Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma

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Abstract | Malignant brain tumours continue to be the cause of a disproportionate level of morbidity and mortality across a wide range of individuals. The most common variants in the adult and paediatric populations — malignant glioma and medulloblastoma, respectively — have been the subject of increasingly intensive research over the past two decades that has led to considerable advances in the understanding of their basic biology and pathogenesis. This Review summarizes these developments in the context of the evolving notion of molecular pathology and discusses the implications that this work has on the design of new treatment regimens.

Malignant glioma
Diffuse glioma of astrocytic, oligodendroglial or mixed lineage with a World Health Organization grade of either III or IV.

Glial
Pertaining to glia, the non-neuronal support cells in the nervous system.

Neuronal
Pertaining to neurons, the primary functional unit of the nervous system.

Histogenesis
The origin of a tissue or tumour especially with regard to its development and formation.

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doi:10.1038/nrc2818

Primary brain tumours consist of a diverse group of neoplasms that are derived from various different cell lineages. Much like those arising from other anatomic sites, tumours of the central nervous system (CNS) have historically been classified on the basis of morphological and, more recently, immunohistochemical features with less emphasis on their underlying molecular pathogenesis. The past two decades, however, have seen striking advances in basic brain tumour biology, especially with regard to malignant glioma and medulloblastoma, the most common CNS cancers of adults and children, respectively. As described more extensively below, malignant gliomas consist of a broad range of histological entities, the plurality of which respond poorly to standard therapeutic regimens. For instance, patients with glioblastoma, the most prevalent and most aggressive glioma variant, have a median survival of only 15 months[1,2]. And although radiation and chemotherapy have been more successful in combating childhood medulloblastoma, with 5-year survival rates now as high as 70–80%[3], the long-term side effects of these conventional treatment modalities can be severe.

The detailed molecular characterization of malignant glioma and medulloblastoma has not only more accurately defined specific subgroupings in individual morphological categories, but has also laid the groundwork for the successful augmentation of standard treatment regimens with rationally designed, targeted therapies. Similarly, and on a more sobering note, increased understanding of the molecular and cellular heterogeneity inherent to these tumour classes has also emphasized the formidable therapeutic challenges still remaining.

Morphological classification

Although microscopic descriptions of brain tumours began in the early nineteenth century, the classification scheme developed by Bailey and Cushing[4] in 1926 formed the foundation on which much of modern pathological diagnosis still rests. Their system proposed that the evolution of CNS neoplasms from glial or neuronal precursors arrested at particular developmental stages and therefore led directly to concepts such as histogenesis and cell of origin. Further refinements, most notably by Kernohan[5] and Ringertz[6], led ultimately to the World Health Organization (WHO) classification, which was first formalized in 1979 (REF. 7) and updated in 2007 (REF. 8). In addition to a morphological grouping of brain tumours on the basis of presumed histogenesis, the WHO schemes have been notable for their grading of individual tumour classes (I, II, III and IV) as a means of reflecting anticipated biological behaviour. In this way, higher grade tumours (grades III and IV), in the absence of treatment, are expected to follow a more aggressive clinical course than their lower grade counterparts (grades I and II). Additionally, although the WHO classification does remain firmly grounded in morphological criteria, relevant molecular information regarding the different tumour classes has been integrated over time.

Most gliomas (presumably derived from either mature glia or their less differentiated precursors (FIG. 1)) diffusely infiltrate surrounding brain tissue and together represent a broad diagnostic group, which the WHO divides into astrocytic, oligodendroglial and mixed (oligoastrocytic) categories[8] (FIG. 2a).

At a glance

- Malignant gliomas and medulloblastomas — the most common brain tumours affecting adults and children, respectively — remain responsible for a disproportionate level of morbidity and mortality among cancer patients.
- The morphological histopathology traditionally used for the subclassification of these brain tumour variants is gradually giving way to more molecularly grounded criteria that better reflect the underlying biology.
- Recent integrated genomics has further implicated specific molecular networks in the pathogenesis of gliomas and medulloblastomas. These most prominently include receptor tyrosine kinase (RTK) signalling through the Ras–MAPK and PI3K–AKT–mTOR pathways, Wnt signalling and sonic hedgehog (SHH) signalling, along with the cell cycle-regulating RB and p53 pathways.
- Expression analysis has recently defined transcriptional subclasses for both malignant gliomas and medulloblastomas that seem to be driven by distinct abnormalities in core signalling pathways. Such findings suggest that tumours in a particular molecular subgroup would preferentially respond to different targeted therapies.
- Malignant gliomas and medulloblastomas also exhibit heterogeneity at the cellular level, with subpopulations of tumour cells harbouring stem-like properties rendering them more resistant to therapy. Such stem-like pools seem to reside in specialized microenvironments that actively maintain their biological characteristics.
- Treatment challenges posed by malignant gliomas and medulloblastomas remain considerable, and many derive from the molecular and cellular heterogeneity inherent to these tumour variants. They include innate and acquired resistance and the obstacle to effective drug delivery posed by the blood–brain barrier.

Additionally, the presence of histological features such as nuclear atypia, increased proliferation, microvascular proliferation and necrosis typically result in higher grade classification as either anaplastic glioma or glioblastoma. Glioblastoma is the most malignant variant of diffuse glioma and, although the generally reliable expression of protein markers such as glial fibrillary acid protein (GFAP) has historically placed it in the confines of the astrocytic class, its precise histogenesis remains unclear despite considerable advances in the understanding of its basic biology (discussed below). Further complicating matters, it has long been known that glioblastomas may evolve from lower grade astrocytic neoplasms over time (secondary glioblastoma), although most of these tumours seem to arise *de novo* (primary glioblastoma)[9–12].

Medulloblastoma is the archetypal primitive neuroectodermal tumour (PNET), which is thought to arise from immature neuronal precursors in the cerebellum (FIG. 1), and is histologically characterized by closely packed small round blue cells, typically exhibiting notable mitotic activity. Although all medulloblastoma variants carry the most aggressive WHO designation (grade IV), their distinct morphological features have been associated with substantial differences in biological behaviour (FIG. 2b). For instance, the nodular or desmoplastic subtype and closely related medulloblastoma with extensive nodularity (MBEN) are associated with relatively favourable prognoses in young children[13,14], whereas the large cell/anaplastic variant tends to exhibit a more aggressive clinical course with a higher incidence of metastatic disease in the neuraxis[15–18]. Recent work has demonstrated, perhaps not surprisingly, that many of the histopathological and clinical distinctions between the aforementioned medulloblastoma and glioma subcategories are grounded in variability at the molecular level, conclusions that have been further bolstered by experiments in mouse model systems (BOX 1).

Variant III deletion
Pathogenic deletion mutant of *EGFR* involving exons 2–7 that leads to a constitutively active truncated protein.

Molecular pathology: diffuse glioma

Numerous molecular abnormalities have been linked with the underlying biology of diffuse glioma (FIG. 3). Mutations in the *TP53* tumour suppressor gene were first implicated in gliomagenesis almost 20 years ago owing to the increased development of gliomas (most commonly astrocytomas) in patients with the rare cancer-predisposing disorder Li–Fraumeni syndrome, which is caused by mutations in *TP53* (REFS 19,20). Subsequent investigations found *TP53* mutations to be a frequent characteristic of sporadic low-grade astrocytic tumours and secondary glioblastomas[21]. By contrast, primary glioblastomas were initially associated with genomic amplifications and activating mutations in the epidermal growth factor receptor (*EGFR*) locus[22–24], the most frequent of these being the variant III deletion (vIII) that was found in 20–30% of all primary glioblastomas and 50–60% of those also exhibiting *EGFR* amplification[25,26].

Contemporaneously, a substantial number of oligodendroglial tumours (60–90%) were found to exhibit a combined loss of chromosome arms 1p and 19q, a genomic abnormality that interestingly predicted less aggressive biological behaviour and a relatively robust response to chemotherapy[27–29]. Despite the fact that 1p/19q deletion analysis is frequently used in the diagnosis and management of oligodendroglial neoplasms, the precise identity of the presumed tumour suppressors resident in these genomic loci remains elusive[30].

In more recent years, additional cancer-related genes and signalling networks have been directly implicated in glioma pathogenesis (FIG. 3). The retinoblastoma (Rb) tumour suppressor pathway has been shown to be defective in a significant number of high-grade gliomas of both astrocytic and oligodendroglial lineage, whether by inactivating mutations in *RB1* itself or amplification of its negative regulators cyclin-dependent kinase 4 (*CDK4*) and, less frequently, *CDK6* (REFS 31–34). Analogously, amplification of the p53 antagonists *MDM2* and *MDM4* have also been found in distinct subsets of *TP53*-intact glioblastomas, as have mutations and/or deletions in the *CDKN2A* locus that encodes both INK4A and ARF, which are crucial positive regulators of RB and p53, respectively. Moreover, recent genome-wide association screens have identified single nucleotide polymorphisms (SNPs) in the *CDKN2A* and adjacent *CDKN2B* loci as risk factors for glioma development[35,36]. These studies have also associated SNPs in other genes, such as regulator of telomere elongation helicase 1 (*RTEL1*) and telomerase reverse transcriptase (*TERT*) with increased glioma incidence, providing the research community with a new set of molecular targets for investigation. These findings emphasize the importance of perturbed cell cycle regulation through the disruption of the p53 and Rb pathways in glioma pathogenesis. Mouse modelling has provided further evidence in this regard by demonstrating that functional loss of either RB or p53 in

Neurofibromatosis type 1
Hereditary cancer-predisposing syndrome caused by mutations in *NF1* and characterized most commonly by neurofibromas, optic gliomas and malignant peripheral nerve sheath tumours.

various experimental contexts can directly drive glioma formation, decrease disease-free latency and/or increase tumour grade[37–46].

The involvement of receptor tyrosine kinases (RTKs) in addition to EGFR in gliomagenesis has also been repeatedly demonstrated. Most notably, enhanced signalling through platelet-derived growth factor receptor-α (PDGFRα) has been found to be a common feature of low-grade astrocytic and oligodendroglial tumours along with a significant subset of glioblastoma (discussed below)[47,48]. Although activating mutations in *PDGFRA* are uncommon[49], frequent co-expression of both the receptor and its ligand, most commonly PDGFB, indicates the potential for autocrine or paracrine loops boosting oncogenic signalling through the PDGF network. Similar findings regarding hepatocyte growth factor (HGF) and its RTK MET (also known as HGFR) have also been reported for glioma[50]. Therefore, enhanced RTK signalling, whether driven by somatic mutagenesis or otherwise, seems to be a foundational oncogenic event in the plurality of malignant gliomas, the effects of which are probably mediated in large part through oncogenic PI3K–AKT–mTOR and Ras–MAPK signalling downstream. Underscoring this fact is the not infrequent dysregulation in malignant gliomas of molecular components in these downstream networks[51,52], the most common of which is functional loss of the tumour suppressor *PTEN*, the primary negative regulator of PI3K–AKT–mTOR signalling[27,53]. Mouse modelling has unequivocally verified a central role for RTK biology in the pathogenesis of malignant glioma, whether signalling is perturbed at the level of the RTK itself[37,38,54–59] or the downstream effector (such as KRAS and/or AKT)[54,60,61]. On a related note, targeted deletions of the neurofibromatosis type 1-associated tumour suppressor neurofibromin 1 (*NF1*), a negative regulator of Ras signalling, have also been shown to drive gliomagenesis in mice[41,46,62,63]. Finally, PTEN loss seems to uniformly facilitate glioma formation across several mouse systems[37,38,44,58,62].

