of the UCSF-based NIH Roadmap Epigenome Mapping Center. Dr. Costello’s laboratory investigates how genetic and epigenetic mechanisms interact in the control of gene expression. His research includes epigenetic technology development and its application to human stem cells and tissues from different developmental stages. His translational research investigates tumour genome and epigenome evolution by integrating treatment, advanced imaging and histologic analyses with patterns of mutations and epimutations.

Antonio Iavarone

Antonio Iavarone received his MD at the Catholic University of Rome, where he also received the Residency degree in Paediatrics and performed a fellowship training in Paediatric Oncology. He worked at UCSF, Memorial Sloan Kettering Cancer Center and Albert Einstein College of Medicine in New York. He is Professor of Neurology and Pathology at Columbia University. One of the first discoveries made by his group is the now widely accepted notion that the most aggressive forms of brain tumours are invariably associated with loss of differentiated features. In more recent years his laboratory identified the master regulators and key genomic drivers (gene fusions and mutations) responsible for initiation, progression and maintenance of malignant glioma.

Kenneth Aldape

Kenneth Aldape is a neuropathologist with an interest in basic and translational research in brain tumours. He has a particular interest in molecular classification of glioma. His research has encompassed expression profiling and use of other genome-wide platforms to identify subclasses of these tumours. He is particularly interested in clinically relevant molecular alterations that can inform treatment and potentially lead to precision medicine for patients with primary and metastatic brain tumours.

Cameron W. Brennan

Cameron Brennan is an oncologic neurosurgeon and a laboratory investigator focused on tumour molecular profiling and brain tumour drug responses. He received his MD and neurosurgery training at New York Hospital – Cornell Medical Center, followed by postdoctoral work in Ronald DePinho's laboratory at the Dana-Farber Cancer Institute. He joined Memorial Sloan-Kettering Cancer Center in 2004 and opened a laboratory in 2009 within the Human Oncology and Pathogenesis Program. He has been a part of The Cancer Genome Atlas from its inception, initially developing methods to identify DNA structural rearrangements and recently as external co-chair of the glioblastoma initiative.

Nada Jabado

Nada Jabado is an Associate Professor with tenure in the Department of Paediatrics and an associate member of the departments of Human Genetics, Oncology, and Experimental Medicine at McGill University. She obtained her MD with a specialization in Paediatrics and a PhD in Immunology from the Université de Paris VI in France. Her work aims to identify molecular alterations underlying paediatric gliomas. She has authored/co-authored 99 peer-reviewed papers in her career and received several awards recognizing her contribution to the field of cancer including the William E. Rawl young investigator award from the National Canadian Cancer Research Council in 2012.

Stefan M. Pfister

Stefan M. Pfister was appointed Head of the Division of Pediatric Neurooncology at the DKFZ in 2012. He is a paediatrician by training, and received his MD from Tübingen University, Germany, and his clinical education at Mannheim and Heidelberg University Hospitals. As a physician scientist, he completed postdoctoral fellowships with Christopher Rudd at the Dana-Faber Cancer Institute/Harvard Medical School, Boston, USA, and with Peter Lichter at the DKFZ, Division of Molecular Genetics, Heidelberg, Germany. His research is focused on the genetic characterization of childhood brain tumours through the application of next-generation profiling methods and subsequently translating novel findings into a clinical context.

Key points

- Glioblastoma is the most frequent and most aggressive malignant primary brain tumour and remains almost universally incurable in both children and adults.
- Comprehensive molecular profiling studies have greatly broadened our knowledge of the underlying genomic and epigenomic aberrations associated with glioblastoma initiation and progression.
- Genetic lesions result in disrupted epigenetic control mechanisms by altering histone modifications, DNA methylation and gene expression patterns in a large proportion of glioblastomas.
- Based on recurrent combinations of genomic and/or epigenomic features with distinct patient characteristics, glioblastomas across all ages are being dissected into meaningful biological subgroups, which are likely to guide future clinical trial design.
- The complex interplay between the glioblastoma genome and epigenome opens the avenue for the development of novel innovative therapeutic strategies that are urgently needed to tackle this deadly brain tumour.

