Chapter 5

Histologic classification of gliomas

ARIE PERRY1* AND PIETER WESSELING2

1Departments of Pathology and Neurological Surgery, University of California San Francisco, San Francisco, CA, USA
2Department of Pathology, VU University Medical Center, Amsterdam, Department of Pathology, Canisius Wilhelmina Hospital, Nijmegen, and Department of Pathology, Radboud University Medical Center, Nijmegen, The Netherlands

Abstract

Gliomas form a heterogeneous group of tumors of the central nervous system (CNS) and are traditionally classified based on histologic type and malignancy grade. Most gliomas, the diffuse gliomas, show extensive infiltration in the CNS parenchyma. Diffuse gliomas can be further typed as astrocytic, oligodendrogial, or rare mixed oligodendroglial-astrocytic of World Health Organization (WHO) grade II (low grade), III (anaplastic), or IV (glioblastoma). Other gliomas generally have a more circumscribed growth pattern, with pilocytic astrocytomas (WHO grade I) and ependymal tumors (WHO grade I, II, or III) as the most frequent representatives. This chapter provides an overview of the histology of all glial neoplasms listed in the WHO 2016 classification, including the less frequent “nondiffuse” gliomas and mixed neuronal-glial tumors. For multiple decades the histologic diagnosis of these tumors formed a useful basis for assessment of prognosis and therapeutic management. However, it is now fully clear that information on the molecular underpinnings often allows for a more robust classification of (glial) neoplasms. Indeed, in the WHO 2016 classification, histologic and molecular findings are integrated in the definition of several gliomas. As such, this chapter and Chapter 6 are highly interrelated and neither should be considered in isolation.

INTRODUCTION

Gliomas are the most frequent primary tumors of the central nervous system (CNS) and form a heterogeneous group of neoplasms with multiple histologic types and malignancy grades (Ostrom et al., 2014) (Fig. 5.1). Gliomas are considered to originate from glial (progenitor) cells or stem cells that develop glial characteristics upon neoplastic transformation (Zong et al., 2012). Until recently, the histologic diagnosis was the gold standard for the classification, conveying important prognostic information and forming the basis for further patient management. Microscopic analysis has been performed for over a century on histochemically, especially hematoxylin and eosin-stained sections. As reflected in subsequent editions of the World Health Organization (WHO) classification of CNS tumors, the ideas about the most adequate taxonomy and definitions of CNS neoplasms have changed over time (Scheithauer et al., 2008; Scheithauer, 2009). For over three decades, ancillary immunohistochemistry has increasingly been used for further improving diagnostic accuracy. Until recently, such immunohistochemical analysis focused mostly on cellular differentiation and indeed largely replaced electron microscopy as a tool for lineage determinations (Dunbar and Yachnis, 2010). However, the recent addition of molecular surrogates has greatly expanded the utility of immunohistochemistry for providing diagnostic, prognostic, and predictive aid in the workup of gliomas.

In order to reach an accurate diagnosis, the (neuro) pathologist often follows a decision tree or diagnostic algorithm. Various nonneoplastic lesions (e.g., reactive astrocytosis, inflammatory lesions such as multiple

*Correspondence to: Arie Perry, M.D., Departments of Pathology and Neurological Surgery, University of California San Francisco (UCSF), 505 Parnassus Avenue, #M551, Box# 0102, San Francisco CA 94143, USA. E-mail: arie.perry@ucsf.edu
sclerosis, and infarcts), metastatic and nonglial, primary CNS tumors (e.g., “embryonal tumors”) need to be ruled out (Fig. 5.2). Clinical information (e.g., patient age, duration of symptoms, previous treatment) and radiologic findings (including location and growth pattern of the tumor, absence/presence of contrast enhancement) provide important clues for narrowing down the differential diagnosis. Macroscopic evaluation of surgical specimens by the pathologist is generally of limited help for reaching a precise diagnosis, particularly when sample size is limited or the tissue is fragmented. In larger intact specimens, a gradual transition of normal-appearing gray or white matter into a lesion with grayish discoloration and blurring of the pre-existent anatomic structures is grossly suggestive of a diffuse glioma. In this context, necrosis usually indicates high-grade malignancy in the absence of prior radio- or chemotherapy. Extensive calcification is more often found in oligodendrogial than in astrocytic tumors, although this is not sufficiently specific. Magnetic resonance imaging (MRI) is now the gold standard for radiologic assessment of CNS tumors and often provides the surrogate for macroscopy, especially in small biopsies (Vincentelli et al., 2012).