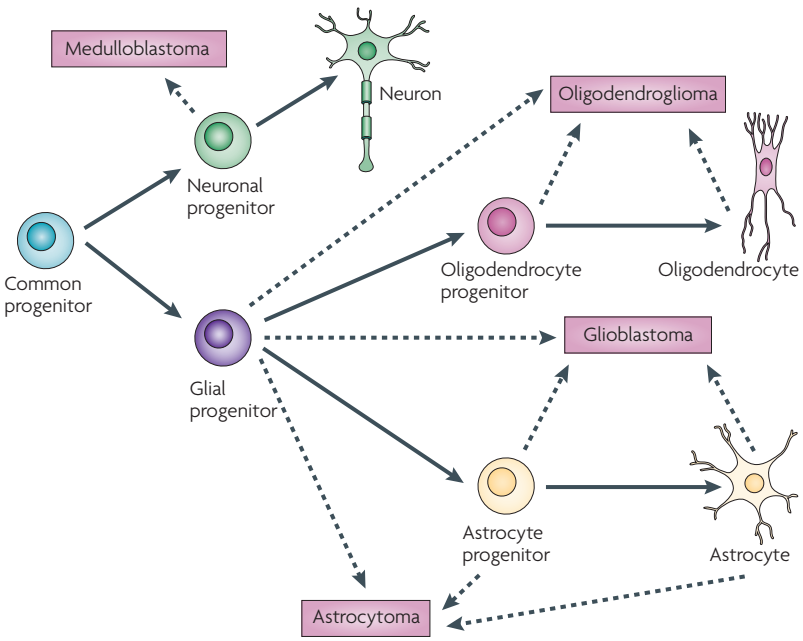

Figure 1 | **The neuroglial lineage tree.** Self-renewing, common progenitors are thought to produce committed neuronal and glial progenitors that eventually differentiate into mature neurons, astrocytes and oligodendrocytes. Although the precise cells of origin for diffuse glioma variants and medulloblastoma remain largely unknown, a selection of likely candidates for each (dashed arrows) is indicated.

Integrated genomics and subsequent advances. The widespread implementation of comparative genomic hybridization (CGH) has resulted in more comprehensive analyses of the molecular aberrations underlying gliomagenesis, as well as insights into their biological heterogeneity[64–71]. CGH profiling of primary and secondary glioblastomas, for instance, has shown strikingly distinct patterns of copy number alteration (CNA), while also demonstrating that secondary glioblastomas themselves consist of two subgroups in which the clinical progression times differ significantly[66]. Coupling array CGH with additional genomic techniques has allowed for increasingly robust, unbiased queries into the identities and functional characteristics of glioma-relevant genes[68,69,71]. Some efforts have been considerably aided by multi-institutional cooperative projects such as the cancer genome atlas (TCGA), which has so far accumulated expression, CNAs and sequencing data from hundreds of histologically confirmed glioblastomas, with additional samples and testing modalities (such as global DNA methylation analysis) currently in progress. These studies have confirmed the importance of the many glioma-associated genes, including those described above, and precisely quantified the extent to which they harbour abnormalities such as mutation, amplification and deletion.

In the context of core signalling pathways, striking patterns emerge. For instance, although the Ras and AKT isoforms themselves are mutated and amplified, respectively, in only 2% of glioblastomas (2 of 91 and 2 of 91), components of the Ras–MAPK and PI3K–AKT–mTOR signalling pathways are affected in the plurality (88%; 80 of 91) of analysed tumours, with *PTEN* representing the most commonly altered gene in either pathway (deleted and/or mutated in ~36% (33 of 91) of all cases)[68]. Intriguingly, mutations and/or deletions in *NF1* are present in 15–18% of sporadic glioblastomas, a much higher number than previously realized[68,71], and they seem to cluster with a specific glioma subtype (discussed below). The p53 and RB tumour suppressor networks are also disrupted in similarly high proportions of glioblastoma: 87% (79 of 91) and 78% (71 of 91), respectively[68]. Such data reinforce the importance of these oncogenic molecular pathways in gliomagenesis, and correlate well with abundant mouse modelling data. Furthermore, computational analyses of the abundant genomic information have led to the development of more effective prognostic stratification algorithms[72].

Integrated genomic analysis has also facilitated the identification and characterization of additional genes involved in glioma pathogenesis. Recently, missense mutations in isocitrate dehydrogenase 1 (*IDH1*) were found in a significant number of glioblastomas that tend to occur mostly in younger patients with more protracted

clinical courses[71]. These point mutations are restricted exclusively to the R132 residue in the active site region of the protein in which they disrupt hydrogen bonding with its substrate[71,73]. Interestingly, a separate group of gliomas harbour mutations in the *IDH1* homologue *IDH2* at the analogous residue (R172). Further investigations have shown that mutations in *IDH1* and *IDH2* are present in high proportions of grade II and III astrocytic and oligodendroglial tumours (72–100%) along with secondary glioblastomas (85%), but are largely absent in primary glioblastomas (5%)[73,74]. Additionally, *IDH* mutations are associated with other genomic abnormalities that are typically seen in lower grade diffuse gliomas, such as *TP53* mutation and 1p/19q deletion; they are also mutually exclusive with *EGFR* amplification and chromosome 10 loss, and multivariate analysis suggests that they are independent favourable prognostic markers[73,75]. These findings suggest that, although *IDH* mutations probably contribute to the early evolution of low-grade gliomas (including those that subsequently progress to higher grade lesions), they seem to have no role in the underlying biology of *de novo* glioblastoma, further emphasizing the fundamental differences in pathogenesis between these two broad diagnostic categories.

The mechanisms by which mutations in *IDH* genes mediate gliomagenesis are still largely unknown. However, one recent study has demonstrated that loss of IDH1 function through point mutation induces hypoxia inducible factor 1α (HIF1α)[76], a component of the hypoxia-responsive transcription factor complex that has been implicated in angiogenesis and tumour growth[77]. By contrast, another recent report has shown that mutant IDH1 proteins exhibit a gain-of-function phenotype by generating *R*(-)-2-hydroxyglutarate (2HG), a toxic metabolite associated with an increased risk of malignant brain tumours in patients with inherited errors of 2HG metabolism[78]. Although much remains to be studied, the identification of *IDH* mutations in diffuse gliomas, and more recently in acute myeloid leukaemia[79], has provided new therapeutic targets and emphasized the increasingly compelling link between cancer biology and basic metabolic processes.

Genomics has also revealed connections between gliomagenesis and microRNA (miRNA) biology. It has recently been shown that amplification of *miR-26a-2* leads to the overexpression of miR-26a in ~12% of glioblastomas, promoting gliomagenesis through direct repression of PTEN, RB and MAP3K2 (REFS 80,81).

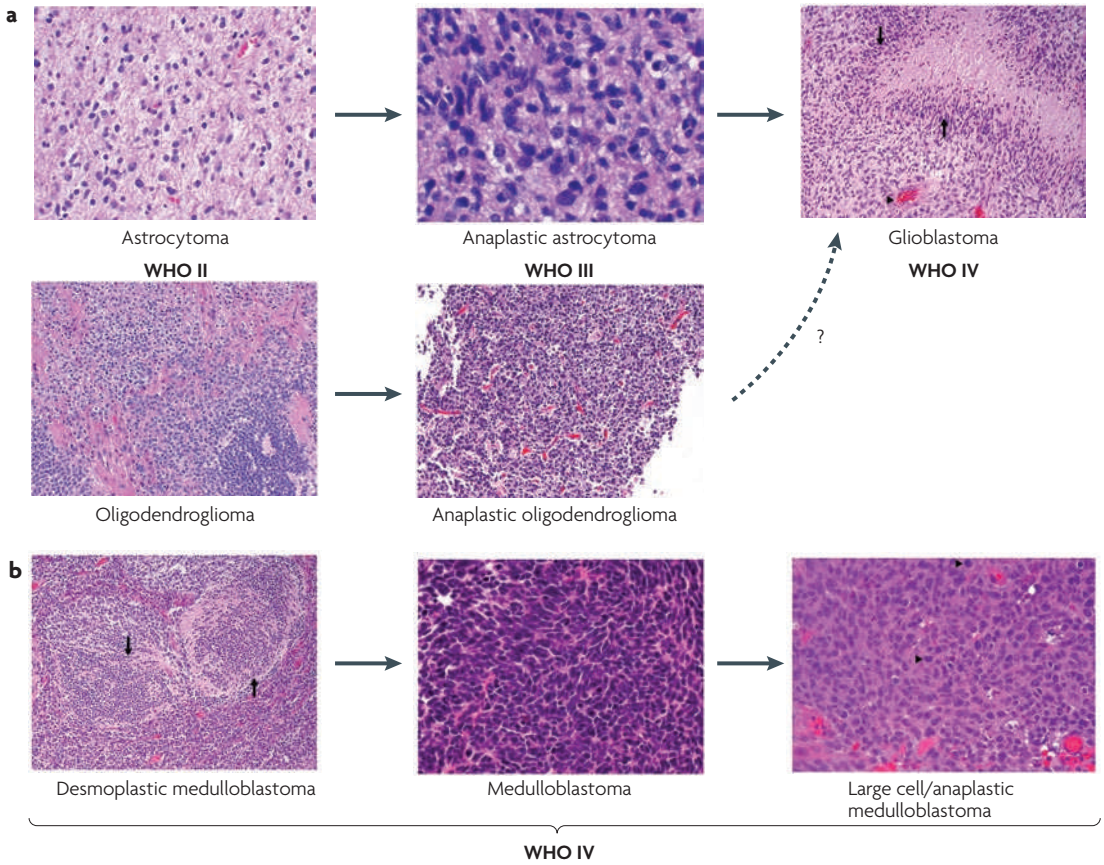

Figure 2 | **Current World Health Organization (WHO) classifications for diffuse glioma and medulloblastoma.** **a** | Schematic showing the classification of diffuse gliomas of astrocytic and oligodendroglial lineages. Representative micrographs for each tumour class are given. The hallmark histological features of glioblastoma, microvascular proliferation (black arrowhead) and pseudopalisading necrosis (black arrows) are also indicated. **b** | Representative micrographs of medulloblastoma histological variants. Differentiated nodules (black arrows) and mitotic figures (black arrowheads) are indicated.

