Molecular Neuropathology and the Ontogeny of Malignant Gliomas

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Introduction

Gliomas are the most common primary parenchymal central nervous system (CNS) neoplasms and form a complex and heterogeneous group of tumors. The classification, grading, and treatment of this diverse group of tumors have been primarily based on morphological criteria, which introduced a certain degree of interpretative subjectivity and moreover provided only suboptimal accuracy for the prediction of treatment response [1]. The discovery of distinct genetic and epigenetic profiles for various glioma subtypes not only contributed to improved understanding of glioma pathogenesis, but also revealed that certain molecular changes are linked to therapeutic response and prognosis. The emergence of molecular signatures challenged the prognostic value of classic morphological grading. Consequently, it became a major goal for contemporary glioma diagnostics to incorporate molecular advances into routine tumor classification, which led to the 'ISN (International Society of Neuropathology) Haarlem consensus guidelines' and a revised World Health Organization (WHO) classification for tumors of the central nervous system in 2016. The new guidelines propose a 'layered' approach, which combines histological classification, WHO grading, and molecular biomarkers to establish an 'integrated' diagnostic assessment of gliomas [2, 3].

This chapter discusses the classification, grading, and molecular features of diffuse malignant gliomas as defined in the revised 2016 WHO classification. It focuses on some of the practical aspects of integrated glioma classification and provides an overview of prognostic and predictive molecular biomarkers, and their importance for the diagnosis and management of malignant gliomas.

2016 WHO Classification—Integrated Diagnostics

Bailey and Cushing's first systematic approach to the classification of gliomas, which was published in 1928, laid the foundation for a classification scheme that was based on the 'histogenesis' of brain tumors [4]. The guiding principle was centered on morphological similarities between tumor cells and various normal constituent glial cell types under the assumption that these would give rise to the different types of glial neoplasms. Subsequent classifications, including the classifications devised by the WHO [5], continued to rely on the assessment of light microscopic criteria for tumor typing and histological grading and presented the 'gold standard' for the diagnosis and management of brain tumor patients [1, 6]. However, over time, it became apparent that the pure morphological classification of gliomas was associated with considerable

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subjectivity and inter-observer variability, particularly in the context of tumor heterogeneity [7]. Furthermore, there is considerable biological and clinical variability, even within morphologically well-defined tumor entities, and it becomes difficult to predict response to therapeutic regimens. Although the morphological classification has its advantages, there are significant limitations. Over the past decade, numerous molecular and translational studies have led to the identification of critical genetic and epigenetic abnormalities in various glioma types [8–11]. They not only provide insight into glioma pathogenesis and allow for a more accurate classification, but also show significant associations with biological behavior, response to therapy, and prognosis. As a result, the 'ISN Haarlem guidelines' and the 2016 WHO classification break with the traditional morphologic approach and institute a new diagnostic concept that merges classic histology with molecular diagnostic testing to create a 'layered' diagnosis (see Fig. 2.1). The layers are formed by histologic classification (tumor type), WHO grading ('malignancy level'), and

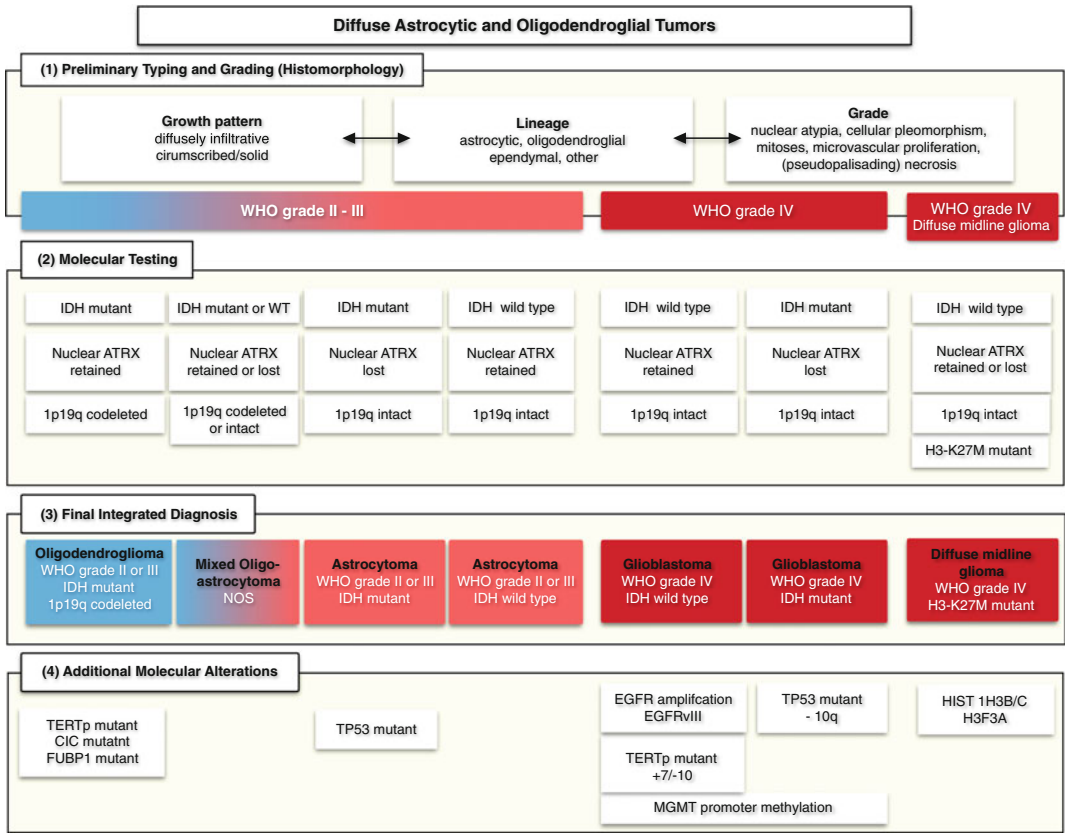

Fig. 2.1 The panels display the new 'layered' approach to the diagnosis of malignant gliomas as suggested by the 'ISN (International Society of Neuropathology) Haarlem consensus guidelines' and revised World Health Organization (WHO) classification. The light microscopic evaluation of gliomas begins the process of glioma classification and grading according to 2016 WHO standards (Preliminary Typing and Grading). Included are lineage-specific immunohistochemical stains such as GFAP. A second step involves molecular testing/biomarker detection for further subclassification and stratification. The results of both, morphology and biomarker analysis, are combined into a 'final integrated diagnosis'. Certain mutational profiles appear to be mutually exclusive and define tumor lineages: The combination of IDH1/1p19q/TERT defines oligodendrogliomas, whereas the combination of IDH1/p53/ATRX is typical for astrocytic tumors. Additional molecular tests are included to add further prognostic and predictive value (e.g., MGMT)

molecular biomarker information, which are combined into the final 'integrated diagnosis'. The purpose of this layered approach is to define individual entities as precisely as possible and to increase and optimize inter-observer diagnostic accuracy. This in turn will optimize predictions for the clinical–pathological behavior of tumors and allow for better prognostic stratification and therapeutic planning [2].

