Genetics and epigenetics of gliomas

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Summary

Gliomas are the most common primary intrinsic tumours. Their classification is based on phenotypic resemblance to normal glial cells (astrocytomas, oligodendrogliomas, mixed oligoastrocytomas) and pathological grading. Whereas this system is clinically relevant and has been the basis for our understanding of gliomas, systematic use of next-generation sequencing has transformed our knowledge of their pathogenesis and has uncovered genetic changes in an unanticipated number of genes and regulatory elements. In the past few years, in-depth analysis of low-grade astrocytomas and glioblastomas in both paediatric and adult populations has clarified our molecular understanding of these diseases, with distinct molecular events occurring in different age groups. In oligodendrogliomas, recent studies have highlighted mutations in candidate tumour suppressor genes located on 1p/19q, chromosome arms frequently deleted in this tumour. In this review, we discuss recent discoveries in the genetics of adult and paediatric gliomas, and highlight how some of the founding genetic mutations reshape the cancer epigenome. These studies provide an in-depth view of the molecular routes leading to brain tumour development and will be key for refining classification systems and improving clinical care.

Key words: adult gliomas; paediatric gliomas; genetics; epigenetics; novel fusion genes; intra-tumoural heterogeneity

Introduction

Despite more than a century of biomedical research, only little progress has been made in the clinical management of diffuse gliomas. Low grade tumour progression to high grade malignancies remains the rule in adult patients, while the most aggressive form, grade IV astrocytoma (glioblastoma), is incurable with the current standard of care therapy [1]. Equally unsuccessful have been molecular therapies targeting angiogenesis [2, 3]. These frustrating results demand a better understanding of the underlying biology, with at least two roads warranting in-depth exploration. *First*, the emergence of molecular therapies targeting cancer-specific genetic events, as evidenced by recent successes in targeting *BRAF* mutant melanoma [4], *EGFR*

mutant lung cancer [5], *HER2* amplified breast cancer [6], *BCR-ABL* translocation in chronic myeloid leukaemias [7] and *EML4–ALK* translocation in lung cancer [8], provide a proof-of principle that histopathologic diagnosis must be complemented with genetic information to exploit tumour dependencies. To that end, recent international efforts in cancer genome sequencing, reviewed here, transformed our understanding of genetic changes in gliomas and should provide a basis for meaningful clinical exploitation. *Second*, the importance of epigenetic modifications in cancer fitness and the rapid emergence of epigenetic drugs offer unprecedented opportunities for novel targeted approaches. In this review, we focus on epigenetic alterations shaped by genetic events relevant to gliomas, such as mutations affecting *IDH1/2*, the histone methyltransferase *SETD2*, as well as histone H3.3 and its chaperones *ATRX/DAXX*.

Recent advances in the genetics of primary glioblastoma, WHO grade IV

Our modelling of the molecular routes leading to primary glioblastoma has been refined by systematic usage of whole-exome sequencing technologies. Adult glioblastoma is characterised by a complex genetic landscape [9]. One of the critical components of these genetic aberrations is amplification and intragenic re-arrangement of receptor tyrosine kinases (RTK), affecting *EGFR* (~50%), *PDGFRA* (~15%) or *MET* (~5%). The most prevalent re-arrangements affect the extracellular domains of *EGFR* (*EGFRvIII* with in frame deletion of exons 2–7) or *PDGFRA* (*PDGFRA8–9* with in frame deletion of exons 8–9). While these genetic events have been known for many years and transduce aberrant signalling, their inhibition has yet to show significant clinical efficacy. This is most likely rooted in the inter- and intra-tumoural heterogeneity observed in glioblastoma. Indeed, recent studies demonstrated a mosaic amplification of multiple RTK in the same tumour sample [10]. This is further supported by recent single cell RNA sequencing efforts that highlighted heterogeneity in expression of different transcriptional programmes and surface receptors in primary glioblastoma cells [11]. In addition, alternate routes to transduce aberrant RTK signalling have recently been discovered: two studies showed that ~3% of glioblastoma, without *EGFR, PDGFRA* or *MET*