By contrast, the p53-induced miR-34a, which is typically downregulated in glioblastoma, suppresses gliomagenesis by directly targeting the expression of the aforementioned oncoproteins MET and CDK6, along with Notch receptor 1 and Notch receptor 2, which seem to have crucial roles in glioma stem cell survival and proliferation (discussed below)[82]. Several other miRNAs have also been implicated in glioma formation, including miR-21, miR-7, miR-124a, miR-137, miR-221, miR-222 and the miR-181 family, with their individual functions reflecting the wide range of mRNAs targeted by each[83]. miRNA-based regulation of the numerous molecular pathways involved in gliomagenesis is undoubtedly complex and this active area of research should produce numerous additional candidates for therapeutic targeting in the near future.

Transcriptional profiling: glioma subclasses. The gene expression profiles of malignant gliomas have been the subject of several published studies. Initial investigations demonstrated that gliomas of different histological classes exhibit relatively distinct transcriptional profiles that can outperform conventional morphological analysis[84,85]. These studies were followed by others that established transcriptional variance between subgroups of glioblastoma and correlated these findings with differences in clinical outcome[86–88]. This work not only revealed an unrealized complexity in the underlying biology of glioblastoma, but also implicated new genes in gliomagenesis, such as fatty acid binding protein 7 (*FABP7*) and *ASPM*[87,89].

Other transcriptionally based classification schemes have uncovered intriguing links between glioma biology and neuroglial developmental stages[88,90,91] (TABLE 1). The first of these segregated a cohort of malignant gliomas, comprised of both WHO grade III and IV varieties, into three subclasses: proneural, proliferative and mesenchymal, the gene expression signatures of which resembled those of fetal and adult brain; haematopoietic stem cells; and various soft tissues (including bone, synovium, smooth muscle and fetal astrocytes), respectively. Remarkably, nearly all WHO grade III tumours (65 of 73) fell into the proneural category, along with a subset of glioblastomas occurring in younger patients with prolonged disease courses. Moreover, recurrent tumours, although mostly retaining their initial transcriptional subclassification, seemed to significantly shift their mRNA signatures towards the mesenchymal profile. On this note, recent work has identified a set of 'master regulator' transcription factors, the most important of which are signal transducer and activator of transcription 3 (STAT3) and CCAAT/enhancer binding protein-β (C/EBPβ), which seem to mediate the expression of the mesenchymal phenotype and so enhance glioma aggressiveness[92].

Additional clustering analysis using transcriptional data from TCGA has established similar, if not entirely overlapping, patterns in primary glioblastomas. Proneural-like and mesenchymal-like signatures have been identified along with additional patterns labelled classic and neuronal (TABLE 1). Remarkably, integrating these findings with copy number, transcriptional, sequencing and proteomic data effectively correlates glioblastoma subgroups with characteristic disturbances in defined core signalling networks[90,91]. The classic, proneural and mesenchymal categories are strongly associated with genomic abnormalities in either *EGFR* (25–30%), *PDGFR*, *IDH1* or *IDH2* (25–30%), or *NF1* (30–40%) (TABLE 1). Interestingly, not all tumours in each group, and in some cases only a minority, exhibit actual somatic alterations in the relevant subclass-defining gene. For example, the proneural subclass contains the most tumours with alterations in *PDGFRA* and *IDH1*, but also includes many cases that are characterized by *MET* amplification and even includes a small number of *EGFR*-mutated tumours. This kind of transcriptional grouping suggests that, despite genomic heterogeneity, gliomas with similar expression profiles are fundamentally driven by alterations in the same basic signalling networks and may therefore be susceptible to the same class of targeted therapeutics. Indeed, exhaustive western blot analysis seems to confirm this conjecture[90], and robust mouse models of each subclass (TABLE 1) should provide a valuable resource for further evaluation. Finally, on a more foundational level, the existence of molecularly defined subgroups of glioblastoma raises the question of whether these categories actually represent separate disease entities rather than the expression of minor variability in a single tumour class, especially if each is fundamentally driven by its own distinct underlying biology.

Molecular pathology: medulloblastoma

Numerous investigations have implicated components of the sonic hedgehog (SHH) signalling cascade in medulloblastoma pathogenesis[93]. The binding of SHH to its receptor patched (PTCH1) relieves tonic inhibition on the downstream effector smoothened (SMO) and allows the release of the Gli family of transcription factors from inhibitory protein complexes that

Box 1 | **Modelling brain tumours in mice**

Correctly identifying which of the many molecular abnormalities in brain tumours actually drive neoplastic processes requires disease-relevant experimental systems both *in vitro* and *in vivo*. The increasingly widespread use of genetically engineered mouse models (GEMMs) over the past decade has provided invaluable insights in this regard[181]. Incorporating basic and conditional transgenic and knockout technologies, GEMMs have allowed for precision testing of several candidate oncogenes and tumour suppressors in various appropriate cellular contexts, both singly and in combination. Furthermore, the use of virally mediated somatic gene transfer in many of these systems has provided a more faithful recapitulation of focal tumorigenesis rather than the field cancerization that is more akin to tumour-predisposing syndromes such as neurofibromatosis.

Numerous GEMMs have been produced to date, particularly for malignant glioma, and are perhaps most notable for their wide variation in genetic design and driving oncogenic mechanism. For example, effective strategies to model glioma have so far included combined loss of neurofibromin 1 (NF1) and p53 (REFS 41,46,62,63), RB depletion[43,44], augmented RTK signalling[37,42,55,57,174,182] and targeted activation of the Ras–MAPK pathway[38,54,56,58,60,61]. Such heterogeneity, as recent data continue to emphasize, accurately reflects the cellular and molecular characteristics of human brain tumours. In this way, the large number of available GEMMs, each with their distinct underlying genetics, offers an invaluable resource for preclinical studies, as targeted therapeutics designed to treat specific glioma or medulloblastoma subclasses can be tested in the most biologically relevant model systems.

Turcot's syndrome
Hereditary cancer-predisposing syndrome caused by mutations in *APC* and most commonly characterized by adenomatous polyposis of the colon and an increased incidence of neuroepithelial tumours.

Supratentorial PNET
A class of PNET arising in the forebrain that is distinct from medulloblastoma.

typically include suppressor of fused (SUFU) (FIG. 4). Genomic alterations in components of the SHH signalling pathway, specifically inactivating mutations of *PTCH1* and *SUFU* and/or activating mutations of *SMO*, have been found in ~15% of sporadic medulloblastomas[94–97]. Additionally, germline mutations in *PTCH1* cause Gorlin's syndrome, a rare congenital condition that is characterized by an increased incidence of several tumour types, including medulloblastoma[98]. SHH signalling is known to drive proliferation in the granule neuron precursors of the cerebellum, and pathway dysregulation resulting from genomic alterations of its components presumably drives medulloblastoma formation through analogous downstream effects[99]. Mouse modelling has further demonstrated the sufficiency of SHH signalling to generate medulloblastomas in multiple cell types in the developing hindbrain (discussed below)[100,101]. On this note, activating the SHH pathway through various genetic strategies remains the causative mechanism underlying the plurality of mouse medulloblastoma models that have been produced to date[100–113].

Dysregulation of the Wnt pathway has also been linked to the development of medulloblastoma. Wnt ligand binds to its receptor frizzled (FZD) leading to the release of its downstream effector β-catenin from an inhibitory complex that includes the tumour suppressor adenomatous polyposis coli (APC) and the axin proteins (FIG. 4). Subsequent nuclear accumulation of β-catenin is thought to mediate its tumorigenic functions, presumably through the activation of target genes such as *MYC*, cyclin D1 (*CCND1*) and RE1-silencing transcription factor (*REST*), which have established roles in cellular proliferation, differentiation and inhibition of apoptosis[114,115]. Approximately 20% of sporadic medulloblastomas harbour mutations in *APC*, *AXIN1*, *AXIN2* or *CTNNB1* (which encodes β-catenin)[116–120], and a similarly sized fraction (18%) has separately been shown to exhibit nuclear β-catenin immunostaining[114]. Furthermore, Turcot's syndrome, which is caused by mutations in *APC*, is characterized by an increased incidence of medulloblastoma and other neuroepithelial tumours. Finally, medulloblastomas that are driven by increased Wnt signalling, as shown by nuclear β-catenin staining, may follow a relatively favourable clinical course[121]. Although *in vivo* models of Wnt pathway-driven medulloblastomas have yet to emerge, one group has successfully generated supratentorial PNETs using, in part, exogenously augmented β-catenin expression[122].

Loss of chromosome 17p, typically in association with gain of 17q (forming isochromosome 17q: i(17)(q10)) is the most common genetic lesion in medulloblastoma, occurring in 30–50% of cases[123–129]. Although the precise mechanism by which this genomic abnormality contributes to tumorigenesis and its prognostic importance remain unclear, the common deletion region of 17p13.2-13.3 includes several confirmed and putative tumour suppressor genes, including *TP53*, the loss of which could presumably facilitate neoplastic behaviour. Germline defects in *TP53*, resulting in Li–Fraumeni syndrome, have been correlated with increased medulloblastoma incidence[19], and although *TP53* mutations in sporadic medulloblastomas are not particularly common, they seem to confer poor clinical outcome[125,130]. Additionally, *Trp53* loss dramatically enhances medulloblastoma formation in SHH pathway-driven mouse models[112,131,132].

Genomic amplification of *MYCN* and *MYC* characterizes a subset of clinically aggressive medulloblastomas that tend to exhibit large cell/anaplastic histological features[130,133]. It has recently been demonstrated that the oncogenic miRNA cluster, *miR-17–92*, is a downstream target of MYC, the expression of which seems to be associated with a wide range of tumour types[134]. Also, MYC or MYCN overexpression facilitates medulloblastoma formation in mice, a characteristic shared with other oncoproteins such as AKT, insulin-like growth factor 2 (IGF2) and BCL-2 (REFS 102,107,110,135).

The Erbb family of RTKs, insulin-like growth factor 1 receptor (IGF1R) and PDGFR have also been directly implicated in medulloblastoma pathogenesis, with IGF1R and PDGFR implicated by their association with poor prognosis[115,136]. One group has documented ERBB2 overexpression in a large proportion of medulloblastomas (28%)[137], in which it is thought to promote tumorigenesis by activating the Ras–MAPK and AKT pathways and by promoting the expression of pro-metastatic genes such as S100 calcium binding protein A4 (*S100A4*), *CCL5* (also known as *RANTES*) and *MAP2K5* (also known as *MEK5*)[138,139]. Additionally, deletion mutants of *ERBB4*

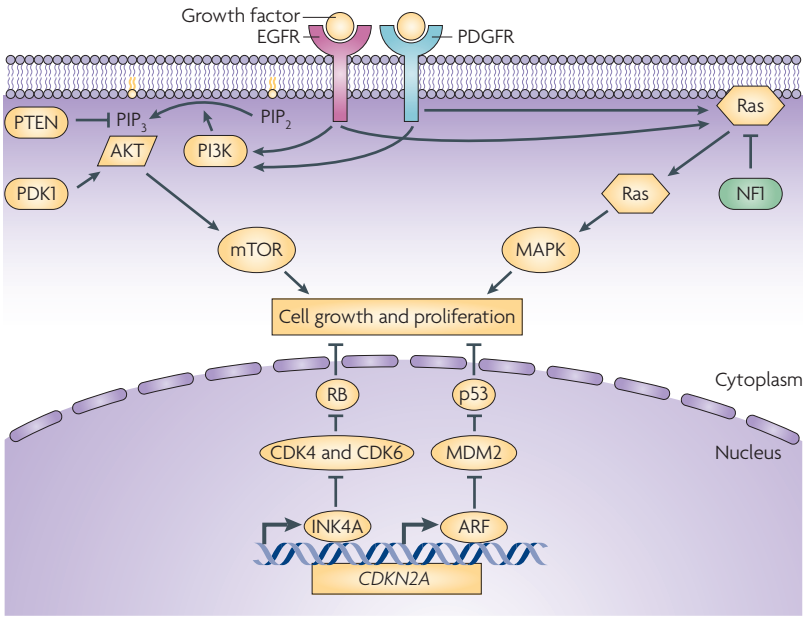

Figure 3 | **Schematic of molecular pathways implicated in the pathogenesis of glioma.** Downstream oncogenic signalling through receptor tyrosine kinases (RTKs) such as epithelial growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) is mediated by the PI3K–AKT–mTOR and Ras–MAPK networks. Defects in the p53 and RB tumour suppressor pathways are also common. Proteins exhibiting alterations at the genomic level that define molecular subclasses of malignant glioma are also indicated: EGFR (red), PDGFR (blue) and NF1 (green). CDK, cyclin-dependent kinase; NF1, neurofibromin 1; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate.