Diffuse Astrocytic and Oligodendroglial Gliomas

The inclusion of molecular markers led to significant changes in the 2016 classification system of gliomas. In prior editions, astrocytic gliomas, oligodendrogliomas, and mixed oligoastrocytic gliomas each formed a separate entity within the larger category of neuroepithelial neoplasms [1]. The 2016 WHO classification (see Table 2.1) merges these gliomas into a single group as '*Diffuse astrocytic and oligodendroglial tumors*' [3]. Aside from their infiltrative growth pattern, diffuse gliomas share frequent isocitrate dehydrogenase (IDH) mutations, a hallmark genetic alteration, which plays a significant role for the stratification of gliomas (please see below). Seminal studies could demonstrate that IDH-mutant gliomas are biologically and clinically distinct from IDH-wild-type gliomas [12, 13].

Diffuse gliomas form the vast majority of glial neoplasms and are primarily classified according to their histopathological appearance as astrocytic, oligodendroglial, or mixed oligoastrocytic

Table 2.1 2016 WHO Classification of Gliomas

Tumor entity/variant	WHO grade
Diffuse astrocytic and oligodendroglial tumors	
Diffuse astrocytoma, IDH-mutant	II
Gemistocytic astrocytoma, IDH-mutant	II
Diffuse astrocytoma, IDH-wild type	II
Diffuse astrocytoma, NOS	II
Anaplastic astrocytoma, IDH-mutant	III
Anaplastic astrocytoma, IDH-wild type	III
Anaplastic astrocytoma, NOS	III
Glioblastoma, IDH-wild type	IV
Giant cell glioblastoma	IV
Gliosarcoma	IV
Epithelioid glioblastoma	IV
Glioblastoma, IDH-mutant	IV
Glioblastoma, NOS	IV
Diffuse midline glioma, H3K27M-mutant	IV
Oligodendroglioma, IDH-mutant and 1p/19q-co-deleted	II
Oligodendroglioma, NOS	II
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-co-deleted	III
Anaplastic oligodendroglioma, NOS	III
Oligoastrocytoma, NOS	II
Anaplastic oligoastrocytoma, NOS	III
Other astrocytic tumors	
Pilocytic astrocytoma	I
Pilomyxoid astrocytoma	II
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumors	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
Papillary ependymoma	II
Clear cell ependymoma	II
Tanycytic ependymoma	II
Ependymoma, RELA fusion-positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Chordoid glioma of the third ventricle	II
Angiocentric glioma	I
Astroblastoma	

tumors. Although the cellular origin is still under investigation, the histological classification (cell lineage) relies on morphological similarities of tumor cells with their presumed non-neoplastic counterpart. Diffuse gliomas are graded in a tiered system as WHO grade II (low-grade), WHO grade III (anaplastic), or WHO grade IV (glioblastoma and variants). The grade is based on histological criteria such as cell density, nuclear atypia, cellular pleomorphism, mitotic activity, vascular proliferation, and necrosis. It can be viewed as 'malignancy scale' that is used to predict the biological behavior of neoplasms [1, 3].

As the name implies, diffuse gliomas display a diffusely infiltrative growth pattern with tumor cells invading brain parenchyma as single cells or small groups of cells. The ability to diffusely disseminate in a single-cell fashion throughout the brain is a rather unique feature among tumor cells and typical of glioma cells. They have the remarkable ability to migrate over long distances along myelinated fiber tracts and not infrequently cross the corpus callosum to infiltrate the contralateral hemisphere ('butterfly glioma') or follow descending fiber tracts. The accumulation of glioma cells around neurons ('perineuronal satellitosis'), around blood vessels and under the pial membrane ('secondary structures of Scherer') are additional classic features [14].

Histological Profiles of Diffuse Astrocytic and Oligodendroglial Tumors

Diffuse Astrocytic Tumors

The incidence of diffuse astrocytomas differs somewhat regionally, but recent estimates suggest an incidence rate of 0.4 per 100,000 people for WHO grade II astrocytomas and an incidence rate of 3.2 per 100,000 people for glioblastomas. The histological grade shows a direct correlation with the age at presentation, as WHO grade II tumors tend to present in younger adults in their 4th or 5th decades, while glioblastomas (WHO grade IV) peak in the elderly (mean age at diagnosis 61 years). Males appear to be more affected than females with a male:female ratio of 1.5:1.0 for all astrocytic tumors [15].

WHO grade II diffuse astrocytomas are morphologically heterogeneous and characterized by a higher degree of cellular differentiation, relatively slow growth, low mitotic activity, diffuse infiltration, and spread into adjacent brain structures (Fig. 2.2a). Tumor cells express GFAP (glial fibrillary acidic protein), a protein typically found in astrocytomas. WHO grade II diffuse astrocytomas can be found at any site within the CNS, but preferentially within the cerebral hemispheres, particularly within the subcortical and deep white matter of frontotemporal lobes. Although these lesions are rare in children, the main site in pediatric patients is the brain stem (so-called brain stem glioma). The 2016 WHO classification removed two variants, *fibrillary* and *protoplasmic* astrocytoma, due to lack of reproducible definition. The *gemistocytic* variant, which shows a very distinct appearance with eccentrically placed nuclei and dense cytoplasm, remains. Further, *gliomatosis cerebri*, previously defined by the diffuse involvement of several cerebral lobes, was also removed as separate entity, and it is simply viewed as an extreme example of widespread dissemination of tumor cells [3, 16].