Histologic typing and grading are relatively straightforward for prototypic tumors. In daily clinical practice, however, gliomas frequently show features in between extremes, e.g., a combination of astrocytic and oligodendroglial characteristics. Furthermore, classification of gliomas can be challenging because of inadequate tissue sampling, imprecise diagnostic criteria, and because the biology of gliomas is not fully represented by its histology alone. During the last two decades, it has become clear that some molecular characteristics correlate better with glioma biology than the histologic diagnosis.

Because of this, in the WHO 2016 classification of CNS tumors (i.e., the update of the fourth edition that was published in 2007), histologic and molecular features are integrated in the definition of multiple glioma types (Louis et al., 2007, 2016).

In this chapter, glial and mixed neuronal-glial neoplasms are discussed as listed in the WHO 2016 classification (Table 5.1 and Fig. 5.2). Of these, the more frequent glial neoplasms (diffuse gliomas, pilocytic astrocytomas, ependymal tumors) are presented and illustrated in greater detail. As the “molecular fingerprint” of some glial neoplasms can now be assessed by practical surrogate immunostains, these tools will be briefly covered as well. For a more indepth discussion of the molecular characteristics of gliomas, however, the reader is referred to Chapter 6.

**DIFFUSE GLIOMAS**

The vast majority of glial neoplasms in adult patients are diffuse gliomas. Such gliomas are characterized by diffusely infiltrative growth within the CNS parenchyma, with tumor cells invading individually or as groups of cells forming a network throughout the neuropil. Only a few other neoplasms (especially lymphomas, histiocytic disorders, and rare examples of metastatic small cell neuroendocrine carcinoma or melanoma) can display this distinctive pattern of diffuse cell invasion (Claes et al., 2007; Osswald et al., 2015). Diffuse glioma growth is often further accompanied by aggregation of neoplastic cells around neurons (perineuronal satellitosis), blood vessels, and under the pial membrane (Fig. 5.3A). Such “secondary structures of Scherer” are nearly pathognomonic of diffuse glioma (Peiffer and Kleihues, 1999). Furthermore, diffuse gliomas tend
to invade over large distances along myelinated fiber tracts, quite frequently crossing the corpus callosum into the opposite hemisphere (“butterfly glioma” pattern). The glioma matrix in especially less cellular/peripheral areas may consist of relatively intact normal gray and white matter, or may show rarefaction with microcystic change and gliosis. Correlation of histologic sections of high-grade diffuse gliomas/glioblastomas with radiologic images revealed that tumor cells are often present several centimeters outside the enhancing area. Occasionally, a widespread diffuse glioma may present with multiple foci of high cellularity, microvascular proliferation (MVP) and/or necrosis (i.e., multifocal or multicentric glioma), radiologically resembling brain metastases or inflammatory lesions (Barnard and Geddes, 1987).

Diffuse gliomas are traditionally typed as astrocytic, oligodendroglial, or mixed oligodendroglial-astrocytic, and graded as WHO grade II (low-grade), III (anaplastic), or IV (glioblastoma) (Fig. 5.4A) (Louis et al., 2007; Kros, 2011). Even though extracranial metastases of gliomas are very rare, most glioblastoma patients die within 1–2 years after diagnosis, despite today’s standard of care (surgery, radiotherapy, and chemotherapy) (Stupp et al., 2009). In contrast, many patients with a WHO grade II glioma survive for over 10 years, although virtually all will eventually progress to a higher-grade malignancy, resulting in fatality.

**Astrocytic, oligodendroglial, and mixed diffuse gliomas (Figs 5.3–5.5)**

Assessment of the subtype of diffuse gliomas is traditionally based on the resemblance of the tumor cells to non-neoplastic glial cells. Most diffuse gliomas can be designated as astrocytic, oligodendroglial, or mixed oligodendroglial and astrocytic (Fig. 5.4A). Those with uniformly rounded nuclei are generally considered oligodendrogliomas while those with nuclear irregularities...
and hyperchromasia are diagnosed as astrocytomas. The conventional fibrillary cell type in diffuse astrocytic neoplasms often displays “naked nuclei” set upon a densely fibrillar background, given that the cytoplasm of such cells appears to blend imperceptibly with the native neuropil of the CNS (Fig. 5.3B). The previously described variant of protoplasmic astrocytoma (with neoplastic cells typically showing small cell bodies and few, flaccid cell processes) is no longer included in the WHO 2016 classification since a reliable and reproducible definition is lacking. A variant of diffuse astrocytic tumor that stood the test of time is gemistocytic astrocytoma, characterized by a substantial gemistocytic component (>20% cells with abundant eccentrically placed cytoplasm) (Fig. 5.3G). This variant often harbors perivascular lymphocytic infiltrates and tends to show more rapid malignant progression than the fibrillary astrocytomas (Ichimura et al., 2015). Gliomatosis cerebri, previously defined as involvement of at least three cerebral lobes by a diffuse (astrocytic) glioma, is in the WHO 2016 classification considered as an extreme example of the infiltrative growth pattern seen in all diffuse glioma subtypes rather than as a separate entity.