Table 1 | **Molecular subclasses of malignant glioma from different studies**

Analysis of WHO grade III and IV glioma variants[88]	Analysis of primary glioblastomas[91]	Analysis of primary glioblastomas, some WHO grade II and III gliomas and secondary glioblastomas[90]	Canonical genomic aberration	Relevant mouse model
Proneural	Proneural	PDGF	• *PDGFR* amplification or mutation • *IDH1* or *IDH2* mutation	Localized PDGFB expression combined with genetically engineered tumour suppression loss[174]
Mesenchymal*	Mesenchymal	NF1	*NF1* deletion or mutation	Localized deletion of *Nf1* and *Trp53* in the mouse SVZ[63]
	Classical	EGFR	*EGFR* amplification or mutation	Localized EGFR vIII expression combined with genetically engineered tumour suppressor loss[59]
Subclass not defined in this study	Neural	Unclassified	Unknown	None

*The relationship between the mesenchymal clusters as defined by Phillips *et al.*[88], and subsequent studies, is not as precisely defined as it is for the proneural clusters. EGFR, epidermal growth factor receptor; IDH, isocitrate dehydrogenase; NF1, neurofibromin 1; PDGF, platelet-derived growth factor; SVZ, subventricular zone; vIII, variant III; WHO, World Health Organization.

have been found in childhood medulloblastomas[140], and overexpression of Erbb proteins has been correlated with unfavourable clinical outcome[141–143].

Transcriptional profiling: medulloblastoma subclasses. As analogous studies have accomplished for glioma, global transcriptional analyses of medulloblastoma have both emphasized and better conceptualized the molecular heterogeneity inherent to this cancer type. Initial efforts in this regard demonstrated gene expression patterns in medulloblastoma that were distinct from other CNS tumours with similar histological features, such as supratentorial PNET and atypical teratoid/rhabdoid tumour (AT/RT)[144]. Furthermore, transcriptional profiles were found to be predictive of clinical outcome and implied tumour cell lineage[136,144]. On a separate note, one study found that PDGFR and Ras–MAPK signalling correlated with metastatic medulloblastoma, supplementing their array analysis with confirmatory experiments *in vitro*[136].

More recently, two integrated genomic investigations have revealed distinct transcriptional subclasses of medulloblastoma that also harbour genomic alterations in oncogenic signalling pathways[145,146]. Both have identified subgroups centred around dysregulated SHH and Wnt signalling that are also characterized by genomic alterations in pathway-relevant genes (TABLE 2). More specifically, the SHH-associated group includes all analysed tumours with mutations in *PTCH1* and *SUFU*, and the Wnt-associated group contains all tumours with mutations in *CTNNB1*. Additional subclasses are less precisely defined but seem to be somewhat distinct with regard to their associated chromosomal abnormalities, patient age distribution and gene expression-related developmental signatures. Interestingly, medulloblastomas with desmoplastic histology mainly cluster in the SHH group, confirming an association that has previously been made[94]. As with similar analyses carried out for glioma, these studies indicate that transcriptional variation in medulloblastoma may reflect basic differences in oncogenic signalling, and in this way invite the implementation of subclass-specific targeted therapy.

Cancer stem cells and cells of origin

Any discussion of molecular and cellular heterogeneity within individual tumours must include the 'cancer stem cell' (CSC) hypothesis, which proposes that neoplastic processes are heavily dependent on a small population of CSCs that possess the distinct properties of self-renewal, multipotency and resistance to conventional therapy[147]. In this scheme, the CSC pool and its unique characteristics are thought to contrast sharply with the bulk of tumour cells of which the lineage is more specified and proliferative capacity presumably more limited. Although considerable controversy still surrounds the existence, behaviour and even the nomenclature of CSCs, populations of cells with stem-like properties have been identified across several solid and liquid tumours, including brain cancers[148–150].

In both human glioblastomas and medulloblastomas, the expression of the neural stem cell marker CD133 (also known as prominin 1) has been associated with both tumour initiation capacity and radioresistance[149–152]. Initial reports indicated that as few as 100 $CD133^+$ cells collected from either glioblastoma or medulloblastoma could form xenografted tumours in immunocompromised mice, in sharp contrast to the 10^5 $CD133^-$ cells required for the same phenotype[150]. Although more recent investigations have called into question the unique

Atypical teratoid/rhabdoid tumour
An aggressive brain tumour variant that occurs in young children and is characterized by loss of the transcription factor integrase interactor 1 (INI1).

Perivascular niche
A specialized microenvironment intimately associated with the microvasculature where the plurality of brain tumour stem-like cells seem to reside.

tumour-initiating properties of the CD133$^+$ subpopulation[153,154], CD133 expression has been linked with poor clinical outcome[155]. Furthermore, it has been argued that the discordant results between studies regarding the stem-like properties of the CD133$^+$ compartment could be attributed to differences in the handling and culture of primary tissue samples, factors that dramatically alter both the biological behaviour of tumour cells and their expression of surface markers such as CD133 (REF. 155). It also remains to be seen whether CD133 expression by stem-like cells is dependent on the molecular subclass of the brain tumour in question.

Related to these ideas is the increasing evidence that CSCs require a specialized microenvironment for the maintenance of their characteristics[156], similar in many ways to normal, non-neoplastic stem cell populations. Indeed, it has been known for some time that stem cells in the adult brain reside specifically in the subgranular zone (SGZ) of the hippocampus and the subventricular zone (SVZ)[157,158], where underlying capillary networks seem to provide both the signals and nourishment that are required for their support[159]. It has recently been shown that CSCs from various brain tumours, including glioblastoma and medulloblastoma, exist in a perivascular niche that is analogous to that of their non-neoplastic counterparts[160]. Stem-like cells from human brain tumours migrate towards, and intimately interact with, endothelial cells in three-dimensional co-cultures. Furthermore, transplantation of these brain tumour CSCs with endothelial cells enhances orthotopic tumour formation in immunocompromised mice, but endothelial cell depletion in xenografts hinders their growth[160]. Additional work has demonstrated that CD133$^+$ cells from glioblastomas secrete high levels of vascular endothelial growth factor (VEGF), which further supports an interactive, functional relationship between brain tumour CSCs and the surrounding microvasculature[161].

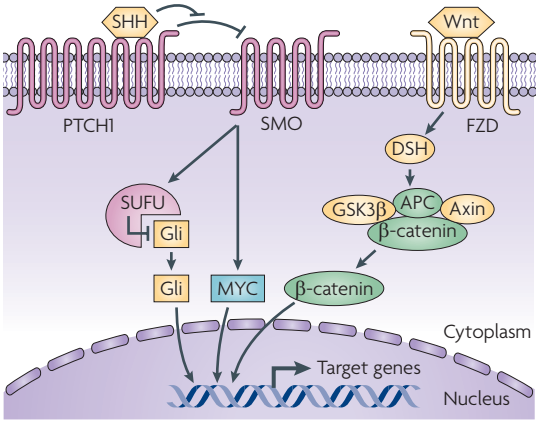

Figure 4 | **The molecular networks implicated in the pathogenesis of medulloblastoma.** Dysregulated signalling through the sonic hedgehog (SHH) and Wnt pathways has been implicated in distinct subclasses of medulloblastoma. Specific proteins in the SHH and Wnt pathways exhibiting alterations at the genomic level are shown in red and green, respectively. *MYC* (blue) amplification has been associated with the large cell/anaplastic medulloblastoma variant. APC, adenomatous polyposis coli; DSH, dishevelled; FZD, frizzled; GSK3β, glycogen synthase kinase-3β; PTCH1, patched; SMO, smoothened; SUFU, suppressor of fused.

Various proteins and signalling cascades that are involved in both tumorigenesis and the maintenance of non-neoplastic stem cell pools have been implicated in the biology of brain tumour CSCs. These include many of the signalling pathways discussed above along with the transcription factors OCT4 (also known as POU5F1), oligodendrocyte lineage transcription factor 2 (OLIG2) and BMI1 (REF. 162). Recent work suggests that the SHH pathway may have an important role in the establishment of the CSC niche in gliomas. Abundant SHH is secreted by perivascular astrocytes in human and mouse glioblastomas and downstream Gli activity correlates well with tumour grade[163]. Similarly, molecular cues responsive to cellular hypoxia, particularly the actions of HIFs, also seem to affect the behaviour of CSCs and the maintenance of the stem cell microenvironment. Experimental depletion of HIFs in glioma CSCs inhibits self-renewal and survival *in vitro* and tumour initiation potential *in vivo*[164]. Furthermore, HIF2α, the transcriptional targets of which include *POU5F1* (REF. 165), induces stem-like behaviour and enhances tumorigenic potential when transduced into non-stem cells derived from human gliomas[166].

The Notch pathway has repeatedly been linked to the biology of normal neural stem cells as well as glioma CSCs[167]. Ligand binding to the Notch receptor results in its cleavage and the release of the Notch intracellular domain (NICD), the subsequent nuclear translocation of which activates various target genes. The Notch pathway is activated in human and mouse gliomas, and forced expression of NICD both induces the stem cell marker nestin and cooperates with KRAS to induce expansion of the SVZ *in vivo*[168]. Furthermore, increased Notch signalling enhances the efflux of cytotoxic drugs through ABC transporters such as ABCG2, a recognized property of stem-like tumour cells that contributes to their resistance to conventional therapies[169,170]. Using fluorescent Hoechst dye, which is also an ABCG2 substrate, stem cells can be effectively sorted by fluorescence-activated cell sorting (FACS) from brain tumours as a 'side population' (SP) that exhibits a lower level of fluorescence than their non-stem cell-like counterparts in the 'main population' (MP)[170,171]. NICD overexpression increases SP cell number, with Notch pathway inhibition having the opposite effect[170]. Extending these findings is a recent study demonstrating that nitric oxide (NO), which is secreted by the tumour vasculature, induces Notch signalling and augments the SP fraction in PDGF-driven gliomas, providing a mechanism by which specific molecular cues can maintain the stem-like character in the perivascular niche[172].