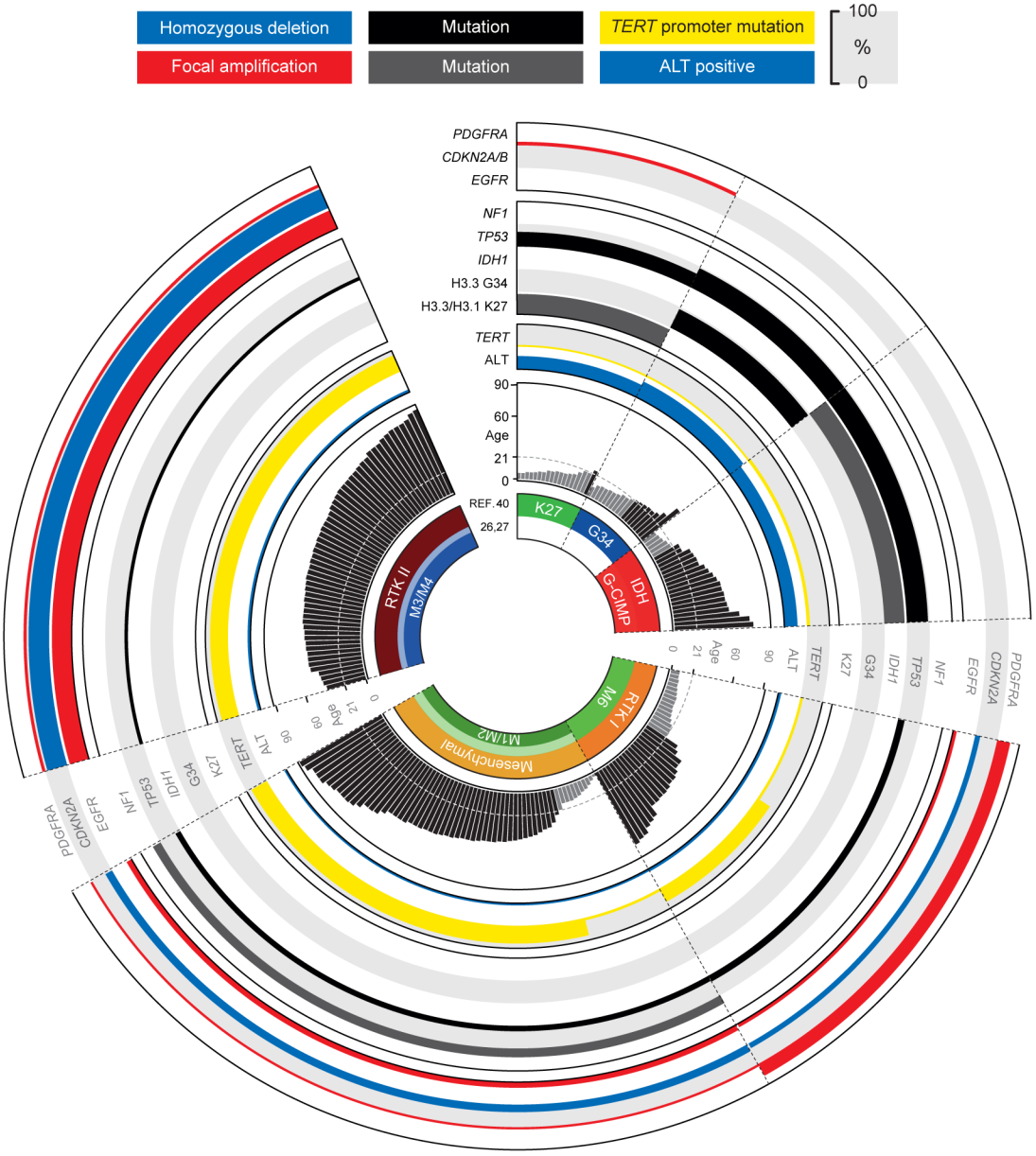

Figure 1. Age-based genomic and epigenomic features of biological glioblastoma subgroups

Simplified schematic overview of glioblastoma subgroups depicting recurrent associations between genomic and epigenomic features: (from inside to outside) DNA methylation subclass affiliation (TCGA methylation [REFS[26, 27]], and dkfz methylation [REF.[40]]), patient age (years), telomere maintenance mechanisms, mutational status (H3.3 and H3.1 K27, H3.3 G34, *IDH1*, *TP53* and *NF1*), and copy-number aberrations, grouped by biological subgroup and sorted by patient age. Copy-number states, presence of mutations and ALT positivity are represented by different colours as indicated. Height of bars represents an estimated percentage of cases positive for a specific feature. ALT, alternative lengthening of telomeres; *CDKN2A* and *B*, cyclin-dependent kinase inhibitor 2A and 2B; *EGFR*, epidermal growth factor receptor; G-CIMP, glioma-CpG island methylator phenotype; *IDH1*, isocitrate dehydrogenase 1; *NF1*, neurofibromin 1; *PDGFRA*, platelet-derived growth factor receptor type alpha; RTK, receptor tyrosine kinase; *TERT*, telomerase reverse transcriptase.

