Genetic alterations of adult and paediatric astrocytic tumours

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GENETIC ALTERATIONS OF ADULT AND PAEDIATRIC ASTROCYTIC TUMOURS

Summary. Astrocytic tumours represent the most frequent adult and paediatric brain tumours. Central nervous system (CNS) tumours are the second most frequent paediatric malignancies. Astrocytic tumours are a heterogeneous group, composed of different histological and biological subtypes organized into four grades of malignancy. Malignant astrocytic tumours occur preferentially in adults, diffusely infiltrate the surrounding brain tissue and have inherited tendency for recurrence and malignant progression. Low-grade astrocytic tumours, particularly grade I tumours, are more prevalent in children and have more benign behaviour. Regardless of the significant advances in surgical techniques, radiation and chemotherapy, the prognosis of affected patients, particularly with high-grade lesions still carry a poor prognosis. Studies of astrocytic tumours, mainly in malignant astrocytic tumours of adults, identified major molecular pathways (e.g. p53 and RB pathways, growth factor signalling and genetic instability), which are frequently disrupted and contribute to astrocytic cell transformation. The identification of the molecular profiles allowed for more objective and reliable classification of tumours and stratification of patients for current and/or new therapeutic approaches. The present review describes relevant genetic alterations of astrocytic tumours in adults and highlights the major differences found in their paediatric counterparts.


AIMS

Central nervous system (CNS) tumours are a significant cause of neurological morbidity and mortality in both children and adults. The incidence of the CNS tumours ranges between 7 to 9 new cases annually per 100,000 population, corresponding to less than 2% of all new cancers [1]. The incidence of CNS childhood or paediatric tumours (age ranging from 0 to 19 years old) is 3.9 new cases per 100,000 population, being the second most frequent paediatric malignancies [2]. Among dozens of CNS neoplasms recognized by the World Health Organization (WHO), the astrocytic tumour type is the most common group in adults (more than 60%) [3] and children (approximately 50%) [4].

Astrocytic tumours are so named because tumour cells phenotypically resemble normal astrocytic cells, nonetheless the tumours may develop from pluripotential precursor cells. The WHO grading system classifies astrocytic tumours into grades I-IV based on the degree of malignancy, as determined by histological criteria (Table I). Diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III) and glioblastoma (WHO grade IV), constitute a distinct group within the astrocytic tumour types, the diffusely infiltrating astrocytomas. Diffusely infiltrating astrocytomas occur preferentially in adults and diffusely disseminate into the surrounding brain tissue, leading almost inevitably to recurrence that often is accompanied by tumour progression to more malignant lesions [3]. These neoplasms should be clearly differentiated from the other astrocytic tumours, namely, pilocytic astrocytoma (WHO grade I), pleomorphic xanthoastrocytoma (WHO grade II) and subependymal giant cell astrocytomas (WHO grade I), that are typically encountered in children and young adults. These frequently benign tumours lack the diffusely infiltrating growth pattern, have different biological ground and a more favourable prognosis [3].

In the last decades the knowledge of the genetic alterations encountered in adult astrocytic tumours has greatly increased. However, the understanding of the pathogenesis of paediatric astrocytic tumours is still limited. The present study aims to review major molecular alteration differences between adult and paediatric astrocytic tumours.

DEVELOPMENT

Clinical and histological features

Pilocytic astrocytomas

Pilocytic astrocytomas (WHO grade I) generally behave in a benign fashion, grow slowly, stabilise spontaneously, and malignant
transformation or meningeal spread rarely happens. It occurs in children and young adults, being the most common paediatric brain tumour (accounting for 23.5% of all paediatric brain tumours) [3,4]. This neoplasm is frequently located in the optic tracts (usually associated with the familial tumour syndrome neurofibromatosis type I), hypothalamus or basal ganglia, and the posterior fossa. Histological features include a biphasic pattern with varying proportion of compacted bipolar cells with Rosenthal fibers and loose textured multilobar cells with microcysts and granular bodies [3].