The AKT pathway has also been identified as a major effector of stem-like behaviour in malignant brain tumours. Increasing AKT signalling through *PTEN* loss increases SP cell number in mouse glioblastomas, at least partially through the direct activation of ABCG2 (REF. 171). Furthermore, in mouse medulloblastoma models, activation of the PI3K–AKT–mTOR pathway seems

Table 2 | **Molecular subclasses in medulloblastoma***

Subclass	Genomic characteristics	Histology	Expression characteristics	Median patient age (range)	Mouse models
A	Wnt signalling: *CTNNB1* mutations	Classic	Notch and PDGF signalling, and increased expression of cell cycle proteins	10.4 years (6–20)	None
B	SHH signalling: *PTCH1 or SUFU* mutations	Enriched for desmoplastic and LC/A	Notch and PDGF signalling, and increased expression of cell cycle proteins	3.0 years (1.5–35.3)	Yes (many)
C	i(17q), -8, -X, +18	Classic	Neuronal markers	7.2 years (3.7–25.6)	None
D	i(17q), -8, -X, +18	Classic or some LC/A	Neuronal markers and photoreceptor markers	5.9 years (3–16.6)	None
E	-X, +18	Classic or some LC/A	Cell cycle proteins and photoreceptor markers	3.8 years (2–15)	None

*Adapted from data in Kool *et al.*[145]. *CTNNB1*, β-catenin; LC/A, large cell/anaplastic; PDGF, platelet-derived growth factor; *PTCH1*, patched; SHH, sonic hedgehog; *SUFU*, suppressor of fused.

to contribute to the relative resistance of perivascular CSCs to therapeutic irradiation[173]. Combining small molecule AKT pathway inhibitors with radiotherapy significantly decreases the survival of this resistant stem cell-like pool, indicating a promising avenue for future treatment strategies. Supporting these findings, another group has recently reported that *PTEN* loss and *TP53* deletion are crucial for the maintenance of self-renewal in neural stem cells and seem to mediate these effects at least in part through the induction of *MYC*[45].

The link between stem cell biology and brain cancer has implications for nascent tumorigenesis and the cells responsible for the initiation of the neoplastic process. More specifically, it is not clear whether malignant brain tumours primarily arise from pluripotent stem cells, their more differentiated progeny, or both. Once again, increasingly sophisticated *in vivo* modelling systems have allowed researchers to begin addressing this question by carefully restricting oncogenic events to small subpopulations of cells within individual mouse brain tumours. Although informative (if somewhat conflicting) observations have been made, the data seem to indicate a considerable degree of complexity underlying this issue that predictably echoes the cellular and molecular heterogeneity of the tumour types in question.

For medulloblastoma, two groups have recently demonstrated that dysregulated SHH signalling is sufficient to generate tumours from multiple distinct precursor cell populations. Indeed, driving oncogenesis in either neural stem cells or more differentiated granule neuron precursors yields medulloblastomas with roughly equal penetrance, although the precise positioning of each tumour does seem to depend on the anatomical distribution of its pool of initiating cells[100,101]. These data indicate that although various cell types of cerebellar lineage are competent to produce medulloblastomas given the appropriate oncogenic stimulus, a certain degree of heterogeneity in the resulting tumours can probably be attributed to differences in cell of origin.

Analogous experiments in glioma mouse models have used viral transduction methods for the precise localization of oncogenic stimuli to specific cellular subpopulations. In one study, cre recombinase-expressing adenovirus (adeno-cre) was used to functionally silence *Nf1*, *Trp53* and/or *Pten* in targeted foci in the brains of mice with floxed alleles for each tumour suppressor[63]. Stereotactic injection of adeno-cre into the SVZ invariably yielded high-grade astrocytomas, and application of the virus into either the cerebral cortex or striatum had minimal results, suggesting that tumour suppressor loss in stem-like progenitor cells is both necessary and sufficient for gliomagenesis. Contrasting sharply with these findings are results from more recent investigations using retrovirus-mediated PDGFB expression in either adult mice or newborn pups that were deficient in $Cdkn2a^{INK4A}$, $Cdkn2a^{ARF}$ and/or *Trp53* (REFS 174,175). These studies demonstrated that driving PDGFB expression in nestin$^+$ progenitor cells, mature GFAP$^+$ astrocytes or committed oligodendroglial precursors is sufficient to generate gliomas. Moreover, gliomas were generated with equal penetrance regardless of whether retrovirus was delivered into the SVZ, cerebral cortex or cerebellum[174]. Similarly, another group has effectively induced high-grade gliomas in the mouse striatum by driving EGFR vIII expression in combination with *Cdkn2a* and *Pten* loss[59], providing another example of gliomagenesis derived from a non-stem-like cellular pool. The discrepancies in these studies indicate that several different cell types probably harbour tumorigenic potential and that their ability to initiate neoplasia may depend on the precise mechanisms governing the underlying oncogenic stimulus (that is, RTK signalling compared with tumour suppressor loss) and/or the molecular subclass of the tumour in question.

To further complicate this issue, additional work has suggested that evolving brain cancers may incorporate large numbers of nominally non-neoplastic cells that are not derived from their cell of origin, and that such recruited elements proliferate and substantially contribute to the pathogenicity of the tumour mass as a whole[176]. Such data argue that determining the precise cellular origins of brain tumours, although fascinating from an academic standpoint, may be less relevant to the development of effective treatment regimens than a fundamental understanding of the striking cellular and molecular complexity that evolves in these neoplasms over time.

Box 2 | **Treatment challenges: the blood–brain barrier**

Achieving adequate delivery of drugs to the malignant cells in brain tumours remains a vexing problem. Indeed, recent work indicates that failure to adequately circumvent the blood–brain barrier (BBB) seems to be at least partially responsible for the lack of tangible progress in the implementation of targeted therapeutics[183]. And although the BBB does seem to be somewhat disrupted in the abnormal vascular networks characterizing most malignant brain tumours[184], it frequently remains intact along the infiltrating edges of the neoplasms where the plurality of recurrences tend to occur[185].

Various strategies have been designed to overcome this problem and are receiving considerable attention from the biomedical community[186]. Simply increasing the dosage of some drugs provides one option, although this is obviously limited by toxicity profiles. Other strategies involve the conjugation of therapies to lipophilic moieties or other vectors (such as antibodies, peptides and viruses), or packaging drugs in carrier systems such as liposomes, micelles and dendrimers. Co-administration with inhibitors of BBB drug-efflux transporters such as ABCG2 is another possibility. Additionally, more invasive approaches such as convection enhanced delivery (CED), for which the anticancer agent is infused directly into the tumour by a catheter or implanted therapies consisting of therapy-infused reservoirs or matrices are also the subject of active research.

Conclusions: implications for therapy

As our understanding of the intricacies of glioma and medulloblastoma biology has progressed, so too has the hope that such breakthroughs will lead to more effective, less toxic, rationally conceived therapeutics. For both disease entities, the mainstays of nonsurgical treatment are notable in their uniformity, consisting of a combination of radiation and cytotoxic chemotherapy. However, as the functional determinants driving brain tumorigenesis continue to be elucidated, revealing among other things the remarkable cellular and molecular heterogeneity discussed above, the opportunities for more targeted, individualized intervention seem to be increasing rapidly.

Indeed, initial efforts towards the stratification of patients with brain tumours into molecularly determined treatment groups have already begun. The discovery that promoter methylation and transcriptional silencing of *O*-6-methlylguanine-DNA methyltransferase (*MGMT*) results in improved chemosensitivity of glioblastomas has now been widely applied as a prognostic indicator for malignant glioma. MGMT repairs *O*-6-methylguanine DNA damage that is induced by alkylating agents such as temozolomide (currently the mainstay of anti-glioma chemotherapy), and strategies to overcome this resistance mechanism are the focus of numerous studies[177,178]. Also relevant to this discussion is the recent finding that glioblastomas driven by EGFR vIII may preferentially respond to the EGFR inhibitor erlotinib when PTEN expression is retained[179], and so forming a molecular subgroup that is presumably more amenable to targeted therapeutics. These two examples foreshadow what will hopefully constitute a paradigm shift in the treatment of brain cancer to a model in which patients will be stratified by molecular characteristics into treatment groups before the initiation of more personalized, biologically grounded therapies. The challenge now becomes how to most efficiently and effectively segregate tumours into treatment-relevant subgroups, and the development of the necessary biomarkers for this purpose.

Innate and acquired resistance to therapeutic regimens will probably continue to frustrate efforts to combat brain cancer, as will issues of drug delivery (BOX 2). In this regard, molecularly targeted therapies have been shown to suffer from the same deficiencies as their more conventional counterparts, as a recent report describing a case of widely metastatic medulloblastoma demonstrates[180]. In this instance, a tumour known to express a PTCH1-W844C mutant (that leads to the pathological activation of SHH signalling) was treated with a specific SMO inhibitor. Although this intervention initially resulted in dramatic remission, the tumour rapidly recurred in ~3 months having acquired a point mutation in *SMO* that rendered it refractory to the drug. Striking examples of acquired resistance mechanisms such as this abound in the literature, are not unique to brain tumours and illustrate the need for combinatorial regimens that are directed at multiple components of disease-implicated pathways simultaneously. Furthermore, the presence of stem-like cells in both glioma and medulloblastoma probably contributes to resistance phenotypes, and argues for the direct targeting of this cellular subpopulation, perhaps through the inhibition of Notch and/or PI3K–AKT–mTOR signalling, in conjunction with more conventional cytotoxic therapies.

In conclusion, although numerous challenges remain, notable progress in the molecular characterization of malignant glioma and medulloblastoma has paved the way for more rationally based treatment strategies that target specific genes and proteins. How best to implement these promising new therapies, particularly in what combinations and patient groups, should be the subject of intensive translational research in the years to come.