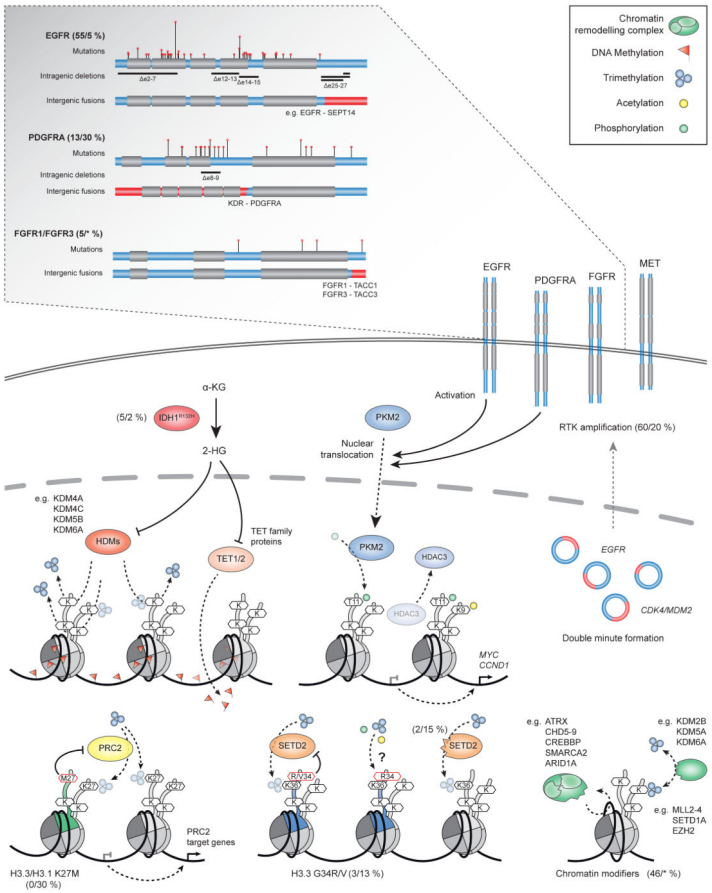

Figure 2. Interplay between the glioblastoma genome and epigenome

Frequent genomic alterations in GBM impacting the epigenomic machinery. Receptor tyrosine kinases (RTKs) are commonly activated by somatic mutations or structural variations (see magnification), leading to PKM2 nuclear translocation and expression of *MYC* and *CCND1*. Recurrent somatic mutations in IDH1, IDH2, histone proteins, SETD2 and various other chromatin modifying proteins result in the disruption of multiple epigenetic regulatory processes by affecting histone modification, DNA methylation and chromatin remodelling. Numbers in brackets represent estimated frequencies of alterations observed in GBM of adults and childhood HGG, respectively (see Table 2 for exact numbers; *unknown). 2-HG, 2-hydroxyglutarate; α-KG, α-ketoglutarate; ARID1A, AT-rich interactive domain-containing protein 1A; ATRX, alpha thalassemia/mental retardation syndrome X-linked; CDK4, cyclin-dependent kinase 4; CHD, chromodomain helicase DNA binding protein; CREBBP, CREB-binding protein; EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homolog 2; FGFR, fibroblast growth factor receptor; HDM, histone demethylase; IDH1, isocitrate dehydrogenase 1; KDM, lysine (K)-specific demethylase; KDR, kinase insert domain receptor; MDM2, mouse double minute 2 homolog; MLL, mixed-lineage leukemia; PDGFRA, platelet-derived growth factor receptor type alpha; PKM2, pyruvate kinase muscle isozyme; PRC2, polycomb repression complex 2; RTK, receptor tyrosine kinase; SEPT14, septin 14; SETD1A, SET domain containing 1A; SETD2, SET domain containing 2; SMARCA2, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2; TACC, transforming acidic coiled-coil.