Anaplastic astrocytomas (WHO grade III) are defined as diffuse astrocytomas with focal or dispersed anaplasia. These tumors are grossly more discernible since they are more cellular and form a more readily identifiable tumor mass. The infiltrative nature tends to create an overall increase in tissue volume without inducing a destructive effect. They are seen to arise from low-grade astrocytomas, but are also frequently diagnosed at first biopsy, without indication of a less malignant precursor lesion. In comparison with low-grade tumors, these neoplasms are microscopically remarkable for increased cellularity and enlarged, irregular hyperchromatic nuclei (Fig. 2.2b). Capillaries are lined by a single layer of endothelium, and frank vascular proliferation and necrosis are not present. Immunoreactivity for GFAP is less consistent than that for grade II lesions. In contrast to low-grade astrocytomas, these lesions display

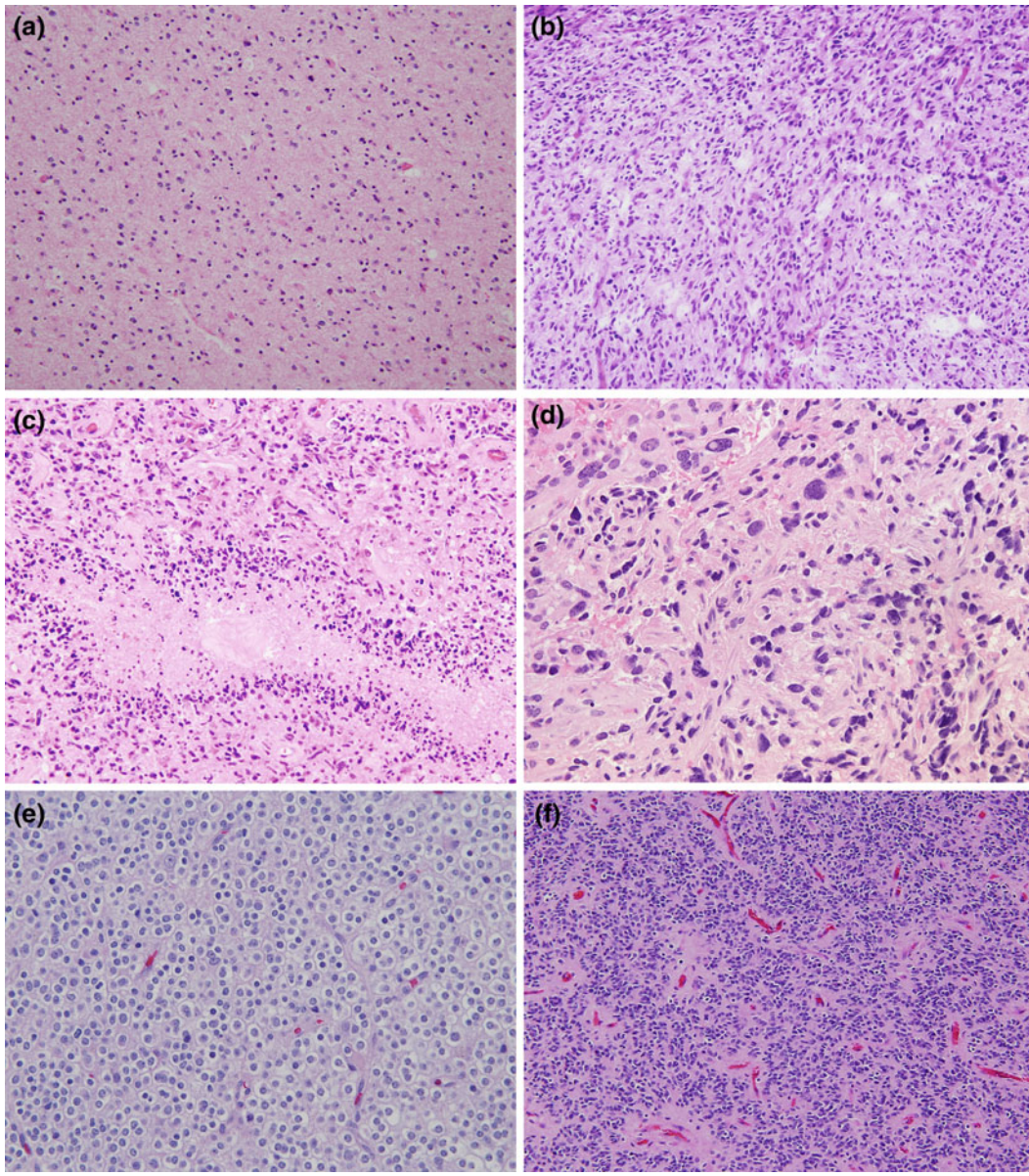

Fig. 2.2 Morphologic appearance of gliomas (hematoxylin- and eosin-stained sections). **a** Diffuse astrocytoma, WHO grade II, characterized by low cellularity and mild nuclear pleomorphism; **b** Anaplastic astrocytoma, WHO grade III with increased cellularity and anaplastic nuclei; **c** Glioblastoma, WHO grade IV with pleomorphic tumor cells, mitoses, and pseudo-palisading necrosis; **d** Diffuse midline glioma with a high degree of pleomorphism; **e** Oligodendroglioma, WHO grade II, with relatively round to oval cell nuclei and typical cytoplasmic clearing ('fried-egg' appearance); **f** Ependymoma, WHO grade II, relative monomorphous appearance of small tumor cells which form perivascular pseudorosettes

increased mitotic activity with a proliferative index (Ki-67/MIB-1 labeling) of 5–10% [3].

Glioblastomas (WHO grade IV) are the most malignant tumors within the spectrum of diffuse astrocytomas and account for up to 60% of all astrocytic tumors [15]. They affect mainly adults with a peak incidence between 40 and 70 years. Less than 10% of glioblastomas arise from a

lesion of lower malignancy grade (secondary glioblastoma) and manifest in younger patients (mean age of 45 years). Most are found de novo (primary glioblastoma) after a short clinical history and are seen in older individuals (mean age 62 years). The majority of tumors are located within the cerebral hemispheres and show a tendency to infiltrate deep nuclei and spread along white matter tracts to the contralateral hemisphere. Brain stem involvement is rare and mainly present in children. Sites such as spinal cord or cerebellum are infrequently involved. Microscopically, glioblastomas are extremely heterogeneous and show a higher degree of cellularity, nuclear atypia, cellular pleomorphism, and mitotic activity, in addition to microvascular proliferation and (palisading) necrosis (Fig. 2.2c). The latter two features are the cardinal diagnostic features of glioblastomas and help distinguish them from grade III astrocytomas. Three distinct glioblastoma variants are part of the 2016 classification, which are giant cell glioblastoma, gliosarcoma, and the recently added variant of epithelioid glioblastoma. [3].