amplification, actually harbour an oncogenic chromosomal translocation fusing the tyrosine kinase coding domains of *FGFR1/3* (fibroblast growth factor receptor 1 or 3) to, respectively, the coding domains of *TACC1/3* (transforming acidic coiled-coil 1 or 3) [12, 13]. A study by Frattini et al. also identified, in rare cases, the novel fusion proteins *EGFR-SEPT14* and *EGFR-PSPH* as mediators of RTK signalling in glioblastoma [14, 15]. Clearly, the complexity of RTK aberrations combined to intra-tumoural heterogenity poses significant challenges for clinical therapies.

Recent advances also highlighted how genetic events affect glioma stem cells self-renewal. In the same study, Frattini et al. identified mutations in *LZTR1*, a gene normally expressed in brain and functioning as adaptor of the cullin3 ubiquitin ligase complex. Through advanced bioinformatics and functional experiments, the study showed that *LZTR1* inactivation promotes glioma stem cells self-renewal and growth. Similarly Morris et al. uncovered the genetic basis for aberrant Wnt signalling in a subset of glioblastoma. The study found that in addition to previously described cases driven by *PLAGL2* amplification/gain, ~20% of glioblastoma harboured recurrent somatic mutations in *FAT1* [16]. The normal function of FAT1 is to bind beta–catenin and antagonise its nuclear localisation. Its mutational inactivation thus promotes Wnt signalling and tumourigenesis. These studies shed light on genetic bases for the reactivation of neurodevelopmental pathways that play a key role in glioblastoma cell properties [17, 18]. Glioblastoma cells self-renewal also requires telomere maintenance and Killela et al. have identified *TERT* gene promoter mutations in ~80% of primary glioblastoma, leading to increased telomerase expression. Of note, these mutations are mutually exclusive with *ATRX* mutations observed in adult secondary glioblastoma or in paediatric populations, and that are correlated with the ALT (alternative lengthening of telomeres) phenotype (see below). Thus it seems that either increased telomerase expression or ALT mechanisms ensure telomeres maintenance in primary or secondary glioblastoma respectively.
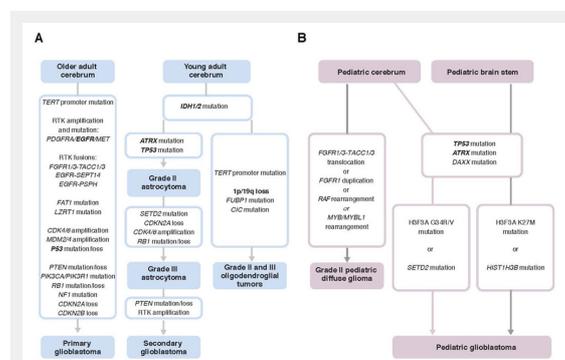
Recent advances in the genetics of adult astrocytic tumours, WHO grade II and III, and secondary glioblastoma, WHO grade IV

Frequent involvement of the tumour suppressor *TP53* in astrocytomas has been known for decades while mutations in *IDH1/2* have been more recently identified [9, 19]. Detection of these mutations, combined with assessment of chromosome 7 copy number, thus represents a sensitive diagnostic panel for low-grade glioma in challenging biopsies [20, 21]. Closely correlated with *IDH1/2* and *TP53* mutations, three studies published in 2012 identified missense mutations in the chromatin regulator *ATRX* (thalassemia/mental retardation syndrome X-linked) in the majority (~70%) of grade II and III astrocytoma [22–24]. *ATRX* mutations were mutually exclusive with 1p/19q co-deletion, the molecular hallmark of oligodendroglioma, and were identified at a lower frequency in mixed oligo-astrocytic tumours. *ATRX* mutations thus could represent a novel candidate marker of astrocytic lineage in diffuse gliomas