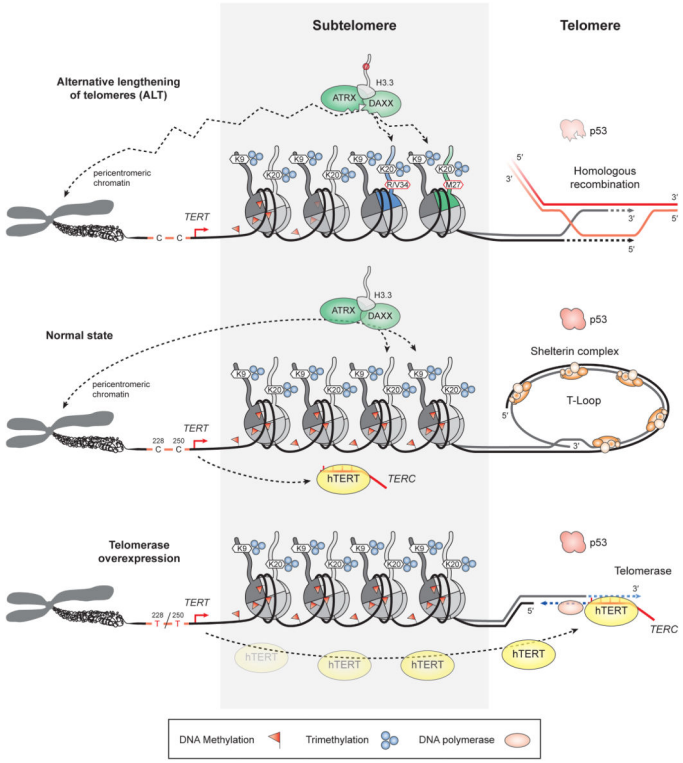

Figure 3. Telomere maintenance mechanisms in glioblastoma

Maintenance of telomere length is accomplished by different mechanisms in GBM. In normal cells (middle panel), chromosome ends are protected from undergoing non-homologous end joining or homologous recombination by forming a T-Loop structure. Alternative lengthening of telomeres (ALT), thought to be mediated by homologous recombination, is more prevalent in the paediatric setting (upper panel). While mutations in ATRX or DAXX (mediating incorporation of histone H3.3 into pericentromeric and subtelomeric regions) are known to promote ALT, the contribution of p53 loss, H3.3 mutations and/or subtelomeric DNA hypomethylation (such as in GBM harbouring H3.3 G34R/V mutation) needs further elucidation. Telomere length in adult GBM (lower panel) in the presence of functional p53 is mainly maintained by upregulated telomerase (hTERT) expression as a consequence of *TERT* promoter hotspot mutations C228T or C250T, for example. ATRX, alpha thalassemia/mental retardation syndrome X-linked; DAXX, death-domain associated protein; T-Loop, telomere loop; TERC, telomerase RNA component; *TERT*, telomerase reverse transcriptase.

Table 1

Frequent chromosomal copy-number alterations in glioblastoma

Genomic region	Copy-number change	Frequency (%)adult GBM*	Frequency (%)childhood HGG¶	Subtype enrichment	References
Chromosome 1q	Single copy gain	9	19–29	G34	26, 36, 38
Chromosome 6q	Single copy loss	22	4–5	non-H3/non-G-CIMP	26, 36, 38
Chromosome 7	Single copy gain	74–83	13–19	non-H3/non-G-CIMP	26, 36, 38, 40
Chromosome 9p	Single copy loss	33–38	11–18	non-K27	26, 36, 38
Chromosome 10	Single copy loss	80–86	16–38	non-H3/non-G-CIMP	26, 36, 38, 40
Chromosome 13q	Single copy loss	31–33	24–34	G34, RTK I	26, 36, 38
Chromosome 14q	Single copy loss	26–27	16–29	RTK I	26, 36, 38
Chromosome 19	Single copy gain	35–40	1–13	Classical, RTK II	26, 36, 38
Chromosome 20	Single copy gain	39	3–6	Classical, RTK II	26, 36, 38
Chromosome 22q	Single copy loss	33	1–16	G-CIMP, MES, RTK II	26, 36, 38

* ≥ 21 years;

¶ < 21 years, diffuse intrinsic pontine glioma (DIPG), anaplastic astrocytoma WHO grade III, GBM; G-CIMP, glioma-CpG island methylator phenotype; RTK, receptor tyrosine kinase.