Giant cell glioblastoma is a variant remarkable for the presence and predominance of many markedly large and bizarre appearing, multinucleated giant cells, within an abundant stromal reticulin network. In spite of their unusual appearance, the consistent expression of GFAP in conjunction with data from genetic profiling confirmed their astrocytic nature.

Gliosarcomas are defined as high-grade astrocytomas with an intermixed sarcomatous component. Gliosarcomas are relatively rare and represent about 2% of all glioblastomas. The clinical features are similar to those of classic glioblastomas. Critical diagnostic parameters are a biphasic growth pattern with areas of glial and mesenchymal differentiation. Molecular changes are variable, but similar to those occurring in glioblastoma; however, tumor histogenesis is still controversial.

Epithelioid glioblastoma is a newly accepted and rare variant, which is characterized by the presence of predominantly epithelioid or focally rhabdoid morphology. This entity poses a diagnostic challenge due to its resemblance to poorly differentiated carcinomas [3, 17].

Diffuse Oligodendroglial Tumors

Oligodendrogliomas form a group of diffusely infiltrative glial tumors with features reminiscent of oligodendrocytes. Oligodendrogliomas account for approximately 5–6% of all glial neoplasms, and overall for 2–3% of all primary brain tumors. The annual estimated incidence rate lies within a range of 0.27–0.35 per 100,000 individuals. Although oligodendrogliomas can develop at any age, the majority of tumors arise within the 4th–5th decade, and less than 2% of oligodendrogliomas are found in children younger than 14 years. Males are more affected than females [15]. Oligodendrogliomas can arise anywhere within the central nervous system, but the majority of tumors are found within the frontal and temporal lobes of the cerebral hemispheres. Other cortical regions are less involved and oligodendrogliomas are rare within deep nuclei or spinal cord. Microscopically, these tumors are composed of a relatively monomorphous population of cells with round-to-oval nuclei with delicate chromatin pattern, and surrounded by perinuclear 'halos' (cytoplasmic clearing), an artifact seen in formalin-fixed and paraffin-embedded sections. The vasculature is typically thin-walled and described by some authors as 'chicken-wire' vasculature (Fig. 2.2e).

The WHO classification assigns two grades to oligodendrogliomas: well-differentiated relatively slow-growing tumors correspond to WHO grade II, whereas oligodendrogliomas with anaplastic features are assigned WHO grade III. Anaplastic oligodendrogliomas are characterized by an increase in cellularity and nuclear atypia, increased cellular pleomorphism, in addition to increased mitotic activity, endothelial proliferation, and necrosis. In contrast to other gliomas, such as astrocytomas and ependymomas, oligodendrogliomas show a more slowly progressive clinical course [3].

Diffuse Oligoastrocytomas

Oligoastrocytomas are defined as diffusely infiltrative glial neoplasms consisting of a mixture of two distinct cell types, which morphologically resemble the tumor cells of diffuse astrocytomas as well as oligodendrogliomas. These two

components coexist either side by side or in a diffusely intermingled fashion. Definitive criteria for identification and classification of these lesions, however, remain somewhat controversial. Oligoastrocytomas are graded as WHO grade II lesions, and the acquisition of anaplastic features will increase the grade to WHO grade III.

Although oligoastrocytomas appear to have a mixed phenotypic appearance, they seem to demonstrate either an astrocytic or oligodendroglial genotype. This indicates that these tumors do not form a separate entity. The new WHO classification recommends molecular testing to assign these tumors a definitive lineage. Therefore, the new WHO classification discourages the diagnoses of oligoastrocytoma and anaplastic oligoastrocytoma. The term 'oligoastrocytoma, NOS' and 'anaplastic oligoastrocytoma, NOS' should be used in cases when gliomas are morphologically mixed or ambiguous and cannot be resolved using molecular testing [2, 18].

Molecular Profiles of Diffuse Astrocytic and Oligodendroglial Tumors

Isocitrate Dehydrogenase (IDH) Mutations

The hallmark genetic alterations in diffuse gliomas are somatic mutations in the gene encoding human cytosolic NADPH-dependent isocitrate dehydrogenase 1 (IDH1), a citric acid cycle component. Less frequently involved are mutations of *IDH2*. The IDH1 enzyme normally catalyzes the oxidative carboxylation of isocitrate to alpha-ketoglutarate (α-KG), resulting in the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. Numerous studies uncovered several mechanisms to explain the tumorigenic potential of IDH proteins. There is convincing evidence that mutant IDH, such as IDH1 (R132H), acquires neomorphic activity that converts alpha-ketoglutarate (α-KG) to 2-hydroxygluturate (2-HG) [19]. 2-HG in turn inhibits α-KG-dependent dioxygenases, including the members of the TET family of 5-methylcytosine hydroxylases and Jumonji-C domain-containing histone lysine demethylases. It has been shown that inhibition of these enzymes increases DNA and histone methylation, which eventually triggers the aberrant methylation of multiple cytosine–phosphate–guanine (CpG) dinucleotide-rich islands across the genome [20]. This 'glioma CpG-island methylator phenotype (G-CIMP)' is a characteristic profile seen in diffuse gliomas [21, 22] and likely contributes to the neoplastic transformation of neural stem or progenitor cells. Additional studies have shown that the production of 2-HG stimulates the activity of prolyl-hydroxylase domain isoform 3 (PHD3/EGLN) and prolyl 4-hydroxylases, which leads to reduced levels of hypoxia-inducible factor (HIF) and consequently to the enhanced proliferation of human astrocytes [23]. Increased oxidative stress due to decreased intracellular NADPH levels as a result of IDH mutations additionally promotes tumorigenesis [24].