[22–24]. As the frequency of *ATRX* mutations is similar in grade II and III astrocytoma, they likely represent early events in the development of the disease, and could provide a genetic mechanism responsible for telomere maintenance. Indeed, ATRX is a SWI/SNF helicase playing a key role in assisting in the deposition of histone variant H3.3 in chromatin, in particular in telomeric and sub-telomeric regions and its mutations are correlated with the ALT phenotype [25]. Involvement of chromatin regulators in gliomagenesis is also illustrated by the identification of missense and truncating mutations in *SETD2*, a histone H3 lysine 36 (H3K36) tri-methyltransferase, observed in 8% of adult high-grade gliomas (WHO grades III and IV). The absence of detectable mutations in *SETD2* in low-grade gliomas suggests a role in glioma progression (see also below) [26]. The role of *BRAF* genetic alterations in diffuse infiltrative gliomas is limited, although the rare diffuse grade II gliomas with *BRAF* V600E mutation may have a better prognosis [27]. *BRAF* V600E hotspot mutations are more commonly found in pleomorphic xanthoastrocytoma, gangliogliomas and extra-cerebellar pilocytic astrocytoma. They can be readily detected by immunohistochemistry or molecular testing, providing an ancillary approach to the diagnosis of such tumours in difficult biopsies.

Recent advances in the genetics of oligodendroglial tumours, WHO grade II and III

Oligodendrogliomas have attracted substantial interest because of their remarkable sensitivity to treatment with alkylating agents. Sensitivity to DNA damaging agents cor-

Figure 1

Genomic alterations underlying the development of gliomas. (A) Gliomagenesis in adults. Secondary glioblastomas (10% of cases) affect younger individuals and develop over a long period of time from lower-grade astrocytomas. Almost all cases are characterised by a mutation in *IDH1/2*, and a majority has mutations in *ATRX*. Primary glioblastomas (90% of cases) occur in older individuals and do not have a known precursor lesion. Almost all cases are wild-type for *IDH1/2* and *ATRX*. Oligodendrogliomas are characterised by mutations in *IDH1/2*, wild-type *ATRX*, and loss of 1p/19q. (B) Paediatric gliomagenesis is molecularly distinct. Low-grade tumours are characterised by a single genetic event affecting *FGFR, MYB/MYBL1* or *RAF*. Paediatric glioblastoma hallmark mutations target *H3F3A* at different residues, depending on tumour location. See text for gene abbreviations. In bold are highlighted proteins routinely tested to guide diagnosis. In addition to this, *MGMT* promoter methylation is frequently tested for therapeutic decisions (see text). Larger panels of genetic mutations are screened in clinical trials.

relates with signature genetic aberrations, in particular with co-deletion of the whole arms of chromosomes 1p and 19q. Although somatic mutation of the *IDH1* or *IDH2* genes may be the earliest and candidate founding event in this cancer, Killela et al. identified mutation in *TERT* promoter in ~80% of oligodendrogliomas, as observed in primary glioblastoma. These mutations lead to increased telomerase expression, as has previously been described in melanoma, thus possibly contributing to early disease pathogenesis. In addition, the frequent co-deletion of 1p/19q suggests that these chromosomal arms may contain tumour suppressor genes adding to disease pathogenesis. In the recent past, three studies described *CIC* (homologue to *Drosophila* gene *capicua*) on chromosome 19q as a candidate tumour suppressor gene, somatically mutated in 70% of oligodendrogliomas with 1p/19q co-deletion [22, 28, 29]. In the same studies, mutations of *FUBP1* (far-upstream element (FUSE) binding protein) on chromosome 1p have been observed in some tumours, although at a lower frequency than *CIC* mutations (fig. 1). Finally, rare cases of *BRAF* gain, by duplication or fusion to *KIAA1549,* classically observed in pilocytic astrocytoma, have been observed in diffuse low grade gliomas, more commonly in oligodendrogliomas [30, 31].