**Pleomorphic xanthoastrocytoma**

Pleomorphic xanthoastrocytoma (WHO grade II) is a rare, usually well-circumscribed tumour that may recur to a more malignant lesion and preferentially develops in the cerebral cortex of children and young adults, accounting for 1.9% of all paediatric brain tumours [4]. Histological hallmarks are pleomorphic and lipidized cells expressing GFAP, a reticulin network and lymphocytic infiltrates [3].

**Subependymal giant cell astrocytoma**

Subependymal giant cell astrocytoma (WHO grade I) is a rare benign, slow growing tumour that occurs typically in paediatric patients, in the context of the familial tumour syndrome tuberous sclerosis. It accounts for 2.5% of all paediatric brain tumours [4]. This neoplasm is a well-circumscribed tumour composed of large ganglioid astrocytes arising in the wall of lateral ventricles [3].

**Diffusely infiltrating astrocytomas**

**Diffuse astrocytomas**

Diffuse astrocytomas (WHO grade II) are slow growing tumours, with a high degree of cellular differentiation, low to moderate cell density, that diffusely infiltrate the normal brain structures and have a tendency for recurrence and progression to anaplastic astrocytoma (WHO grade III) and ultimately glioblastoma multiforme (WHO grade IV) [3]. Diffuse astrocytoma represents 10-15% of all astrocytic brain tumours and typically manifest in young adults with a peak of incidence between 30 and 40 years of age. It represents 5% of all paediatric brain tumours [4]. Histologically three different variants can be distinguished: fibrillary (the most common), gemistocytic (particularly prone to malignant progression) and protoplasmic astrocytomas (rare variant) [3].

**Anaplastic astrocytoma**

Anaplastic astrocytoma (WHO grade III) may arise from progression of diffuse astrocytoma or occur de novo without evidence of a less malignant precursor lesion. It is characterised by a high cellularity, considerable morphologic heterogeneity, presence of mitotic figures and nuclear atypia. The incidence is highest in patients between 40 and 50 years old [3,5]. It represents 7.2% of all paediatric brain tumours [4].

**Glioblastoma**

Glioblastoma (WHO grade IV) is the most malignant and most common brain tumour, accounting for approximately 12-15% of all intracranial neoplasms and 50 to 60% of all astrocytic tumours [3,6]. Histologically, glioblastoma is characterised by poorly differentiated neoplastic astrocytes, nuclear atypia and presence of high mitotic activity. The presence of necrosis and exuberant microvascular proliferation, are histological hallmarks of glioblastomas. The histology reveals an architectural pleomorphism, not only between patients but also within the same tumour. There are two histological glioblastoma variants, giant-cell glioblastoma and gliosarcoma [3,7]. Despite multimodal treatment, including surgery, radiotherapy, and chemotherapy, the prognosis of glioblastoma patients remains dismal. The mean survival time is less than 1 year and has changed little over the past two decades [3,6,8]. Less than 2% of patients survive more than 3 years [9].

In 1940, Scherer [10] used the terms primary and secondary glioblastoma to subdivide glioblastoma based on their clinical and biological features. Primary (de novo) glioblastoma constitutes the vast majority of glioblastomas (approximately 80%). They develop rapidly, with a short clinical history (usually less than 3 months) and without clinical, histologic or radiologic evidence of a less malignant precursor lesion. Secondary glioblastomas are less frequent; develop more slowly, through progression from diffuse astrocytoma or anaplastic astrocytoma [3,10]. Primary glioblastoma occur in older patients (mean, 55 years), whereas secondary glioblastoma arise in younger patients (mean, 40 years) [3,11,12]. In children, glioblastoma is less frequent, accounting for less than 7.5% of all paediatric malignancies, usually develop de novo and are managed as the adult counterpart [3,4].

**Genetic alterations of adult astrocytic tumours**

Tumourigenesis is a multistep process characterised by the accumulation of a series of genetic alterations. These genetic lesions lead to deregulation of growth, impaired apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion resulting in the progressive conversion of normal human cells into tumour cells [13]. In particularly, disruption of cell-cycle control, growth factor signalling, and genetic instability are important mechanisms in the development of astrocytic tumours.