It has been postulated that IDH mutations likely represent an initiating event, but that they are probably not sufficient to induce tumor growth on their own, instead they have to be accompanied by additional genetic mutations. Mutations involving tumor protein 53 (TP53) and ATRX genes play a role in diffuse and anaplastic astrocytomas. Co-deletions of 1p/19q and mutations involving the promoter region of telomerase reverse transcriptase (TERT) have been described for oligodendrogliomas [25].

These mutational profiles appear to be mutually exclusive and define tumor lineages: The combination of IDH1/p53/ATRX and IDH1/1p19q/TERT are mutational signatures for astrocytic tumors and oligodendrogliomas, respectively [26] (see Fig. 2.1). The new WHO classification requires the demonstration of both IDH1 mutation and 1p19q co-deletion for the diagnosis of oligodendroglioma and anaplastic oligodendroglioma. Similarly, the diagnosis of diffuse or anaplastic astrocytoma requires molecular testing for IDH mutations, with additional demonstration of ATRX mutation or loss of nuclear ATRX expression confirming an astrocytic lineage [27, 28].

Numerous studies established that IDH1 mutations are present at high frequency in

secondary glioblastomas that originate from prior low-grade gliomas (~85%), which is contrasted by the fact that these mutations rarely occur in primary or de novo glioblastomas (<1%), which are found in the absence of low-grade precursor lesion. IDH1 mutations are further identified in the vast majority of diffuse low-grade (WHO grade II) and anaplastic (WHO grade III) astrocytomas (~70–80%), oligodendrogliomas (80%), anaplastic oligodendrogliomas (85%), and mixed oligoastrocytomas (100%). The IDH1 mutation frequency appears to be similar for WHO grade II and WHO grade III tumors [29]. Interestingly, the mutation rate in pilocytic astrocytomas (WHO grade I), ependymal tumors, or other less common glial tumors is extremely low or absent [13]. It was further demonstrated that IDH1 mutations do not exist in reactive conditions related to cerebral ischemia or infarctions, viral infections, or radiation change [30]. These findings are of particular diagnostic value as they enable the distinction of reactive gliosis from low-grade diffuse astrocytoma, a diagnostically challenging task, especially in the context of small biopsy samples.

It is of clinical importance that IDH 1 and IDH 2 mutations are found to be associated with a favorable prognosis and overall prolonged survival time independent of treatment. The survival of patients with the mutant form of IDH1 in astrocytomas or oligodendrogliomas (WHO grade II-III) and glioblastoma is longer than that of their IDH1 wild-type counterparts. Interestingly, patients with IDH1-mutated glioblastomas (WHO grade IV) show better survival than patients with wild-type anaplastic astrocytomas (WHO grade III). The IDH status, however, does not predict treatment-specific responses of patients with glioma [31].

In 2010, an antibody (Fig. 2.3a) was developed which is able to specifically recognize the mutant IDH1-R132H protein, which represents the majority (90%) of glioma-associated hotspot mutations [32, 33]. In the case of IDH1-R132H-negative immunostaining, testing for other IDH1 or IDH2 mutations is required for WHO grade II and III gliomas as well as glioblastomas from young patients and secondary glioblastomas [34]. This is usually accomplished by direct DNA sequencing or pyrosequencing using DNA extracted either from frozen tissue or more commonly formalin-fixed tissue [35, 36].

Co-deletion of 1p/19q

The combined deletion of the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) together with IDH1 mutations defines oligodendrogliomas and anaplastic oligodendrogliomas [37]. Mechanistically, this co-deletion results from of an unbalanced centromeric translocation and leads to loss of entire chromosomal arms t(1;19) (q10;p10). The frequency of 1p/19q co-deletions has been estimated to be 80–90% in WHO grade II oligodendrogliomas and 50–70% in WHO grade III oligodendrogliomas. In spite of a strong association between 1p/19q loss and classic oligodendroglioma morphology, morphology alone cannot predict the 1p/19q status. Interestingly, the chromosomal regions of 1p and 19q have been mapped in great detail; however, no definitive candidate genes have been identified which could explain the tumorigenic effect. Although the genes on 1p/19q remain enigmatic, numerous correlations have been established demonstrating that many tumors with 1p/19q co-deletions also show IDH1/IDH2 mutations; however, 1p/19q loss appears to be absent in cases with tumor protein p53 (TP53) mutations or EGFR amplifications. Notably, the combined loss of 1p/19q is also found in mixed glial tumors (oligoastrocytomas), but extremely rare in non-glial malignancies.

In the 2016 classification, co-deletion of 1p/19q serves a diagnostic biomarker. It was originally described in oligodendrogliomas in 1994, and a few years later, it was noted that a high proportion of oligodendrogliomas with 1p/19q loss demonstrated a favorable response to chemotherapeutic agents, in addition to substantially improved survival times [38]. Long-term follow-up data from the RTOG 9402 and EORTC 26951 phase III trials also pointed toward a role of 1p/19q loss in predicting long-term survival following aggressive