Recent advances in the genetics of paediatric diffuse gliomas, WHO grade II

Adult and paediatric diffuse gliomas have very different underlying genetic events and clinical behaviour, despite similar histopathological aspects. For example, *IDH1* or *IDH2* mutations, found in the majority of grade II and III diffuse gliomas in adults are very rare in children. Paediatric gliomas also less frequently progress toward higher-grade than adult tumours. While these differences have been known for many years, it was only in 2013, using whole-genome sequencing, that signature genetic events were identified in paediatric populations [32]. One of the major findings was that most paediatric tumours only harbour a single somatic event affecting protein coding sequences, suggesting that few oncogenic hits are required for transformation. The most common genetic event identified is a duplication of the tyrosine kinase domain of *FGFR1,* present in approximately one quarter of cases (fig. 1). This duplication results in FGFR1 autophosphorylation and activation of the downstream MAPK/ERK and PI3K pathways. *FGFR* was also subject to other rearrangements, albeit at much lower frequency. These include *FGFR1–TACC1* and *FGFR3–TACC3* translocations, as reported in rare cases of adult glioblastoma (see above). A total of 25% of cerebral gliomas, including a few cases of angiocentric gliomas, harboured recurrent genetic rearrangements and amplifications affecting *MYB* or *MYBL1*. These cases also showed higher MYB protein expression levels detectable by immunohistochemistry. Several abnormalities affecting *RAF* were also identified, including different fusions (*FXFR1–BRAF, QKI-RAF1, BRAF-MACF1, FAM131B-BRAF*). Finally recurrent genetic events affecting histones and chromatin regulators were present in a subset of tumours, affecting *ATRX, EP300, H3F3A, CHD2*

or *WHSC1*. The functionality of some of these genes is detailed below.

Recent advances in the genetics of paediatric high-grade gliomas, WHO grade III-IV

Paediatric high-grade gliomas also have genetic underpinnings distinct from low-grade lesions and from their adult counterparts. Using whole-genome or whole-exome sequencing, two independent studies [33, 34] have discovered recurrent mutations in the *H3F3A* gene, encoding histone H3.3, in approximately 30% of cases of paediatric glioblastoma. These mutations affect either lysine 27 (K27M), or less commonly glycine 34 (G34R/V) of histone H3.3. Notably, in a minority (~20%) of cases of diffuse intrinsic pontine gliomas (DIPGs), an anatomical variant of high-grade glioma, K27M mutations were also identified in the canonical H3.1 (*HIST1H3B*). All histone alterations identified are heterozygous, suggesting a gain-of-function mutation. Genetic mutations affecting many different residues were also discovered in *ATRX* and *DAXX* (death domain associated protein), chaperones which mediate deposition of H3.3 in telomeric regions. Overall, in cohorts of hundreds of cases, approximately 40% of paediatric high-grade gliomas had mutations either in *H3F3A*, *ATRX* or *DAXX*. Most mutations in *ATRX* correlate with loss of its expression and with an ALT phenotype. Subsequent studies highlighted that distinct *H3F3A* mutations define epigenetic and clinicopathological entities and are mutually exclusive with *IDH1* mutations [35]. In particular, K27M mutations exclusively affect the brain stem or thalamus [36], while G34R/V mutations are found in hemispheric cases. The mutations in *H3F3A* and *HIST1H3B* discovered in these studies are located in the histone tail that is subject to extensive post-translational modification (see below).

Genetics events that reshape the epigenome of gliomas

Links between chromatin and cancer were originally evidenced by oncogenic fusions containing chromatin modifying proteins and have been recently strengthened by whole-exome and whole-genome sequencing studies that have identified mutations in histone modifying enzymes and chromatin remodelers. In 2012, these links were further reinforced by the discovery of somatic mutations directly affecting modifiable residues in histone H3 proteins themselves in paediatric glioblastoma. A number of studies have together provided growing insight into the interplay between histone modifications and tumourigenesis. The human histone H3 family consists of H3.1 and H3.2 that both represent "canonical" H3 as well as the H3.3 variant, the testes-specific variants H3t and H3.5 and finally the centromeric variant CenH3 (reviewed in [37]). In humans, there are two genes, *H3F3A* and *H3F3B* that produce identical H3.3 proteins, differing only by their mRNA untranslated regions. H3.3 is preferentially deposited into genomic regions displaying active or poised transcriptional status as well as in telomeric and pericentromeric regions. Unlike H3.1 and H3.2, H3.3 deposition is replication in-