**Alterations of genes involved in cell-cycle control**

**Rb pathway (p16INK4a/CDK4/6/Rb)**

A complex network co-ordinates the cell cycle. In a simplified manner, progression of cells from G1 to S phase is positively regulated by proteins such as cyclins (cyclin D and E) and cyclin-dependent kinases (CDKs) (CDK4, CDK6 and CDK2), and negatively by their inhibitors that can be of two major families; the
CIP/Kip family, that includes the p21^{Waf1/Cip1}, p27^{kip1}, and p57^{kip2} proteins; and the INK4 family, including the founding member p16^{INK4a} and the other closely related proteins p15^{INK4b}, p18^{INK4c}, and p19^{INK4d}. These positive and negative factors regulate ultimately the phosphorylation status of the retinoblastoma protein (Rb). The phosphorylation of Rb protein induces the release of the E2F transcription factor family that in turn activates genes involved in the late G1 and S phase leading to cell-cycle progression (Fig. 1) [14].

The p16^{INK4a} (p16, CDKN2A, MTS1) and p15^{INK4b} genes map to chromosome 9p21, a locus commonly deleted in astrocytic tumours. This locus also encodes another distinct protein through alternative reading frame, the p14^{ARF} protein [15]. p16^{INK4a} homozygous deletion has been frequently detected in glioblastomas, principally primary glioblastomas (30-40%). In anaplastic astrocytomas the frequency is lower (13-25%) and absent in diffuse astrocytomas [3,16,17]. Recently, studies have reported that loss of expression of p16^{INK4a} can be due to methylation of the CpG island in the promoter or first exon region. This mechanism is associated with delayed replication, condensed chromatin and inhibition of transcription initiation, resulting ultimately in gene silencing [18]. Promoter methylation is responsible for p16^{INK4a} loss of expression in 7-20% of diffuse astrocytomas, in 17% of anaplastic astrocytomas and in 25% of non-specified glioblastomas [16,17,19]. Recently, Nakamura et al [19], found that promoter methylation is preferentially associated with the secondary glioblastoma variant. These results suggest that p16^{INK4a} deletion is a late event, while p16^{INK4a} promoter methylation constitutes an early event in diffusely infiltrating astrocytoma tumourigenesis.

The Rb tumour suppressor gene is located on chromosome 13q14, a locus that is altered in about one-third of high-grade astrocytic tumours [3]. Rb inactivation can be due to a variety of mechanisms, such as mutations, homozygous deletion and promoter methylation [3,17,18]. Genetic alteration of Rb has been found to occur in 14-43% of glioblastomas, approximately 20% of anaplastic astrocytomas, whereas in diffuse astrocytomas no alteration in the Rb gene was found [3,16,17]. A clear correlation has been found between loss of expression of Rb by immunohistochemistry, and promoter methylation, which was found to occur more frequently in secondary than primary glioblastomas (43 and 14%, respectively) [20].

CDK4 and CDK6 genes map to chromosome 12q13-14 and 7q21-22, respectively. Amplification of CDK4 has been detected in approximately 10% in high-grade astrocytic tumours (anaplastic astrocytomas and/or glioblastomas) [3,16,17]. No amplification of CDK4 has been found in diffuse astrocytomas [3,16,17]. CDK6 amplification has been detected in 6% of high-grade astrocytic tumours [21], and overexpression of CDK6 protein has been found in 44% of glioblastomas [22]. No amplification or overexpression of CDK6 has been detected in diffuse astrocytomas, suggesting that both CDK4 and CDK6 amplification overexpression are late events in astrocytic tumourigenesis [21,22].