In low-grade oligodendrogliomas, the nuclei are round and uniform with a crisp nuclear membrane, delicate chromatin, and small to inconspicuous nucleoli, while in more anaplastic examples, cell size is increased and often shows epithelioid features, along with

### Table 5.1

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<th>Diffuse astrocytic and oligodendroglial tumors</th>
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Fig. 5.3. See legend on next page.
increased pleomorphism, a more vesicular chromatin pattern, and more prominent nucleoli (Fig. 5.3H, K). Nevertheless, anaplastic oligodendrogliomas generally still maintain an overall sense of regularity and nuclear roundness at lower magnifications. The classic perinuclear halo is a useful additional feature when present. It is this halo that gives oligodendrogial cells the “fried egg” appearance and makes clusters of such cells look like a honeycomb. Of note, this halo formation is in fact a reproducible artifact elicited by formalin fixation and can thus be absent in specimens that are more promptly fixed or used without fixation for frozen-section diagnosis. A subset of oligodendrogliomas contains gliofibrillary or minigemistocytic tumor cells. Other oligodendrogial tumors may show scattered cells with marked pleomorphism (“polymorphic oligodendroglial tumors may show scattered cells resembling medulloblastoma (Fig. 5.5J, K). Rarer patterns include nodular foci of primitive neuronal cells resembling medulloblastoma (Fig. 5.5L–M) and granular cells with histiocyte-like cytoplasmic granularity and vacuolation (Fig. 5.5N, O).

**Grade II, III, and IV diffuse gliomas**

For grading of diffuse gliomas, the histologic features of mitotic activity (Fig. 5.3K), MVP (Fig. 5.3L), and necrosis (Fig. 5.3A) are used. A diffuse astrocytoma without these features is diagnosed as low-grade (WHO grade II). With increased mitotic activity, the diagnosis of anaplastic astrocytoma is rendered, although a specific mitotic cutoff has not been officially endorsed and this accounts for some grading discordances. The presence of necrosis and/or MVP leads to a diagnosis of glioblastoma. Often, necrosis in these tumors consists of irregular, serpiginous foci surrounded by densely packed, somewhat radially oriented tumor cells (“pseudopalisading necrosis”; Fig. 5.5A).

In oligodendrogial tumors and mixed gliomas, the malignancy grade is assessed using the same set of histologic features but in a somewhat different way. In one larger study the number of mitoses required for the
Bendroglioma with an oligodendroglial component. (cytoma, respectively; GBM, glioblastoma; GBM-O, glioblastoma with oligodendroglial component (GBM-O) (Louis et al., 2007; Miller and Perry, 2007). However, molecular studies will typically show astrocytoma-like, oligodendroglioma-like, or glioblastoma-like changes in such seemingly mixed gliomas, such that most GBM-O cases would be converted into one of three prognostically distinct categories when using WHO 2016 integrated diagnoses: (1) GBM, IDH wild-type, WHO grade IV; (2) GBM, IDH-mutant, WHO grade IV; or (3) anaplastic oligodendroglioma, IDH-mutant, lp19q-co-deleted (Fig. 5.4).

In daily clinical practice, not only typing but also grading of gliomas can be difficult (Kros, 2011). Diffuse gliomas often show marked phenotypic heterogeneity with spatial differences in cellular phenotype and degree of anaplasia. Cell density and nuclear atypia do not always correlate with overall malignancy, such that low-grade oligodendrogliomas are often highly cellular while glioblastomas may show areas of limited cellularity. Similarly, nuclear atypia is occasionally greater in low-grade gliomas than in glioblastomas. Evaluation of mitotic activity should be performed in the context of sample size: a single mitosis in a large resection specimen is insufficient for the diagnosis of anaplastic change, whereas it may well indicate anaplasia (WHO grade III) in a small sample.

Glioblastomas typically show necrosis and prominent or even “glomeruloid” MVP. MVP is a term used for the presence of multilayered microvessels with hypertrophy and hyperplasia of endothelial cells and pericytes in the wall of the proliferating microvessels (Hardee and Zagzag, 2012). However, the minimum diagnostic criteria for this phenomenon are not clear. Even identification of necrosis may be troublesome in biopsy samples that are small or poorly preserved. Also, in recurrent gliomas discrimination of native tumor from therapy-induced necrosis may be difficult (Perry and Schmidt, 2006).