dependent. It is incorporated into chromatin by two major histone chaperones complexes: ATRX/DAXX (see above) that mediates deposition in pericentromeric and telomeric regions and HIRA (histone regulator A) that incorporates H3.3 in active regions of the genome. The reported mutations in *ATRX/DAXX* in paediatric glioblastoma, adult low grade gliomas [22] and in neuroblastoma [38], suggests that impaired deposition of H3.3 in pericentromeric and telomeric regions would represent a critical axis leading to gliomas. *H3F3A* alterations are also likely to interfere with transcriptional regulation. Indeed, Lewis et al. identified that introduction of H3K27M leads to global decrease in H3K27 methylation and global increase in H3K27 acetylation, a mark associated with activated regulatory elements [39]. Introduction of K27M peptide allosterically inhibits the methytransferase activity of EZH2 (enhancer of zeste homologue), a sub-unit of PRC2 (polycomb repressive complex 2) complex, thus representing a dominant-negative mechanism to alter gene expression [39]. This loss of H3K27 trimethylation in cancer cells may be visualised by immunohistochemistry and may find use in diagnostic applications [40]. Therefore H3.3 K27M mutation alters gene regulation by affecting global levels of H3K27me3 through inhibition of PRC2 in regions where the mutant histone is deposited. Deregulation of the PRC2 complex has been involved as an underlying mechanism in many malignancies, including adult gliomas [41, 42]. Both gain and loss-of-function mutations of the polycomb complex have been shown to contribute to transformation in a variety of cancers. However, additional genetic events are likely to be required in paediatric gliomas, as the introduction of H3.3K27M into p53–null, nestin-expressing progenitors in the neonatal mouse brainstem was insufficient to generate gliomas [39]. The role of mutations affecting G34 is less well established. Introduction of the H3G34R/V mutant results in decrease H3K36me3 in the same and nearby nucleosomes, through inhibition of the H3K36 tri-methyltransferase SETD2. Notably, Fontebasso et al. identified mutations in *SETD2* in up to 15% of paediatric high-grade gliomas, supporting the notion that a disruption of H3K36 through either *SETD2* or *H3F3A* mutations plays a key role in gliomagenesis [26]. A suggested mechanism could be that H3G34 mutations cause profound upregulation of MYCN, potentially driving glioblastoma when expressed in the correct developmental context [43]. Importantly, nearly all G34 mutant tumours also bear alterations in *ATRX/DAXX*, and display the ALT phenotype. The association between *TP53/H3F3A* mutations and *TP53/IDH1* mutations in paediatric and adult gliomas, respectively, suggests at least a partial functional overlap between *IDH1* and *H3F3A* genetic alterations.