Notably, alterations of p16^{INK4a}, Rb and CDK4 genes are almost mutually exclusive, suggesting that alteration of any one of these components is adequate to sufficiently abrogate the G1/S checkpoint [3,16,17]. Altogether, deregulation of the Rb pathway occurs in 7-20% of diffuse astrocytomas, a significant proportion of anaplastic astrocytomas (20 to 40%), and in the majority of glioblastomas (50 to 70%) [3,16,17]. The remaining high-grade astrocytic tumours that lack p16^{INK4a}-Rb-CDK4/CDK6 detectable alterations may harbour disruption of other components of this pathway. In fact, amplification and overexpression of cyclin D family, has been found in a small number (approximately 3%) of diffusely infiltrating astrocytomas [23].

p53 pathway (TP53/MM2/p14^{ARF})

The TP53 tumour suppressor gene, encodes a transcriptional factor, p53, which is involved in a diversity of cellular processes in mammalian cells, including growth arrest, induction of apoptosis, DNA repair, and inhibition of angiogenesis [24].

Inactivation of TP53 by mutation is a key molecular event, detected in approximately 50% of all neoplasms, including brain tumours [25]. In most of the cases, mutations in one allele are associated with loss of the other allele. These mutations impair the ability of mutant protein to carry out its activities and in some cases bestow a new dominant negative or gain of function properties [24]. TP53 mutations have been reported to occur in approximately 40% of diffusely infiltrating astrocytomas of all grades. An extremely high frequency of TP53 mutations, (60-82%) and 85%, has been observed in gemistocytic astrocytomas and giant cell glioblastomas, respectively [3].

There is compelling evidence that TP53 mutations constitute an early event in astrocytic tumourigenesis. Although TP53 mutations are rare in primary glioblastomas (< 10%), they are frequent in secondary glioblastomas (> 65%) and in 90% of cases the mutation was already present in the first biopsy [3,5]. Patients with Li-Fraumeni syndrome (due to germline TP53 mutations) are characterised by the frequent occurrence of brain tumours in early life, including diffusely infiltrating astrocytomas [3]. The extensive analysis of TP53 mutation of a diffuse astrocytoma whole brain section, showed the presence of the same somatic mutation in all tumour regions (Reis RM et al, unpublished results). These findings suggest that in astrocytic tumours TP53 can act as a gatekeeper gene, directly preventing tumour initiation.

The MDM2 gene is localised on chromosome 12q14.3-1q.4. MDM2 establishes an autoregulatory feedback loop with p53 protein (Fig. 1) [24]. Amplification of MDM2 is absent in diffuse astrocytomas and anaplastic astrocytomas, but present in up to 10% of primary glioblastomas that lack a TP53 mutation [26]. However, overexpression of MDM2 has been observed in more than 50% of primary glioblastomas and less frequently in secondary glioblastomas (< 10%) [3,27]. Thus, alteration of MDM2 by amplification/overexpression constitutes an alternative mechanism for escaping the p53-regulated cell-cycle control.
The p14arf has been identified as another important regulator in this pathway. The expression of p14arf is down-regulated by p53, which would establish an autoregulatory feedback loop between p53, MDM2 and p14arf (Fig. 1) [24]. p14arf expression is activated by abnormal mitogenic signals induced by overexpression of oncoproteins such as Myc, Ras and E2F1 [24]. In this manner p14arf induces p53 activity under abnormal mitogenic signals and serves to connect the RB pathway with the p53 pathway [28]. Homozygous deletion and promoter methylation of p14arf have been reported in astrocytic brain tumours. Homozygous deletions were reported in 40 to 58% of glioblastomas, in 13% of anaplastic astrocytomas but in none of diffuse astrocytomas [19,29]. In contrast, promoter methylation has been found at high frequency in diffuse astrocytomas (33%) and secondary glioblastomas (31%) [19]. Overall, impairment of p53 pathway occurs in the majority of glioblastomas [3,16,17,29]. Unlike the RB pathway, several studies suggest that alterations of the p14arf, MDM2 and TP53 are not always mutually exclusive [3,16,17].

Alterations of growth factors and growth factor receptors

Normal cells require mitogenic growth signals to proliferate. These growth factors bind to their respective receptors and their signal is transmitted into the cells. One of the most important classes of receptors belongs to the family of receptor tyrosine kinase (RTK) that have an intracellular tyrosine kinase domain. After binding to a growth factor, RTK, undergo receptor dimerization, autophosphorylation and activation of downstream cellular cascades, including RAS-MAPK and PI3K-AKT pathways. Alteration of this homeostatic mechanism is frequently observed in diffusely infiltrating astrocytoma, leading to self-sufficiency in growth signals [17].