**Ancillary immunohistochemistry**

Immunohistochemical analysis can be very helpful for adequate recognition of diffuse and other glioma subtypes. A key marker for recognition of glial differentiation is glial fibrillary acidic protein (GFAP) (Dunbar and Yachnis, 2010). GFAP is an intermediate cytoskeletal filament expressed by normal glial cells, especially by astrocytes and ependymal cells. Normal and neoplastic neuronal, epithelial, and lymphoid cells are almost always GFAP-negative. Except for the minigemistocytic and gliofibrillary elements (Fig. 5.3J), oligodendrogliomas with MVP and/or necrosis are still considered as WHO grade III lesions. In contrast, according to the WHO 2007 classification, MVP in mixed/oligoastrocytic tumors was still compatible with WHO grade III, while the presence of necrosis justified a diagnosis of glioblastoma with oligodendroglial component (GBM-O) (Louis et al., 2007; Miller and Perry, 2007). However, molecular studies will typically show astrocytoma-like, oligodendroglioma-like, or glioblastoma-like changes in such seemingly mixed gliomas, such that most GBM-O cases would be converted into one of three prognostically distinct categories when using WHO 2016 integrated diagnoses: (1) GBM, IDH wild-type, WHO grade IV; (2) GBM, IDH-mutant, WHO grade IV; or (3) anaplastic oligodendroglioma, IDH-mutant, lp19q-co-deleted (Fig. 5.4).

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Fig. 5.5. See legend on next page.
often resemble oligodendroglioma at first glance. The glioma marker, however, is OLIG2, a murine basic helix–loop–helix transcription factor expressed in neural progenitors and oligodendroglia and considered to be essential for oligodendrocyte development. This marker does not allow for unequivocal discrimination between oligodendrogliomas and diffuse astrocytomas, but may be of help in the differential diagnosis of a diffuse glioma and a neuronal, ependymal, or nonglial tumor type, as OLIG2 staining is typically limited or absent in the latter (Ligon et al., 2004; Preusser et al., 2007).

Immunohistochemical staining for synaptophysin, a synaptic vesicle protein, has long been considered as sound evidence for neuronal rather than glial differentiation. However, synaptophysin staining may also occur in bona fide gliomas, including oligodendrogliomas, indicating that the progenitor cells from which these tumors are derived are less strictly committed to glial lineage than previously thought (Perry et al., 2002). This may also explain that occasionally oligodendrogliomas are reported to display neurocytic or ganglioglioma-like maturation and that differentiation from other, more typical glioneuronal tumors can be challenging. Immunohistochemistry for neurofilament protein (a cytoskeletal protein of neurons especially present in their axons) can be useful for discriminating diffuse from nondiffuse gliomas, with widespread presence of entrapped neurofilament-positive axons supporting a diffusely infiltrative growth pattern. Other markers used for immunohistochemical demonstration of neuronal rather than (only) glial differentiation of tumor cells are NeuN (an antigen expressed in the nuclei and to a lesser extent in the cytoplasm of native neurons) and microtubule-associated protein 2 (MAP2), although the latter is often also useful in distinguishing immunoreactive glioma cells from immunonegative reactive astrocytes. Furthermore, CD34 staining of not only endothelium, but also of lesional ganglion cells and/or other dysplastic cells is present in the vast majority of gangliogliomas, pleomorphic astrocytomas, and some forms of cortical dysplasia (Blumcke et al., 1999; Blumcke and Wiestler, 2002).

Expression of cytokeratins, the epithelial group of intermediate cytoskeleton filaments, usually indicates epithelial differentiation. Occasionally diffuse gliomas, in particular the epithelioid variant of glioblastoma and gliosarcomas, may show bona fide cytokeratin expression as well. Of note though, the pan-cytokeratin cocktails, including AE1/3, often cross-react with GFAP and should therefore not be overinterpreted as showing epithelial differentiation. Epithelial membrane antigen (EMA) is a cell surface-associated glycoprotein widely expressed in nearly all epithelial cells, but also in (neoplastic) meningothelial cells. In glial tumors, EMA staining can be helpful in supporting ependymal differentiation (with luminal or dot-like intracytoplasmic staining, the latter corresponding to microlumina in these cells) (Dunbar and Yachnis, 2010). The Ki-67 immunostain (typically the MIB1 clone in paraffin) targets a nuclear protein present during all phases of the cell cycle except G0; therefore, the fraction of positive tumor nuclei reflects the level of proliferative activity. The Ki-67 labeling index (LI) in diffuse gliomas generally increases with malignancy grade (roughly <5% in low-grade diffuse gliomas, 5–10% in anaplastic gliomas, and >10% in glioblastomas) (Giannini et al., 1999). Although reactive processes typically have a low Ki-67