Table 2

Recurrent somatic alterations in glioblastoma

Target gene(s)	Alteration type	Frequency (%)adult GBM*	Frequency (%)childhood HGG¶	Subtype enrichment	References
EGFR	Focal amplification	43	1–11	Classical, Neural, RTK II	25, 26, 36, 38, 40, 67, 74
	Point mutation	18–26	4	Classical	26, 27, 41
	Intragenic deletion (Δe2-7; EGFRvIII)	10–64	17	Classical	26, 74, 76
	Intragenic deletion (Δe25-27)	3–9	NA	Classical	26
	Intragenic deletion (Δe12-13/Δe14-15)	29/3	NA	Classical	26
	Fusion	8	NA	Classical, Mesenchymal	26, 32
PDGFRA	Focal amplification	11–26	8–39	Proneural non-G-CIMP, RTK I	25, 26, 36, 38, 40, 84, 217
	Mutation	3–4	4–9		4, 26, 41, 79
	Intragenic deletion	7–18	4		26, 78, 79
PTEN	Focal deletion	8–10	0–1		26, 36, 38
	Mutation	23–31	1–12	non-G-CIMP	26, 41, 218, 219
NF1	Focal deletion	1	0–1	Mesenchymal	25, 26, 36, 38
	Mutation	9–10	24	Mesenchymal	25, 26, 41
RB1	Focal deletion	3–4	0–3		26, 36, 38
	Mutation	7–9	10	Mesenchymal	26, 41
CDKN2A* and *CDKN2B	Focal deletion	62	10–19	non-H3/non-G-CIMP	25, 26, 36, 38, 217
TERT	Promoter mutation	55–83	3–11	non-H3/non-G-CIMP	26, 91–93, 95
TP53	Mutation	20–29	34–37	K27, G34, G-CIMP	25–27, 72, 220

Target gene(s)	Alteration type	Frequency (%)adult GBM*	Frequency (%)childhood HGG¶	Subtype enrichment	References
CDK4	Focal amplification	13–18	3–4	Proneural non-G-CIMP	26, 36, 38
FGFR1* or *FGFR3	Fusion	3–8	NA		82, 83
IDH1	Mutation	5–12	0–16	G-CIMP	25–27, 36, 40, 41, 86, 113, 221
PIK3CA	Mutation	7–21	8–20		26, 41, 222,§
PIK3R1	Mutation	6–11	10–12		26, 41,§
***H3F3A* G34R/V**	Mutation	0–3	12–14	G34	26, 40, 41, 101
***H3F3A* K27M**	Mutation	0–1	23–43	K27	26, 40, 41, 101,§,‡
***HIST1H3B* K27M or *HIST1H3C* K27M**	Mutation	0	12–31¥		26, 67, 101,§,‡
ATRX	Mutation	6	14–29	G34, G-CIMP	26, 41, 97
SETD2	Mutation	1–2	15		26, 110
ACVR1	Mutation	0	20–22¥		26,§,‡
MYCN	Focal amplification	3	3–5		26, 36, 38, 217
MYC	Focal amplification	2	3–4	G-CIMP	26, 36, 38
***BRAF*V600E**	Mutation	2	10–25		26, 41, 223, 224

*≥ 21 years;

¶< 21 years, diffuse intrinsic pontine glioma (DIPG), anaplastic astrocytoma WHO grade III, GBM; *ACVR1*, activin a receptor type I; *ATRX*, alpha thalassemia/mental retardation syndrome X-linked; *CDK4*, cyclin-dependent kinase 4; *CDKN2A* and *B*, cyclin-dependent kinase inhibitor 2A and 2B; *EGFR*, epidermal growth factor receptor; *FGFR*, fibroblast growth factor receptor; G-CIMP, glioma-CpG island methylator phenotype; *IDH1*, isocitrate dehydrogenase 1; *NF1*, neurofibromin 1; *PDGFRA*, platelet-derived growth factor receptor type alpha; *PIK3CA*, phosphatidylinositol 3-kinase catalytic subunit alpha; *PIK3R1*, phosphatidylinositol 3-kinase regulatory subunit alpha; *PTEN*, phosphatase and tensin homolog; *RB1*, retinoblastoma 1; RTK, receptor tyrosine kinase; *SETD2*, SET domain containing 2; *TERT*, telomerase reverse transcriptase;

¥DIPG only;

§C.J., J.G., O.B., C.H., J.M., N.J., unpublished observations;

‡Suzanne J. Baker, personal communication.