Epidermal growth factor (EGF) / Epidermal growth factor receptor (EGFR)

EGFR, a RTK, has as major ligands the epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) [30]. EGF gene is located on chromosome 7p12-1-7p12.3, and it was the first gene to be found amplified in astrocytic tumours. Approximately 40% of primary glioblastomas show EGF amplification, which results in overexpression of the EGF transcript [31,32]. EGFR amplification has rarely been detected in diffuse astrocytoma, anaplastic astrocytoma and secondary glioblastoma [3,17,33]. The observation of both ligands and receptors expression in gliomas suggest an autocrine stimulation loop. Approximately half of glioblastomas with gene EGFR amplification also contain gene rearrangements [32]. The most common rearrangements, called EGFRvIII or D EGFR, result in the elimination of exon 2-7 from the extracellular domain, which leads to a truncated receptor [34,35]. This mutant variant displays constitutive ligand-independent tyrosine kinase activity [36] and overexpression of this variant mutant in human glioblastoma cell lines leads to enhanced tumourigenicity in vivo by stimulating proliferation and inhibiting apoptosis [37]. The use of EGFR antagonist holds much promise for improve treatment of high-grade astrocytomas. In particular, the use of small molecule tyrosine kinase inhibitors, such as ZD1839 (Iressa) has raised great expectations [38].

Platelet-derived growth factor (PDGF) / Platelet-derived growth factor receptor (PDGFR)

For almost two decades, PDGF homodimers (PDGF-AA and PDGF-BB) and the heterodimer (PDGF-AB) were thought to be the only ligands for the PDGF α-receptor (PDGFR-α) and PDGF β receptor (PDGFR-β) [39]. Recently, two additional ligands were identified, PDGF-C and PDGF-D [40,41]. Like EGF, PDGFRs belong to the RTK family. Despite the low frequency of PDGFR-α amplification [17,42], overexpression is frequently observed in all grades, suggesting that this alteration is important for the development of diffusely infiltrating astrocytomas [42]. The mechanism that leads to such overexpression remains unclear. Tumours frequently express both ligands and receptors, suggesting autocrine growth stimulation [17,42]. The newly identified PDGF-C and PDGF-D have been involved in signalling regulating angiogenesis, survival and mitogenic pathways in cancer cells [43,44]. Like for EGF, specific PDGFR kinase inhibitors are being tested for suppression of astrocytic tumours growth. In particular, Imatinib mesylate (Gleevec), which is used for treatment of chronic myeloid leukaemia, has demonstrated activity on glioma cell cultures, in mouse model of glioblastoma and is currently in phase I and II clinical trials [38,45].

Genetic instability

Genetic instability is a critical phenomenon in the development of malignant human neoplasms and occurs at least in two different forms, microsatellite instability (MSI) and chromosomal instability (CIN) [46,47]. MSI is characterised by widespread insertions and deletions in microsatellite repeats [46,47]. Tumours with MSI usually have a normal complement of chromosomes, i.e. a diploid or near-diploid karyotype [46]. In adult astrocytomas the frequency of microsatellite instability is very low, approximately 3% [48,49]. In astrocytic tumours, as well as in the majority of other neoplasms, the most common form is CIN, which is characterised by gains and/or losses of whole chromosomes, referred as aneuploidy [46,50,51]. At molecular level, aneuploidy is reflected in allelic imbalance and is often associated with loss of heterozygosity (LOH) [46].

One of the possible mechanisms leading to aneuploidy is the inactivation of the mitotic spindle checkpoint [46,52]. This is a highly conserved mechanism that prevents improper segregation of sister chromatids to the two daughter cells, avoiding in this way abnormal chromosomal segregation and CIN [53,54]. Although some studies suggested that hUTB1, hUTB2, hMAD2 mutations are involved in the evolution of a fraction of human neoplasms with aneuploidy [55,56], no hUTB1, hUTB2 and hUTB3 inactivation mutations were recently reported in glioblastomas [57].