1. Stupp, R. *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **352**, 987–996 (2005).
2. Wen, P. Y. & Kesari, S. Malignant gliomas in adults. *N. Engl. J. Med.* **359**, 492–507 (2008).
3. Gilbertson, R. J. Medulloblastoma: signalling a change in treatment. *Lancet Oncol.* **5**, 209–218 (2004).
4. Bailey, P. & Cushing, H. *A Classification of Tumours of the Glioma Group on a Histogenic Basis* (J. B Lippincott, Philadelphia, 1926).
5. Kernohan, J. W., Mabon, R. F. & Svien, H. J. A simplified classification of gliomas. *Proc. Staff Meetings Mayo Clinic* **24**, 71–74 (1949).
6. Ringertz, N. Grading of gliomas. *Acta Pathol Microbiol Scand.* **27**, 51–65 (1950).
7. Zülch, K. J. (ed.) *Histological typing of tumours of the central nervous system* (World Health Organization, Switzerland, 1979).
8. Louis, D. N. *et al.* The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol.* **114**, 97–109 (2007).
This paper establishes the current morphological and molecular criteria for the subclassification of brain tumours.
9. Dropcho, E. J. & Soong, S. J. The prognostic impact of prior low grade histology in patients with anaplastic gliomas: a case-control study. *Neurology* **47**, 684–690 (1996).
10. Ohgaki, H. & Kleihues, P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J. Neuropathol. Exp. Neurol.* **64**, 479–489 (2005).
11. Ohgaki, H. *et al.* A case history of glioma progression. *Acta Neuropathol.* **97**, 525–532 (1999).
12. Scherer, H. J. Cerebral astrocytomas and their derivatives. *Am. J. Cancer* **40**, 159–198 (1940).
13. Rutkowski, S. *et al.* Treatment of early childhood medulloblastoma by postoperative chemotherapy alone. *N. Engl. J. Med.* **352**, 978–986 (2005).
14. Sure, U. *et al.* Staging, scoring and grading of medulloblastoma. A postoperative prognosis predicting system based on the cases of a single institute. *Acta Neurochir. (Wien)* **132**, 59–65 (1995).
15. Brown, H. G. *et al.* "Large cell/anaplastic" medulloblastomas: a Pediatric Oncology Group Study. *J. Neuropathol. Exp. Neurol.* **59**, 857–865 (2000).
16. Giangaspero, F. *et al.* Large-cell medulloblastomas. A distinct variant with highly aggressive behavior. *Am. J. Surg. Pathol.* **16**, 687–693 (1992).
17. Giangaspero, F. *et al.* Stratification of medulloblastoma on the basis of histopathological grading. *Acta Neuropathol.* **112**, 5–12 (2006).

18. McManamy, C. S. *et al.* Morphophenotypic variation predicts clinical behavior in childhood non-desmoplastic medulloblastomas. *J. Neuropathol. Exp. Neurol.* **62**, 627–632 (2003).
19. Malkin, D. *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* **250**, 1233–1238 (1990).
20. Srivastava, S., Zou, Z. Q., Pirollo, K., Blattner, W. & Chang, E. H. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature* **348**, 747–749 (1990).
21. Louis, D. N. The p53 gene and protein in human brain tumors. *J. Neuropathol. Exp. Neurol.* **53**, 11–21 (1994).
22. Libermann, T. A. *et al.* Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature* **313**, 144–147 (1985).
23. Libermann, T. A. *et al.* Expression of epidermal growth factor receptors in human brain tumors. *Cancer Res.* **44**, 753–760 (1984).
24. Wong, A. J. *et al.* Structural alterations of the epidermal growth factor receptor gene in human gliomas. *Proc. Natl Acad. Sci. USA* **89**, 2965–2969 (1992).
25. Frederick, L., Wang, X. Y., Eley, G. & James, C. D. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Res.* **60**, 1383–1387 (2000).
26. Sugawa, N., Ekstrand, A. J., James, C. D. & Collins, V. P. Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. *Proc. Natl Acad. Sci. USA* **87**, 8602–8606 (1990).
27. Cairncross, J. G. *et al.* Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J. Natl Cancer Inst.* **90**, 1473–1479 (1998).
28. Reifenberger, G. *et al.* Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. *Am. J. Pathol.* **145**, 1175–1190 (1994).
29. Smith, J. S. *et al.* Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. *Oncogene* **18**, 4144–4152 (1999).
30. Barbashina, V., Salazar, P., Holland, E. C., Rosenblum, M. K. & Ladanyi, M. Allelic losses at 1p36 and 19q13 in gliomas: correlation with histologic classification, definition of a 150-kb minimal deleted region on 1p36, and evaluation of CAMTA1 as a candidate tumor suppressor gene. *Clin. Cancer Res.* **11**, 1119–1128 (2005).
31. Costello, J. F. *et al.* Cyclin-dependent kinase 6 (CDK6) amplification in human gliomas identified using two-dimensional separation of genomic DNA. *Cancer Res.* **57**, 1250–1254 (1997).
32. Henson, J. W. *et al.* The retinoblastoma gene is involved in the malignant progression of astrocytomas. *Ann. Neurol.* **36**, 714–721 (1994).
33. Reifenberger, G., Reifenberger, J., Ichimura, K., Meltzer, P. S. & Collins, V. P. Amplification of multiple genes from chromosomal region 12q13–14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. *Cancer Res.* **54**, 4299–4303 (1994).
34. Watanabe, T. *et al.* Concurrent inactivation of RB1 and TP53 pathways in anaplastic oligodendroglioma. *J. Neuropathol. Exp. Neurol.* **60**, 1181–1190 (2001).
35. Shete, S. *et al.* Genome-wide association study identifies five susceptibility loci for glioma. *Nature Genet.* **41**, 899–904 (2009).
36. Wrensch, M. *et al.* Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility. *Nature Genet.* **41**, 905–908 (2009).
References 35 and 36 are large genome-wide association screens identifying low-penetrance susceptibility alleles that increase glioma risk.
37. Dai, C. *et al.* PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes *in vivo*. *Genes Dev.* **15**, 1913–1925 (2001).
38. Hu, X. *et al.* mTOR promotes survival and astrocytic characteristics induced by Pten/AKT signaling in glioblastoma. *Neoplasia* **7**, 356–368 (2005).
39. Uhrbom, L., Nerio, E. & Holland, E. C. Dissecting tumor maintenance requirements using bioluminescence imaging of cell proliferation in a mouse glioma model. *Nature Med.* **10**, 1257–1260 (2004).
40. Marino, S., Vooijs, M., van Der Gulden, H., Jonkers, J. & Berns, A. Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. *Genes Dev.* **14**, 994–1004 (2000).
41. Reilly, K. M., Loisel, D. A., Bronson, R. T., McLaughlin, M. E. & Jacks, T. Nf1; Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. *Nature Genet.* **26**, 109–113 (2000).
42. Weiss, W. A. *et al.* Genetic determinants of malignancy in a mouse model for oligodendroglioma. *Cancer Res.* **63**, 1589–1595 (2003).
43. Xiao, A., Wu, H., Pandolfi, P. P., Louis, D. N. & Van Dyke, T. Astrocyte inactivation of the pRb pathway predisposes mice to malignant astrocytoma development that is accelerated by PTEN mutation. *Cancer Cell* **1**,157–168 (2002).
44. Xiao, A. *et al.* Somatic induction of Pten loss in a preclinical astrocytoma model reveals major roles in disease progression and avenues for target discovery and validation. *Cancer Res.* **65**, 5172–5180 (2005).
45. Zheng, H. *et al.* p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation. *Nature* **455**, 1129–1133 (2008).
46. Zhu, Y. *et al.* Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer Cell* **8**, 119–130 (2005).
47. Di Rocco, F., Carroll, R. S., Zhang, J. & Black, P. M. Platelet-derived growth factor and its receptor expression in human oligodendrogliomas. *Neurosurgery* **42**, 341–346 (1998).
48. Westermark, B., Heldin, C. H. & Nister, M. Platelet-derived growth factor in human glioma. *Glia* **15**, 257–263 (1995).
49. Clarke, I. D. & Dirks, P. B. A human brain tumor-derived PDGFR-α deletion mutant is transforming. *Oncogene* **22**, 722–733 (2003).
50. Abounader, R. & Laterra, J. Scatter factor/hepatocyte growth factor in brain tumor growth and angiogenesis. *Neuro Oncol.* **7**, 436–451 (2005).
51. Guha, A., Feldkamp, M. M., Lau, N., Boss, G. & Pawson, A. Proliferation of human malignant astrocytomas is dependent on Ras activation. *Oncogene* **15**, 2755–2765 (1997).
52. Wang, H. *et al.* Analysis of the activation status of Akt, NFkB, and Stat3 in human diffuse gliomas. *Lab. Invest.* **84**, 941–951 (2004).
53. James, C. D. *et al.* Clonal genomic alterations in glioma malignancy stages. *Cancer Res.* **48**, 5546–5551 (1988).
54. Ding, H. *et al.* Oligodendrogliomas result from the expression of an activated mutant epidermal growth factor receptor in a RAS transgenic mouse astrocytoma model. *Cancer Res.* **63**, 1106–1113 (2003).
55. Shih, A. H. *et al.* Dose-dependent effects of platelet-derived growth factor-B on glial tumorigenesis. *Cancer Res.* **64**, 4783–4789 (2004).
56. Uhrbom, L. *et al.* Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. *Cancer Res.* **62**, 5551–5558 (2002).
57. Uhrbom, L., Hesselager, G., Nister, M. & Westermark, B. Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. *Cancer Res.* **58**, 5275–5279 (1998).
58. Wei, Q. *et al.* High-grade glioma formation results from postnatal pten loss or mutant epidermal growth factor receptor expression in a transgenic mouse glioma model. *Cancer Res.* **66**, 7429–7437 (2006).
59. Zhu, H. *et al.* Oncogenic EGFR signaling cooperates with loss of tumor suppressor gene functions in gliomagenesis. *Proc. Natl Acad. Sci. USA* **106**, 2712–2716 (2009).
60. Ding, H. *et al.* Astrocyte-specific expression of activated p21-ras results in malignant astrocytoma formation in a transgenic mouse model of human gliomas. *Cancer Res.* **61**, 3826–3836 (2001).
61. Holland, E. C. *et al.* Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. *Nature Genet.* **25**, 55–57 (2000).
62. Kwon, C. H. *et al.* Pten haploinsufficiency accelerates formation of high-grade astrocytomas. *Cancer Res.* **68**, 3286–3294 (2008).
63. Llaguno, S. A. *et al.* Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell* **15**, 45–56 (2009).
64. Burton, E. C. *et al.* Genetic aberrations defined by comparative genomic hybridization distinguish long-term from typical survivors of glioblastoma. *Cancer Res.* **62**, 6205–6210 (2002).
65. Hui, A. B., Lo, K. W., Yin, X. L., Poon, W. S. & Ng, H. K. Detection of multiple gene amplifications in glioblastoma multiforme using array-based comparative genomic hybridization. *Lab. Invest.* **81**, 717–723 (2001).
66. Maher, E. A. *et al.* Marked genomic differences characterize primary and secondary glioblastoma subtypes and identify two distinct molecular and clinical secondary glioblastoma entities. *Cancer Res.* **66**, 11502–11513 (2006).
67. Misra, A. *et al.* Array comparative genomic hybridization identifies genetic subgroups in grade 4 human astrocytoma. *Clin. Cancer Res.* **11**, 2907–2918 (2005).
68. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **455**, 1061–1068 (2008).
This study reports large-scale, integrated genomic analysis revealing the central importance of certain core signalling pathways in the pathogenesis of malignant glioma.
69. Nigro, J. M. *et al.* Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. *Cancer Res.* **65**, 1678–1686 (2005).
70. Nishizaki, T. *et al.* Clinical evidence of distinct subgroups of astrocytic tumors defined by comparative genomic hybridization. *Hum. Pathol.* **31**, 608–614 (2000).
71. Parsons, D. W. *et al.* An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**, 1807–1812 (2008).
This study reached many of the same conclusions as reference 68 but also identified pathogenic mutations in *IDH1*.
72. Bredel, M. *et al.* A network model of a cooperative genetic landscape in brain tumors. *JAMA* **302**, 261–275 (2009).
73. Yan, H. *et al.* IDH1 and IDH2 mutations in gliomas. *N. Engl. J. Med.* **360**, 765–773 (2009).
74. Hartmann, C. *et al.* 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.* **118**, 469–474 (2009).
75. Sanson, M. *et al.* Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J. Clin. Oncol.* **27**, 4150–4154 (2009).
76. Zhao, S. *et al.* Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1α. *Science* **324**, 261–265 (2009).
This study identifies a potential pathogenic mechanism for mutations in IDH proteins relating to a loss of enzymatic function.
77. Jensen, R. L. Brain tumor hypoxia: tumorigenesis, angiogenesis, imaging, pseudoprogression, and as a therapeutic target. *J. Neurooncol.* **92**, 317–335 (2009).
78. Dang, L. *et al.* Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462**, 739–744 (2009).
This study identifies the abnormal production of 2-HG as a potential pathogenic mechanism for IDH mutations in glioma.
79. Mardis, E. R. *et al.* Recurring mutations found by sequencing an acute myeloid leukemia genome. *N. Engl. J. Med.* **361**, 1058–1066 (2009).
80. Huse, J. T. *et al.* The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis *in vivo*. *Genes Dev.* **23**, 1327–1337 (2009).
81. Kim, H. *et al.* Integrative genome analysis reveals an oncomir/oncogene cluster regulating glioblastoma survivorship. *Proc. Natl Acad. Sci. USA* **107**, 2183–2188 (2010).
82. Li, Y. *et al.* MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res.* **69**, 7569–7576 (2009).
83. Novakova, J., Slaby, O., Vyzula, R. & Michalek, J. MicroRNA involvement in glioblastoma pathogenesis. *Biochem. Biophys. Res. Commun.* **386**, 1–5 (2009).
84. Godard, S. *et al.* Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. *Cancer Res.* **63**, 6613–6625 (2003).