Up to 80%–90% of adult grade II/III gliomas and secondary glioblastomas harbour a genetic mutation in *IDH1* or *IDH2* affecting Arg residues 132 or 172 respectively [19, 44]. Glioblastomas bearing the mutations have significantly better prognosis than IDH wild-type tumours [45]. These *IDH* mutations are sufficient to establish the CpG island methylator phenotype (CIMP), a distinct subclass of tumours in a number of human malignancies, including in glioblastoma [46]. CIMP is associated with extensive and coordinated hypermethylation at specific genomic loci, one of which is the O^{6-}methylguanine-DNA-methyltransferase (*MGMT*) promoter (see below). The underlying mechanism relies on the fact that mutant IDH enzymes are not catalytically inactive, but rather have altered activity and produce the oncometabolite (R)-2–hydroxyglutarate [(R)-2HG], normally only present in a very low quantity of cells [44, 47]. Several mechanisms of (R)-2HG mediated transformation have been suggested, mostly relying on the structural similarity between (R)-2HG and 2OG. The dominant model is that (R)-2HG transforms cells by competitively inhibiting tumour suppressor enzymes that depend on 2OG. While there are many candidate enzymes that could be the target of (R)-2HG, a lot of the attention has been drawn towards *TET2*, in the context of myeloid tumours [44]. TET enzymes are thought to be epigenetic regulators of gene expression by mediating demethylation of DNA. Specifically, TET family members hydroxylate 5–methylcytosine (5mC) to generate 5–hydroxymethylcytosine (5hmC). It would thus be expected that loss or inhibition of TET2 activity would result in DNA hypermethylayion. However, the studies in AML have been variable and contradictory, reporting both hyper- and hypo-methylation in TET2 mutant cases [48, 49]. A unifying hypothesis could be that TET2 mutations alter the methylation status of DNA in specific genomic sites while having variable effects on the global level of DNA methylation [44]. Very strong evidence for a link between *IDH* and *TET2* mutations derives from the observation that they appear to be mutually exclusive in acute myeloid leukaemia, suggesting they act on the same leukomogenic pathway [48]. While TET family member mutations have been identified in myeloid tumours, they are not present in gliomas. However, rare cases of *TET2* promoter methylation have been reported in IDH1 WT grade II and III gliomas [50]. This would suggest that TET2 inhibition by *IDH1* mutation or its down-regulation by promoter methylation play similar roles in gliomagenesis. Other enzyme candidate targets of (R)-2HG include JmJC histone demethylases that regulate gene expression by modulating methylation of histone tail residues and appear to function as tumour suppressors [44]. Affecting their activity through *IDH* mutations would suggest a functional overlap with the mutually exclusive *H3F3A* mutations observed in paediatric populations.

As mentioned above, *MGMT* promoter methylation is part of the CIMP phenotype associated with *IDH1/2* mutations. It is also observed in ~40% of primary (*IDH1/2* wild-type) glioblastoma, but the underlying mechanisms of its silencing are less clear [51]. In clinical settings, *MGMT* promoter methylation is predictive of clinical response to alkylating agents, most notably in the elderly (65–70 years), *MGMT* promoter methylated patients benefitting the most from temozolomide therapy [52]. Importantly, MGMT silencing is also thought to increase genetic instability, by facilitating G>A transition mutations. This hypothesis is supported by the more frequent mutation in *TP53* and *PTEN* observed in MGMT-methylated glioblastoma than in MGMT-nonmethylated cases [53]. It is also supported by the frequent induction of a "hypermutator phenotype" due to mutational inactivation of mismatch repair genes in temozolomide treated MGMT-methylated cases [53]. While

MGMT somatic mutations have been observed in certain malignancies, they are exceptional in gliomas [51].

Conclusion

The studies reviewed here have certainly furthered our understanding of brain tumour development. In addition to the wealth of genomic alterations previously identified in gliomas (fig. 1), these recent genetic and epigenetic studies further underscore that gliomas are very complex ecosystems with significant inter- and intra-tumoural genetic diversity. The prominent involvement of histone genes and chromatin regulators mutations, and their impact on the epigenome, emphasises the critical roles of epigenetic mechanisms in cancer development [54]. While a comprehensive catalogue of genetic and epigenetic events in cancer will require further efforts, we have already gained significant information about major molecular events and their significance. These discoveries challenge our grading systems, with certain mutations such as *IDH1/2* being powerful predictors of clinical outcome, so that we have begun to integrate them into our classifications schemes [55]. More systematic genomics and epigenomics approaches will likely become part of future clinical assessment. The novel findings provide a rational basis for renewed attempts at improving clinical care of brain tumour patients.