LOH and inactivation of tumour suppressor genes

Chromosome 10, PTEN and other tumour suppressor genes LOH of chromosome 10 (LOH#10) is the most frequent alteration in glioblastomas, occurring in approximately 80% of this neoplasm [3,16,58]. In anaplastic astrocytomas the frequency of LOH is high (about 30%), while in diffuse astrocytomas it is rarely observed [3,16,58,59]. Most glioblastomas exhibit loss of an entire copy of chromosome 10, and in cases with partial deletion, a complex pattern of LOH occurs on both arms of the chromosome [3,16,59]. Loss of the entire chromosome 10 was found to occur associated with primary glioblastomas, whereas, secondary glioblastoma only exhibit deletion in the long arm of chromosome 10 (10q) [60]. A frequent deleted locus is the 10q25-pter and the 10q23-24 [3,16,59]. These frequent and complex deleted regions indicate the presence of several tumour suppressor genes on chromosome 10.
The tumour suppressor gene PTEN (MMAC1 or TEP1) that maps to the common deleted region, 10q23.3 was found mutated in high-grade astrocytic tumours [62,63]. PTEN encodes a phosphatase that negatively regulates PKB/Akt [64]. Multiple studies have shown that PTEN negatively regulates cell survival, is involved in G1 cell-cycle arrest, down-regulation of focal adhesion kinase (FAK) and inhibition of angiogenesis [65]. Germline mutations of PTEN are found in several rare inherited benign tumour (hamartoma) syndromes [65,66]. In addition, sporadic PTEN mutations are frequent in several types of tumours, including primary glioblastoma (24-44%) and anaplastic astrocytomas (approximately 23%), while in diffuse astrocytomas they are generally absent [3,67,68]. In addition to deletion/mutations, current findings suggest that PTEN inactivation can also be due to PTEN promoter methylation [69].

A putative tumour suppressor gene KLF6, which maps on 10q15, was found mutated in small subset of diffusely infiltrating astrocytomas [70]. Several other putative tumour suppressor genes have been identified on 10q, including the DMBT1 on the region 10q25.3-26.1 [71] and LGG1 at 10q24 [72], however, their actual role in astrocytic tumourigenesis is still unclear.

**Other common deleted chromosomal regions**

Allelic loss of 1p (common deleted region on 1p36), 11p (common deleted region on 11p15), 19q, and 22q (defined minimal region on 22q13) are frequently found in these tumours. LOH of 1p, 11p and 19q are commonly associated with high-grade lesions, whereas, LOH of 22q occur at similar frequencies, regardless of malignancy grade [3,16,17,59,73].

**Primary and secondary glioblastoma genetic profile**

Recent studies showed that primary and secondary glioblastomas have not only distinct clinical features, but also evolve through distinct genetic pathways that lead ultimately to the same phenotype [3,16,17,73]. During the last years these molecular pathways started to be unveiled, although the whole picture of the molecular events is far from complete (Fig. 2).

Primary glioblastoma is characterised by high frequency of EGFR amplification, P16INK4A deletion, and PTEN mutations, while secondary glioblastoma is characterised by high frequency of TP53 mutations, LOH19q, and Rb and MGMT methylation. However, some genetic alterations can occur at similar extension in both subtypes, such as homozygous deletion of P14ARF. Another observation that can be drawn from this picture is the frequent association between abnormal promoter methylation mechanisms with secondary glioblastomas (Fig. 2).

**Genetic alterations of paediatric astrocytic tumours**

**Paediatric diffusely infiltrating astrocytomas**

Compared with their adult counterparts, the number of molecular studies is considerably lower. The majority of paediatric diffusely infiltrating astrocytomas studies are focused on the high-grade lesions. The limited studies available for paediatric diffuse astrocytomas, found absence of most current abnormalities encountered in their adult counterparts, with the exception of low frequency of TP53 mutations (approximately 15%) [74,75].