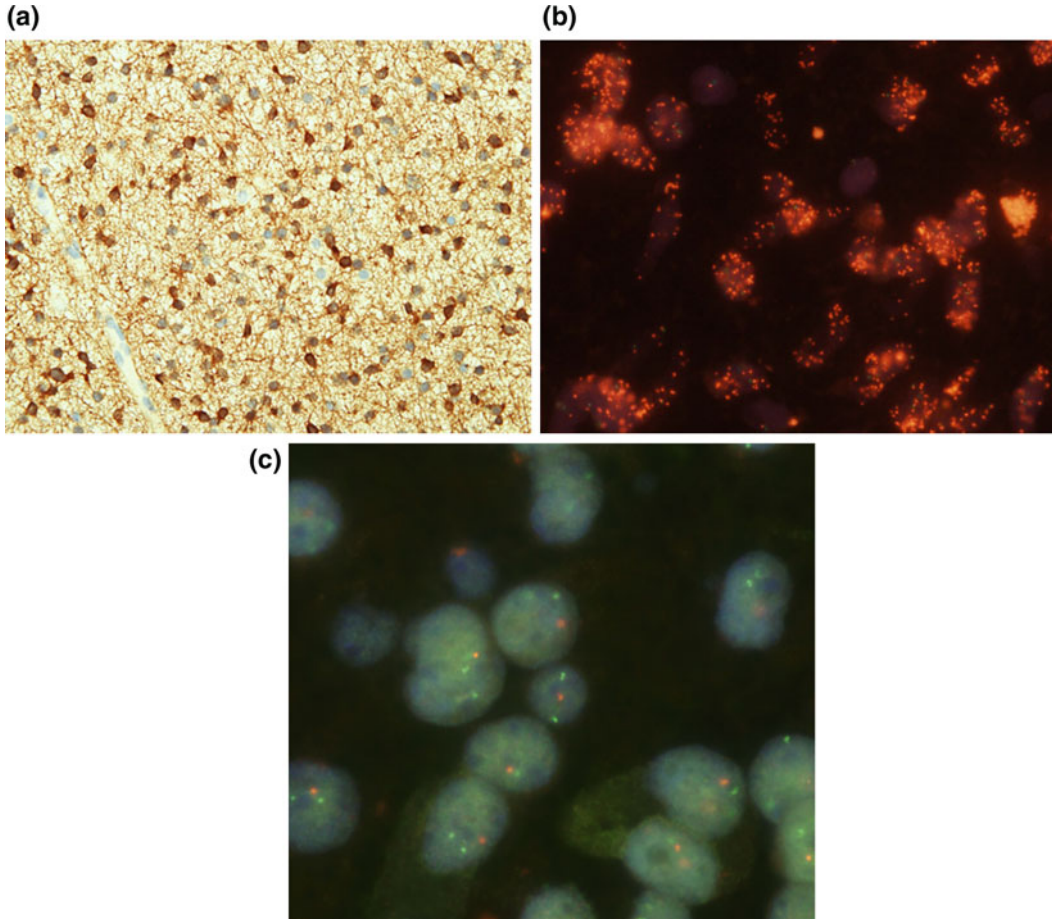

Fig. 2.3 Molecular biomarkers detected in FFPE (formalin-fixed paraffin-embedded) tissue **a** Immunohistochemistry for IDH1 shows mainly cytoplasmic, to a lesser extent nuclear, staining. This mutation-specific antibody against the most common IDH1 mutation, R132H, allows the identification of more than 90% of all IDH-mutant diffuse gliomas. **b** FISH (fluorescent in situ hybridization) for EGFR amplification is recognized by innumerable interphase FISH signals in red (probe set for gene region 7p11). The green signal is a SE7 gene region probe to facilitate chromosome identification. **c** FISH (fluorescent in situ hybridization) to demonstrate loss of the short arm of chromosome 1 (1p loss) as indicated by the presence of only *one red signal* (probe binds to gene region on 1p). The *green signal* serves as control and is a centromeric enumeration probe for chromosome 1 (CEP1). The presence of *2 green signals* indicates both chromosomes (paternal and maternal) are present

multimodal treatment (surgery and upfront combined radio- and chemotherapy with procarbazine, CCNU, and vincristine). In contrast, patients with 1p/19q deleted tumors, who undergo tumor resection alone without receiving any adjuvant chemotherapy or radiation, do not show longer progression-free survival, suggesting that 1p/19q loss characterizes a group of tumors with greater sensitivity to genotoxic agents. Subgroup analyses have further shown that cases of anaplastic oligodendrogliomas with 1p/19q co-deletion and IDH mutation had significantly longer median survival times when treated upfront with radiotherapy and vincristine as compared to treatment with radiotherapy alone. The lack of 1p/19q co-deletion in anaplastic oligodendrogliomas, in contrast, led to a significantly shorter survival times and showed no difference between radio-chemotherapy and radiotherapy-only arms [39]. These findings have

been replicated numerous times over the past decade and extended to the use of additional chemotherapeutic drugs such as temozolomide in conjunction with radiation therapy. The molecular mechanisms, however, that underlie the association between 1p/19q loss, chemosensitivity and favorable prognosis remain to be elucidated.

Due to the well-accepted prognostic significance of 1p/19q loss in conjunction with adjuvant chemotherapy, testing for 1p/19q has become routine many institutions. Commonly used methods for 1p/19q co-deletion testing include fluorescent or chromogenic in situ hybridization (FISH/CISH) (Fig. 2.3c), microsatellite analysis for loss of heterozygosity (LOH), and multiplex ligation-dependent probe amplification (MLPA).

Importantly, 1p/19q co-deletion refers to whole-arm deletions of both chromosome arms that are typically due to an unbalanced translocation [t(1;19)(q10;p10)]. If testing for IDH mutation and 1p/19q co-deletion is not possible or remains inconclusive, tumors with classic oligodendroglial histology should be diagnosed as 'oligodendroglioma, NOS' or 'anaplastic Oligodendroglioma, NOS'.

MGMT Methylation Status

The gene encoding the O^6-methylguanine-DNA methyltransferase (*MGMT*) at 10q26 has become one of the most widely studied molecular markers in neurooncology, because it has the potential to counteract the efficacy of chemotherapy with temozolomide (TMZ). MGMT is a suicide DNA repair enzyme that protects cells against damage from ionizing radiation and alkylating agents [40]. Alkylating chemotherapeutic drugs, such as temozolomide, have been used for years in the treatment of patients with glioblastoma. Mechanistically, these drugs methylate the O^6 position of the DNA nucleotide guanine leading to cell death. MGMT is constitutively expressed in cells and part of an inherent DNA repair mechanism that can counteract the effects of alkylating agents. It catalyzes DNA repair by transferring this methyl group from the O^6 position of the DNA nucleotide guanine to a cysteine residue of the MGMT protein, acting against the cytotoxic effects of chemotherapy [41].

A significant proportion of glioblastomas have been found to express decreased levels of MGMT, which makes these tumors more susceptible to the effects of alkylating agents. The primary mechanism of MGMT downregulation is via aberrant DNA methylation of the promoter of the MGMT gene at its 5′-associated CpG-island. The MGMT promoter methylation represents an epigenetic regulatory mechanism, which consequently leads to transcriptional silencing and is found in 40% of IDH-wild-type glioblastomas as well as the vast majority of IDH-mutant and G-CIMP-positive gliomas. Consequently, glioblastoma cells with MGMT promoter (hyper) methylation respond better to temozolomide, as they lack the ability to efficiently repair the damage introduced by alkylation.