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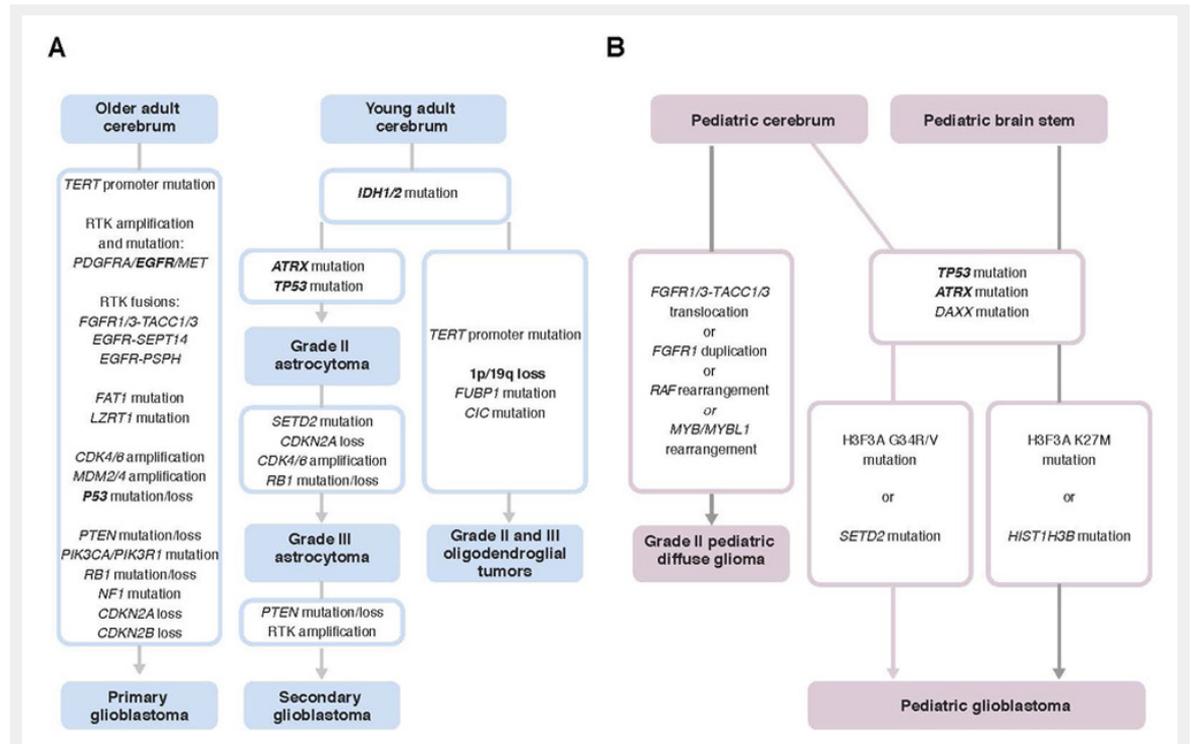
Figures (large format)

Figure 1

Genomic alterations underlying the development of gliomas. (A) Gliomagenesis in adults. Secondary glioblastomas (10% of cases) affect younger individuals and develop over a long period of time from lower-grade astrocytomas. Almost all cases are characterised by a mutation in *IDH1/2*, and a majority has mutations in *ATRX*. Primary glioblastomas (90% of cases) occur in older individuals and do not have a known precursor lesion. Almost all cases are wild-type for *IDH1/2* and *ATRX*. Oligodendrogliomas are characterised by mutations in *IDH1/2*, wild-type *ATRX*, and loss of 1p/19q. (B) Paediatric gliomagenesis is molecularly distinct. Low-grade tumours are characterised by a single genetic event affecting *FGFR, MYB/MYBL1* or *RAF*. Paediatric glioblastoma hallmark mutations target *H3F3A* at different residues, depending on tumour location. See text for gene abbreviations. In bold are highlighted proteins routinely tested to guide diagnosis. In addition to this, *MGMT* promoter methylation is frequently tested for therapeutic decisions (see text). Larger panels of genetic mutations are screened in clinical trials.