The most important pathway deregulated in paediatric high-grade astrocytomas is p53 pathway. TP53 mutation is a frequent event in these tumours, ranging from 28-38% [3,75-78]. Pollack et al [76], analysing an extensive number of paediatric tumours (148 cases) showed that overexpression of p53 was strongly associated with adverse outcome, independently of clinical and histological features. Low frequency or absence (0-10%) of MDM2 amplification was observed [26,75,79,80], but overexpression of MDM2 has been reported in more than 50% of the cases [79]. P14ARF alterations have been found in up to 14% of tumours [79]. The exact role of Rb pathway in paediatric astrocytomas remains unclear. Alterations of the Rb pathway

| Table II. Clinical and genetic features of common adult and paediatric astrocytic tumours. |
|-----------------------------------------------|-----------------|------------------|
| Clinical onset | De novo | Secondary |
| Gender | Male | Male |
| Location | Supratentorial | Supratentorial |
| p53 mutation | ~10% | >65% |
| PTEN mutation | ~30% | <5% |
| P16INK4A deletion | ~35% | <5% |
| MDM2 amplification | <10% | 0% |
| EGFR amplification | ~40% | 0% |
| CDKN2A deletion | <5% | ~10% |
| MSI | ~3% | 0% |

*Glioblastoma subtyping not available.*

![Figure 2. Summary of some major genetic alterations in primary and secondary glioblastomas. Adapted from [3].](image-url)
were detected in less than 15% of high-grade astrocytomas, with deletion of $p16^{INK4A}$ gene accounting for most of the genetic lesions [75,79,80].

Despite contradictory results, most studies showed absence of $EGFR$ amplification/overexpression [3,74,75,78,81]. PDGFR-α amplification has been recently reported in 1 out of 17 cases (<6%) [75]. An important feature of paediatric high-grade astrocytic tumours is that they have an increased frequency (approximately 30%) of microsatellite instability (MSI) [49,82]. Results on chromosomal instability (CIN) are also contradictory. However, there are some genetic lesions shared with their adult counterparts, such as loss of 10q, 17p, 22q, and others found only on paediatric tumours, such as gain of 1q, 2q, 9p and losses of 16p [83-86]. Analysis of $PTEN$ gene showed absence to low frequency (0-20%) of mutations [68,74,78], and when present, they were associated with worse prognosis [74].

Pilocytic astrocytoma / Pleomorphic xanthoastrocytoma / Subependymal giant cell astrocytoma

The bulk of genetic studies are centred on pilocytic astrocytomas. Although pilocytic astrocytomas are the principal CNS tumours of NF1, the disease, there is no evidence of NF1 gene mutations in sporadic tumours [3]. Pilocytic astrocytomas are characterized by the absence or very low frequency of $TP53$ and $PTEN$ mutations, and alterations of $p16^{INK4A}$, $p14^{ARF}$, CDK4, MDM2, $EGFR$ and PDGFR-α genes [3,75,87-89]. A recent study showed no evidence of aberrant promoter methylation of a panel of genes, including $p14^{ARF}$, $Rb$, $APC$, $CDH1$, $GSTP1$, $TGFBR2$, $THBS1$, $TIMP3$, $PTGS2$, and $CTNNB1$ [90]. At variance with paediatric diffuse infiltrating astrocytomas, no MSI phenotype was observed and chromosomal abnormalities are not a frequent event in pilocytic astrocytoma [49,87].

Genetic studies of pleomorphic xanthoastrocytoma have shown low frequency (5%) of $TP53$ mutations, rare or absence of $EGFR$, $MDM2$, $CDK4$, $p16^{INK4A}$, $p14^{ARF}$ genetic alterations, and no LOH of 10q and 19q [91,92]. The rare number of subependymal giant cell astrocytoma genetic studies reported normal karyotypes [91,93].

Adult versus paediatric astrocytic tumours

The comparative analysis of adults and paediatric tumours shows that they have different clinicopathological and molecular features (Table II). Despite the increasing knowledge, the picture is far from complete. This is particularly true for paediatric astrocytic tumours, in part, due to their relative low frequency.