85. Nutt, C. L. *et al.* Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res.* **63**, 1602–1607 (2003).
86. Li, A. *et al.* Unsupervised analysis of transcriptomic profiles reveals six glioma subtypes. *Cancer Res.* **69**, 2091–2099 (2009).
87. Liang, Y. *et al.* Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc. Natl Acad. Sci. USA* **102**, 5814–5819 (2005).
88. Phillips, H. S. *et al.* Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* **9**, 157–173 (2006).
This study uses transcriptional profiling to subdivide malignant gliomas into distinct categories that differ in their neurobiological characteristics and clinical prognoses.
89. Horvath, S. *et al.* Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proc. Natl Acad. Sci. USA* **103**, 17402–17407 (2006).
90. Brennan, C. *et al.* Glioblastoma subclasses can be defined by activity among signal transduction pathways and associated genomic alterations. *PLoS ONE* **4**, e7752 (2009).
This study uses an integrated genomics analysis of TCGA data coupled with proteomics in a second large tumour set to link glioma transcriptional subclasses with defined core signalling abnormalities.
91. Verhaak, R. G. W. *et al.* An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* **17**, 98–110 (2009).
This study uses TCGA data and an integrated genomics approach to associate glioma transcriptional subclasses with distinct abnormalities in specific genes.
92. Carro, M. S. *et al.* The transcriptional network for mesenchymal transformation of brain tumours. *Nature* **463**, 318–325 (2010).
93. Entz-Werle, N. *et al.* Medulloblastoma: what is the role of molecular genetics? *Expert Rev. Anticancer Ther.* **8**, 1169–1181 (2008).
94. Pietsch, T. *et al.* Medulloblastomas of the desmoplastic variant carry mutations of the human homologue of *Drosophila patched*. *Cancer Res.* **57**, 2085–2088 (1997).
95. Raffel, C. *et al.* Sporadic medulloblastomas contain PTCH mutations. *Cancer Res.* **57**, 842–845 (1997).
96. Reifenberger, J. *et al.* Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. *Cancer Res.* **58**, 1798–1803 (1998).
97. Taylor, M. D. *et al.* Mutations in SUFU predispose to medulloblastoma. *Nature Genet.* **31**, 306–310 (2002).
98. Hahn, H. *et al.* Mutations of the human homolog of *Drosophila patched* in the nevoid basal cell carcinoma syndrome. *Cell* **85**, 841–851 (1996).
99. Pomeroy, S. L. & Sturla, L. M. Molecular biology of medulloblastoma therapy. *Pediatr. Neurosurg.* **39**, 299–304 (2003).
100. Schuller, U. *et al.* Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell* **14**, 123–134 (2008).
101. Yang, Z. J. *et al.* Medulloblastoma can be initiated by deletion of *Patched* in lineage-restricted progenitors or stem cells. *Cancer Cell* **14**, 135–145 (2008).
102. Browd, S. R. *et al.* N-myc can substitute for insulin-like growth factor signaling in a mouse model of sonic hedgehog-induced medulloblastoma. *Cancer Res.* **66**, 2666–2672 (2006).
103. Goodrich, L. V., Milenkovic, L., Higgins, K. M. & Scott, M. P. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**, 1109–1113 (1997).
104. Hallahan, A. R. *et al.* The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. *Cancer Res.* **64**, 7794–7800 (2004).
105. Hatton, B. A. *et al.* The Smo/Smo model: hedgehog-induced medulloblastoma with 90% incidence and leptomeningeal spread. *Cancer Res.* **68**, 1768–1776 (2008).
106. Lee, Y. *et al.* Patched2 modulates tumorigenesis in patched1 heterozygous mice. *Cancer Res.* **66**, 6964–6971 (2006).
107. McCall, T. D., Pedone, C. A. & Fults, D. W. Apoptosis suppression by somatic cell transfer of Bcl-2 promotes Sonic hedgehog-dependent medulloblastoma formation in mice. *Cancer Res.* **67**, 5179–5185 (2007).
108. Pazzaglia, S. *et al.* High incidence of medulloblastoma following X-ray-irradiation of newborn Ptc1 heterozygous mice. *Oncogene* **21**, 7580–7584 (2002).
109. Pazzaglia, S. *et al.* Linking DNA damage to medulloblastoma tumorigenesis in patched heterozygous knockout mice. *Oncogene* **25**, 1165–1173 (2006).
110. Rao, G. *et al.* Sonic hedgehog and insulin-like growth factor signaling synergize to induce medulloblastoma formation from nestin-expressing neural progenitors in mice. *Oncogene* **23**, 6156–6162 (2004).
111. Rao, R. D. *et al.* Disruption of parallel and converging signaling pathways contributes to the synergistic antitumor effects of simultaneous mTOR and EGFR inhibition in GBM cells. *Neoplasia* **7**, 921–929 (2005).
112. Wetmore, C., Eberhart, D. E. & Curran, T. Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. *Cancer Res.* **61**, 513–516 (2001).
113. Zurawel, R. H., Allen, C., Wechsler-Reya, R., Scott, M. P. & Raffel, C. Evidence that haploinsufficiency of Ptch leads to medulloblastoma in mice. *Genes Chromosom. Cancer* **28**, 77–81 (2000).
114. Eberhart, C. G., Tihan, T. & Burger, P. C. Nuclear localization and mutation of beta-catenin in medulloblastomas. *J. Neuropathol. Exp. Neurol.* **59**, 333–337 (2000).
115. Rossi, A., Caracciolo, V., Russo, G., Reiss, K. & Giordano, A. Medulloblastoma: from molecular pathology to therapy. *Clin. Cancer Res.* **14**, 971–976 (2008).
116. Baeza, N., Masuoka, J., Kleihues, P. & Ohgaki, H. AXIN1 mutations but not deletions in cerebellar medulloblastomas. *Oncogene* **22**, 632–636 (2003).
117. Huang, H. *et al.* APC mutations in sporadic medulloblastomas. *Am. J. Pathol.* **156**, 433–437 (2000).
118. Koch, A. *et al.* Mutations of the Wnt antagonist AXIN2 (Conductin) result in TCF-dependent transcription in medulloblastomas. *Int. J. Cancer* **121**, 284–291 (2007).
119. Koch, A. *et al.* Somatic mutations of WNT/wingless signaling pathway components in primitive neuroectodermal tumors. *Int. J. Cancer* **93**, 445–449 (2001).
120. Zurawel, R. H., Chiappa, S. A., Allen, C. & Raffel, C. Sporadic medulloblastomas contain oncogenic β-catenin mutations. *Cancer Res.* **58**, 896–899 (1998).
121. Ellison, D. W. *et al.* β-catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee. *J. Clin. Oncol.* **23**, 7951–7957 (2005).
122. Momota, H., Shih, A. H., Edgar, M. A. & Holland, E. C. c-Myc and β-catenin cooperate with loss of p53 to generate multiple members of the primitive neuroectodermal tumor family in mice. *Oncogene* **27**, 4392–4401 (2008).
123. Biegel, J. A. *et al.* Prognostic significance of chromosome 17p deletions in childhood primitive neuroectodermal tumors (medulloblastomas) of the central nervous system. *Clin. Cancer Res.* **3**, 473–478 (1997).
124. Bigner, S. H., Mark, J., Friedman, H. S., Biegel, J. A. & Bigner, D. D. Structural chromosomal abnormalities in human medulloblastoma. *Cancer Genet. Cytogenet.* **30**, 91–101 (1988).
125. Cogen, P. H. & McDonald, J. D. Tumor suppressor genes and medulloblastoma. *J. Neurooncol.* **29**, 103–112 (1996).
126. Lamont, J. M., McManamy, C. S., Pearson, A. D., Clifford, S. C. & Ellison, D. W. Combined histopathological and molecular cytogenetic stratification of medulloblastoma patients. *Clin. Cancer Res.* **10**, 5482–5493 (2004).
127. Nicholson, J., Wickramasinghe, C., Ross, F., Crolla, J. & Ellison, D. Imbalances of chromosome 17 in medulloblastomas determined by comparative genomic hybridisation and fluorescence *in situ* hybridisation. *Mol. Pathol.* **53**, 313–319 (2000).
128. Steichen-Gersdorf, E., Baumgartner, M., Kreczy, A., Maier, H. & Fink, F. M. Deletion mapping on chromosome 17p in medulloblastoma. *Br. J. Cancer* **76**, 1284–1287 (1997).
129. Vagner-Capodano, A. M. *et al.* Detection of i(17q) chromosome by fluorescent *in situ* hybridization (FISH) with interphase nuclei in medulloblastoma. *Cancer Genet. Cytogenet.* **78**, 1–6 (1994).
130. Tomlinson, F. H. *et al.* Aggressive medulloblastoma with high-level N-myc amplification. *Mayo Clin. Proc.* **69**, 359–365 (1994).
131. Lee, Y. *et al.* Loss of suppressor-of-fused function promotes tumorigenesis. *Oncogene* **26**, 6442–6447 (2007).
132. Uziel, T. *et al.* The tumor suppressors Ink4c and p53 collaborate independently with Patched to suppress medulloblastoma formation. *Genes Dev.* **19**, 2656–2667 (2005).
133. Aldosari, N. *et al.* MYCC and MYCN oncogene amplification in medulloblastoma. A fluorescence *in situ* hybridization study on paraffin sections from the Children's Oncology Group. *Arch. Pathol. Lab. Med.* **126**, 540–544 (2002).
134. Mendell, J. T. miRiad roles for the miR-17-92 cluster in development and disease. *Cell* **133**, 217–222 (2008).
135. Rao, G., Pedone, C. A., Coffin, C. M., Holland, E. C. & Fults, D. W. c-Myc enhances sonic hedgehog-induced medulloblastoma formation from nestin-expressing neural progenitors in mice. *Neoplasia* **5**, 198–204 (2003).
136. MacDonald, T. J. *et al.* Expression profiling of medulloblastoma: PDGFRA and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nature Genet.* **29**, 143–152 (2001).
137. Gilbertson, R. *et al.* Clinical and molecular stratification of disease risk in medulloblastoma. *Br. J. Cancer* **85**, 705–712 (2001).
138. Calabrese, C., Frank, A., Maclean, K. & Gilbertson, R. Medulloblastoma sensitivity to 17-allylamino-17-demethoxygeldanamycin requires MEK/ERKM. *J. Biol. Chem.* **278**, 24951–24959 (2003).
139. Hernan, R. *et al.* ERBB2 up-regulates S100A4 and several other prometastatic genes in medulloblastoma. *Cancer Res.* **63**, 140–148 (2003).
140. Gilbertson, R. *et al.* Novel ERBB4 juxtamembrane splice variants are frequently expressed in childhood medulloblastoma. *Genes Chromosom. Cancer* **31**, 288–294 (2001).
141. Gajjar, A. *et al.* Clinical, histopathologic, and molecular markers of prognosis: toward a new disease risk stratification system for medulloblastoma. *J. Clin. Oncol.* **22**, 984–993 (2004).
142. Gilbertson, R. J., Pearson, A. D., Perry, R. H., Jaros, E. & Kelly, P. J. Prognostic significance of the c-erbB-2 oncogene product in childhood medulloblastoma. *Br. J. Cancer* **71**, 473–477 (1995).
143. Gilbertson, R. J., Perry, R. H., Kelly, P. J., Pearson, A. D. & Lunec, J. Prognostic significance of HER2 and HER4 coexpression in childhood medulloblastoma. *Cancer Res.* **57**, 3272–3280 (1997).
144. Pomeroy, S. L. *et al.* Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* **415**, 436–442 (2002).
145. Kool, M. *et al.* Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PLoS ONE* **3**, e3088 (2008).
146. Thompson, M. C. *et al.* Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J. Clin. Oncol.* **24**, 1924–1931 (2006).
References 145 and 146 establish transcriptional subclasses of medulloblastoma and links each to specific genomic and/or transcriptional characteristics.
147. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **414**, 105–111 (2001).
148. Galli, R. *et al.* Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res.* **64**, 7011–7021 (2004).
149. Singh, S. K. *et al.* Identification of a cancer stem cell in human brain tumors. *Cancer Res.* **63**, 5821–5828 (2003).
150. Singh, S. K. *et al.* Identification of human brain tumour initiating cells. *Nature* **432**, 396–401 (2004).
151. Bao, S. *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**, 756–760 (2006).
152. Blazek, E. R., Foutch, J. L. & Maki, G. Daoy medulloblastoma cells that express CD133 are radioresistant relative to CD133- cells, and the CD133+ sector is enlarged by hypoxia. *Int. J. Radiat. Oncol. Biol. Phys.* **67**, 1–5 (2007).