Numerous studies found an association between MGMT promoter hypermethylation and response of malignant gliomas to alkylating agents. In the EORTC/NCIC trial, Hegi et al. found that patients with hypermethylated MGMT promoters who were treated with temozolomide and radiation showed significantly increased survival times when compared to patients whose tumors were hypomethylated [42]. Interestingly, when treated with radiation alone, there was no significant extension of survival times, emphasizing a predictive role for MGMT hypermethylation and a favorable response to chemotherapy. The MGMT promoter methylation status is at the moment viewed as one of the most significant predictors of clinical outcome and response to treatment with temozolomide. Analyses by Gorlia et al. go as far as to suggest a stratification of all patients according to MGMT status as soon as they are enrolled in glioblastoma trials that use alkylating agents [43]. Also, a retrospective analysis could show that MGMT promoter methylation patterns can change between initial tumor diagnosis and later recurrence, particularly in MGMT-methylated cases [44]. This implies that MGMT methylation is only of prognostic value for the initial assessment, and it is not predictive of outcome for recurrences [45].

Further, MGMT promoter methylation is detectable in the vast majority of IDH-mutant gliomas, including both, astrocytic and oligodendroglial tumors, and associated with longer survival, independent of chemo- or radiation therapy. MGMT methylation appears to be frequent in low grade and anaplastic gliomas (up to 90%), which show 1p/19q co-deletion. Treatment with temozolomide correlated positively with longer progression-free survival in those patients. It should be pointed out that in the absence of alternative treatments, temozolomide is often applied as first-line agent, even without a methylated MGMT promoter, as these patients appear to benefit from this drug [46].

The MGMT status is most commonly being tested by methylation-specific PCR (MSP) (Fig. 2.4) or methylation-specific pyrosequencing, whereby both approaches are based on bisulfite conversion of unmethylated cytosines

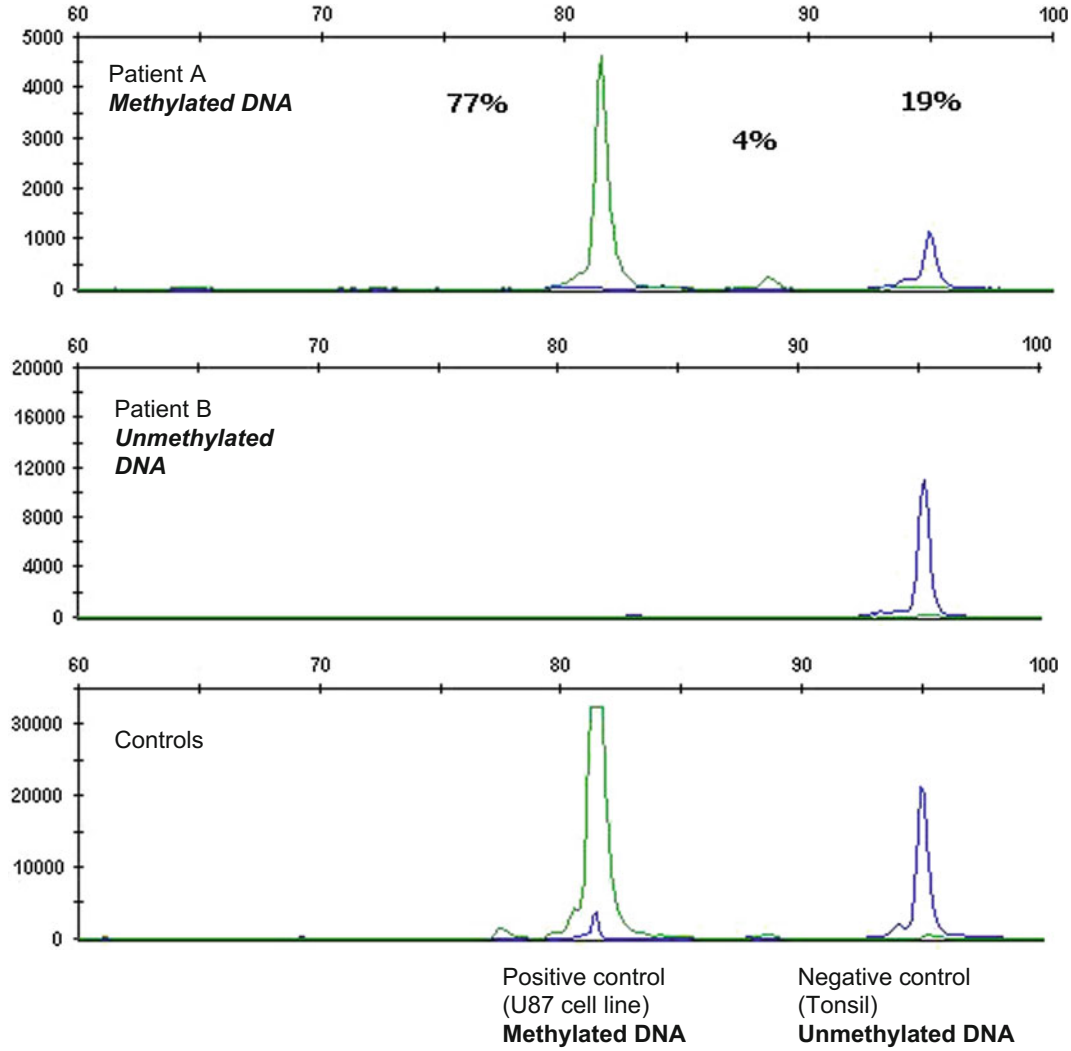

Fig. 2.4 O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status. The graphs represent the result of capillary gel electrophoresis after sodium bisulfite conversion and methylation-specific multiplex PCR. The DNA was extracted from FFPE (formalin-fixed paraffin-embedded) tumor tissue. The *upper panel* shows a patient whose tumor DNA is methylated, and the *middle panel* shows a different patient whose tumor DNA is unmethylated. The *bottom panel* shows the controls for reference

into uracil [47]. Other techniques, like methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), combined bisulfite restriction analysis (COBRA), or methylation-specific high-resolution melting (HRM) analysis, are less commonly used [48, 49].