By location, paediatric tumours are predominantly infratentorial, whereas they are supratentorial in adults. Pilocytic astrocytoma is the most frequent histological type in paediatric patients, while high-grade astrocytic tumours predominate in adults. The biological behaviour is different for similar histological types depending on the age at diagnosis: diffuse astrocytomas in adults very often progress to higher-grade tumours, whereas this is uncommon in their paediatric tumour counterparts [3,4,94].

There are also some interesting genetic differences occurring in diffuse infiltrating astrocytomas. $TP53$ mutations frequencies in paediatric glioblastomas lies between those found in primary and secondary glioblastoma subtypes of adult, despite the clinical onset being de novo in paediatric glioblastoma [3,76,77,94]. Interestingly, $MDM2$ amplification seems to be absent in paediatric GBM and secondary GBM of adults. Alterations of $Rb$ pathway are less frequently found in paediatric glioblastoma than in glioblastoma of adults; no cases of $CDK4$ amplification described and less than 15% of the cases with $p16^{INK4A}$ deletion [79-81]. $PTEN$ mutations frequency in paediatric tumours seems to be similar to that of primary glioblastoma of adults. $EGFR$ amplification is less frequently found in paediatric tumours than is primary glioblastoma of adults [68,74,78]. Finally, MSI phenotype is much more frequently found in paediatric patients than in adults with glioblastoma [49,82].

CONCLUSIONS

Astrocytic neoplasm treatment and predictive prognosis is highly dependent on precise tumour classification. The actual methods of astrocytic tumours classification are essentially based on histological criteria of the biopsy or resection specimen, and do not distinguish tumours that will respond to treatment from those that will recur and/or progress to higher grades. Genetic alterations are becoming increasingly useful in the classification and have potential for patients stratification for current and/or new therapeutic approaches of astrocytic tumours. Differences in tumour location and of frequencies of genetics alterations (e.g. MSI phenotype, $EGFR$ amplification), support different pathogenetic pathways involved in paediatric and adult astrocytomas, and should be considered in the diagnosis and planning of treatment for these tumours. The advent of microarrays technologies that can simultaneously monitor expression of tens of thousands of genes represents a seminal tool to analyse the complex biology of adult and paediatric astrocytic tumours. Their application to CNS tumours has started to show their value in a more accurate stratification of glioblastomas and prediction of CNS embolonal tumours outcome [95,96]. The challenge of coming times will be to translate laboratory results into efficient clinical practice.

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Nouvelles techniques d'imagerie pour le diagnostic et la prise en charge thérapeutique des tumeurs cérébrales

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Compléments de l’IRM morphologique et de la tomodensitométrie, les nouvelles techniques d’imagerie telles que l’IRM de diffusion et le tenseur de diffusion, l’IRM de perfusion, la spectroscopie, l’IRM fonctionnelle, ont modifié, depuis une décennie, la prise en charge radiologique des tumeurs cérébrales. Elles interviennent à toutes les étapes du diagnostic: positif ou différentiel, étiologique, topographique et du planning opératoire. Elles sont indispensables à une approche thérapeutique plus performante et moins mutilante et sont aussi primordiales dans l’appréciation du suivi thérapeutique. Leur rôle se limite, dans le cadre du diagnostic différentiel d’une masse cérébrale, à éliminer un abcès ou un infarctus atypique (diffusion, spectroscopie) ou à différencier un kyste arachnoïdien d’un épidérmide (diffusion). En revanche, leur rôle est important dans le diagnostic étiologique permettant une meilleure caractérisation des tissus, de leur néovascularisation et de l’extension tumorale. Ainsi, la diffusion est restreinte dans les tumeurs hypercellulaires comme les PNET (tumeurs et méastases) par rapport aux tumeurs pédiatiques d’autre nature.

L’IRM de perfusion, grâce aux cartographies du volume sanguin cérébral (CBV) permet de différencier une méastase d’un gliome, d’apprécier le grade d’une tumeur gliale en localisant les zones de néo-angiogenèse particulières aux tumeurs de haut grade et ainsi d’orienter les biopsies stéréotaxiques.