153. Ogden, A. T. *et al.* Identification of A2B5 + CD133- tumor-initiating cells in adult human gliomas. *Neurosurgery* **62**, 505–515 (2008).
154. Wang, J. *et al.* CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. *Int. J. Cancer* **122**, 761–768 (2008).
155. Cheng, J. X., Liu, B. L. & Zhang, X. How powerful is CD133 as a cancer stem cell marker in brain tumors? *Cancer Treat Rev.* **35**, 403–408 (2009).
156. Gilbertson, R. J. & Rich, J. N. Making a tumour's bed: glioblastoma stem cells and the vascular niche. *Nature Rev. Cancer* **7**, 733–736 (2007).
157. Altman, J. & Das, G. D. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124**, 319–335 (1965).
158. Lois, C. & Alvarez-Buylla, A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons an glia. *Proc. Natl Acad. Sci. USA* **90**, 2074–2077 (1993).
159. Riquelme, P. A., Drapeau, E. & Doetsch, F. Brain micro-ecologies: neural stem cell niches in the adult mammalian brain. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **363**, 123–137 (2008).
160. Calabrese, C. *et al.* A perivascular niche for brain tumor stem cells. *Cancer Cell* **11**, 69–82 (2007).
This study demonstrates that brain tumour cells with stem cell-like characteristics localize in a perivascular distribution and require microvasculature for their tumour-initiating capacity.
161. Bao, S. *et al.* Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res.* **66**, 7843–7848 (2006).
162. Li, Z., Wang, H., Eyler, C. E., Hjelmeland, A. B. & Rich, J. N. Turning cancer stem cells inside out: an exploration of glioma stem cell signaling pathways. *J. Biol. Chem.* **284**, 16705–16709 (2009).
163. Becher, O. J. *et al.* Gli activity correlates with tumor grade in platelet-derived growth factor-induced gliomas. *Cancer Res.* **68**, 2241–2249 (2008).
164. Li, Z. *et al.* Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell* **15**, 501–513 (2009).
165. Covello, K. L. *et al.* HIF-2α regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev.* **20**, 557–570 (2006).
166. Heddleston, J. M., Li, Z., McLendon, R. E., Hjelmeland, A. B. & Rich, J. N. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle* **8**, 3274–3284 (2009).
167. Wang, Z., Li, Y., Banerjee, S. & Sarkar, F. H. Emerging role of Notch in stem cells and cancer. *Cancer Lett.* **279**, 8–12 (2009).
168. Shih, A. H. & Holland, E. C. Notch signaling enhances nestin expression in gliomas. *Neoplasia* **8**, 1072–1082 (2006).
169. Bhattacharya, S., Das, A., Mallya, K. & Ahmad, I. Maintenance of retinal stem cells by Abcg2 is regulated by notch signaling. *J. Cell Sci.* **120**, 2652–2662 (2007).
170. Fan, X. *et al.* Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res.* **66**, 7445–7452 (2006).
171. Bleau, A. M. *et al.* PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell* **4**, 226–235 (2009).
This study demonstrates that PI3K–AKT–mTOR signalling regulates the expression of stem-like characteristics in glioma cells.
172. Charles, N. *et al.* Nitric oxide drives notch signaling and stem cell-like character via PKG in the perivascular niche of PDGF gliomas. *Cell Stem Cell.* **6**, 141–152 (2010).
173. Hambardzumyan, D. *et al.* PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma *in vivo*. *Genes Dev.* **22**, 436–448 (2008).
174. Hambardzumyan, D., Amankulor, N. M., Helmy, K. Y., Becher, O. J. & Holland, E. C. Modeling Adult Gliomas Using RCAS/t-va Technology. *Transl. Oncol.* **2**, 89–95 (2009).
175. Lindberg, N., Kastemar, M., Olofsson, T., Smits, A. & Uhrbom, L. Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. *Oncogene* **28**, 2266–2275 (2009).
176. Assanah, M. *et al.* Glial progenitors in adult white matter are driven to form malignant gliomas by platelet-derived growth factor-expressing retroviruses. *J. Neurosci.* **26**, 6781–6790 (2006).
177. Bleau, A. M., Huse, J. T. & Holland, E. C. The ABCG2 resistance network of glioblastoma. *Cell Cycle* **8**, 2936–2944 (2009).
178. Wick, W., Platten, M. & Weller, M. New (alternative) temozolomide regimens for the treatment of glioma. *Neuro Oncol.* **11**, 69–79 (2009).
179. Mellinghoff, I. K. *et al.* Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N. Engl. J. Med.* **353**, 2012–2024 (2005).
This trial of EGFR kinase inhibitors suggests that patients with both the EGFR vIII mutation and intact PTEN may be a molecularly defined subgroup that is more likely to respond to targeted kinase inhibition.
180. Yauch, R. L. *et al. Smoothened* mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. *Science* **326**, 572–574 (2009).
181. Huse, J. T. & Holland, E. C. Genetically engineered mouse models of brain cancer and the promise of preclinical testing. *Brain Pathol.* **19**, 132–143 (2009).
182. Charest, A. *et al.* ROS fusion tyrosine kinase activates a SH2 domain-containing phospatase-2/ phosphatidylinositol 3-kinase/mammalian target of rapamycin signaling axis to form glioblastoma in mice. *Cancer Res.* **66**, 7473–7481 (2006).
183. Lassman, A. B. *et al.* Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from North American Brain Tumor Consortium Trials 01–03 and 00–01. *Clin. Cancer Res.* **11**, 7841–7850 (2005).
184. Liebner, S. *et al.* Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. *Acta Neuropathol.* **100**, 323–331 (2000).
185. de Vries, N. A., Beijnen, J. H., Boogerd, W. & van Tellingen, O. Blood-brain barrier and chemotherapeutic treatment of brain tumors. *Expert Rev. Neurother* **6**, 1199–1209 (2006).
186. Laquintana, V. *et al.* New strategies to deliver anticancer drugs to brain tumors. *Expert Opin. Drug Deliv.* **6**, 1017–1032 (2009).

Acknowledgements
The authors would like to thank M. Rosenblum for providing representative tumour micrographs.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
ASPM | *AXIN1* | *AXIN2* | *CCL5* | *CCND1* | *CDK4* | *CDK6* | *CDKN2A* | *CDKN2B* | *EGFR* | *ERBB4* | *FABP7* | *IDH1* | *IDH2* | *MAP2K5* | *MDM2* | *MDM4* | *MGMT* | *miR-26a-2* | *MYC* | *MYCN* | *NF1* | *PTEN* | *RB1* | *REST* | *RTEL1* | *S100A4* | *TERT* | *TP53*
miRBase: http://www.mirbase.org/
miR-137 | miR-21 | miR-221 | miR-222 | miR-34a
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/
erlotinib | temozolomide
OMIM: http://www.ncbi.nlm.nih.gov/omim
Gorlin's syndrome | Li–Fraumeni syndrome
Pathway Interaction Database: http://pid.nci.nih.gov/
Rb
UniProtKB: http://www.uniprot.org
β-catenin | ABCG2 | APC | BCL-2 | BMI1 | CD133 | C/EBPβ | ERBB2 | GFAP | HIF2α | IGF1R | IGF2 | KRAS | MAP3K2 | MET | nestin | OCT4 | OLIG2 | PDGFB | PDGFRα | PTCH1 | SHH | SMO | STAT3 | SUFU

FURTHER INFORMATION
Eric C. Holland's homepage:
http://www.mskcc.org/mskcc/html/10561.cfm
Jason T. Huse's homepage:
http://www.mskcc.org/mskcc/html/94171.cfm

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