Role of Epidermal Growth Factor Receptor (EGFR) Pathway Aberrations

Malignant glial neoplasms, particularly glioblastomas, have been found to upregulate several growth factors and their receptors. The epidermal growth factor receptor (EGFR) gene at 7p12 has been described as the most frequently amplified and overexpressed gene in about 60% of glioblastomas and has been associated with shorter survival times. Further, about one-half of glioblastomas that overexpress wild-type EGFR also express EGFR mutant alleles, such as the EGFR variant III (EGFRvIII), which constitutes an 801-bp-in-frame deletion of exons 2–7 and leads to a truncated receptor protein that lacks the ligand-binding domain. This mutation ultimately leads to a constitutively activated EGFR-phosphoinositide 3-kinase pathway and appears to be unique to glial cells [50, 51].

The identification of EGFR amplifications and mutations, especially EGFRvIII, has been associated with poorer prognosis and in general are considered indicative of high-grade malignancy. However, the prognostic value of this information is somewhat ambiguous as several studies produced rather contradictory results. The EGFRvIII mutation, however, might be helpful in the identification of a subgroup of tumors with more malignant behavior than suggested by their histopathology alone. Further, gene expression profiling approaches for glioblastomas with EGFR amplifications enabled a subclassification of morphologically indistinguishable tumors based on their gene expression signatures [52].

Although EGFR pathway aberrations represent attractive therapeutic targets for molecular inhibition, the clinical benefits thus far have been rather disappointing. Attempts to impact tumor growth with the use of EGFR inhibitors, such as erlotinib and gefitinib, failed in spite of sufficient bioavailability and activity to dephosphorylate the EGFR in the tumor tissue. The overall progression-free survival was not prolonged, and only a subset of patients showed some response. Additional missense mutations have been identified in exons that encode extracellular EGFR domains, which appear to drive oncogenesis in vitro and potentially could convey sensitivity to small molecule tyrosine kinase inhibitors [53].

In general, a network of complex and redundant signal transduction pathways that bridge cell surface bound epidermal growth factor receptors with its oncogenic effects in the nucleus likely prevents rather simplistic therapeutic approaches from being successful. In addition, glioblastoma cells often show activation of multiple growth factor pathways, suggesting that a panel of targeting drugs might be necessary to interfere with tumor growth. At this stage, assessments of EGFR signaling pathways for glioblastomas is academically interesting, but clinically not indicated due to a lack of standard drug regimens that specifically target these pathways.

The characterization of EGFR amplification in glioblastoma is typically based on the detection of double-minute chromosomes, which are small fragments of extrachromosomal DNA by fluorescent in situ hybridization (FISH) (Fig. 2.3b). Other techniques such as real-time PCR and MLPA are also used to identify EGFR amplification. MLPA may also detect EGFRvIII rearrangement in EGFR-amplified tumors but appears to be less sensitive.

Diffuse Midline Glioma

Diffuse Midline Glioma is a high-grade glioma with predominantly astrocytic differentiation (Fig. 2.2d), which is mainly seen in children, but can also occur in young adults [3]. The most common locations are brain stem, thalamus, and spinal cord. The diffuse midline glioma was previously known as ‘brain stem glioma’ and ‘diffuse intrinsic pontine glioma (DIPG)’. Histologically, it shows divergent patterns. Approximately, 10% of cases have a histologically low-grade appearance, whereas the

remainder is of higher grade with features of anaplasia such as mitoses, vascular proliferation, and necrosis [54]. Sequencing studies have demonstrated that diffuse midline gliomas typically carry the H3F3A K27M mutation, which correlates with poor prognosis independent of histologic grade. Consequently, the K27M-mutated diffuse midline gliomas are now introduced as a separate entity in the WHO 2016 classification [55, 56].

Ependymal Tumors

Ependymomas are defined as slowly growing glial neoplasms, which can arise anywhere along the walls of the cerebral ventricles or within the spinal canal. The group of ependymal tumors is comprised of the classic ependymoma (plus variants) and anaplastic ependymoma (malignant variant). The benign variants subependymoma and myxopapillary ependymoma will not be discussed in this chapter.

Ependymomas (Fig. 2.2f) account for approximately 5–6% of all gliomas, and for 2.5% of all primary intracranial neoplasms in adults. In children below 14 years, these tumors play a significant role and form about 7–8% of all primary intracranial neoplasms with an adjusted annual incidence rate of 5–6 per 1 million individuals [15]. Overall, ependymomas are the third most common pediatric tumor after astrocytomas and medulloblastomas. Ependymomas can develop at any age; however, there are two distinct incidence peaks: one in children before the age of 14 years and a second one in adults between 35 and 45 years. These tumors can arise anywhere along the ventricular system within brain and spinal canal, but approximately 60% of lesions are located in the 4th ventricle, particularly in pediatric patients. In the spinal cord, it is the most common type of glial neoplasm affecting adults. Males are in general slightly more affected than females.

Morphologically, classic ependymomas are composed of a relatively monotonous population of cells, which tend to form characteristic rosette-like structures, so-called perivascular pseudo-rosettes and ependymal rosettes that have been recognized as diagnostic hallmark features (Fig. 2.4). Recent studies suggest that they might arise from radial glial cells [57].

The WHO classification [3] separates ependymal tumors into three grades, whereby subependymoma and myxopapillary ependymoma correspond to WHO grade I, and classic ependymoma and related variants (cellular, papillary, clear cell, and tanycytic ependymoma) correspond to WHO grade II, and anaplastic ependymomas are WHO grade III.

Anaplastic ependymomas are the malignant variant of classic ependymomas, characterized by high cell density, high mitotic activity, microvascular proliferation, and necrosis. Anaplastic ependymomas are associated with rapid disease progression and unfavorable outcome.

Molecular Profiles of Ependymal Tumors

Until recently, there was very limited information of molecular pathogenesis of ependymal tumors. Frequent NF2 gene mutations and chromosome arm 22q deletion had been described in spinal intramedullary ependymomas [58]. Recent studies led to the discovery of a highly recurrent fusion gene involving the NF-kB downstream intermediate transcription factor p65 (RELA) and an anonymous gene (C11 or f95) in a significant number of supratentorial ependymomas [59, 60]. These RELA fusion-positive supratentorial ependymomas are associated with unfavorable prognosis and form a new entity in the WHO classification of 2016.

A smaller subgroup of supratentorial ependymomas is characterized by gene fusions involving the YES-associated protein 1 gene (YAP1). DNA methylation profiling revealed further subtypes in an evolving field [61].

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