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**Fig. 5.5.** Glioblastoma (GBM) and its variants. (A) Pseudopalisading necrosis is highly characteristic of GBM. (B) Giant cell GBM with large, highly pleomorphic, multinucleated giant cells. (C) Gliosarcoma with alternating islands of fibrillary-appearing glioma (upper half of image) and fascicular spindled sarcomatous component (lower half of image). The sarcomatous element is typically reticulin-rich (D), while the glial component is glial fibrillary acidic protein (GFAP)-immunoreactive (E). (F) The epithelioid GBM often appears more demarcated and resembles metastatic carcinoma or melanoma. (G) Roughly half of epithelioid GBMs are immunopositive for BRAF V600E-mutant protein. (H) The small cell GBM consists of oval monomorphic nuclei that often resemble oligodendroglioma at first glance. (I) Despite their bland cytology, they generally show markedly elevated proliferative indices on Ki-67 immunostain. (J) GBM with a primitive neuronal component often shows sharply demarcated hypercellular clones (left) arising within an otherwise characteristic diffuse glioma (right side). Evidence of a primitive neuronal component includes Homer Wright rosettes (K), loss of glial markers such as GFAP or OLIG2 (L), and gain of neuronal marker expression, including synaptophysin (M). (N) The granular cell variant of GBM is deceptively bland-appearing, with abundant clear to granular eosinophilic cytoplasm often mimicking macrophages. (O) In contrast to macrophages, however, glial lineage is evident with immunohistochemistry for GFAP (not shown) or nuclear staining for OLIG2.
LI, proliferation of endothelial and inflammatory cells may artificially raise the perceived LI if one is not aware of this diagnostic pitfall. Furthermore, no specific cutoffs are advocated by the WHO, given substantial overlap in Ki-67 LI within the three malignancy grades and variable staining results among laboratories (Johannessen and Torp, 2006).

**Diagnostic challenges**

The decision tree provided in this chapter for typing and grading of gliomas (Fig. 5.2) only partially reflects the many diagnostic challenges that often occur. For instance, gliomas not infrequently have indeterminate cytologic features, causing frustratingly high interobserver discordance rates, even among experienced neuropathologists (Coons et al., 1997; Giannini et al., 2001; Kros et al., 2007). In cases in which (due to, e.g., indeterminate histology or limited sample size) the subtype of glioma is difficult to assess, the diagnosis “(malignant) glioma, not otherwise specified/NOS” is sometimes rendered (Fig. 5.1).

This explains at least part of the conflicting results in the literature with regard to clinical characteristics, prognosis, and molecular underpinnings of glioma subtypes. The introduction of signature molecular alterations, such as IDH mutation and 1p/19q co-deletion in the WHO 2016 classification of diffuse gliomas, allows for more robust glioma diagnosis in adults (Fig. 5.4). This paradigm shift necessitates further study of how histologic grade and/or additional molecular markers can be used for prognostication (Louis et al., 2014; Reuss et al., 2015b). Furthermore, while diffuse gliomas in children and adults may look the same histologically, their molecular features are often completely different. For example, the so-called “diffuse midline gliomas” occur mostly in children and young adults (including diffuse intrinsic pontine glioma) and histologically look like the glioblastomas or lower-grade diffuse astrocytomas in older adults. However, these diffuse midline gliomas typically carry the *H3F3A* (or, less commonly, *HIST1H3B/C*) K27M mutation. As this mutation correlates with poor prognosis independent of histologic grade (Sturm et al., 2012; Buczkowicz et al., 2014; Korshunov et al., 2015), the K27M-mutated diffuse midline gliomas are now introduced as a separate entity in the WHO 2016 classification (Table 5.1).

Discriminating diffuse glioma from other lesions by histology alone can also be challenging (Omuro et al., 2006). For example, it may be difficult to appreciate the infiltrative growth in a highly cellular, more solid part of a diffuse glioma, raising the differential diagnosis with “nondiffuse” glioma variants such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and ganglioglioma. Furthermore, several primary CNS tumors other than oligodendroglial neoplasms may show the fried-egg appearance as well, examples being pilocytic astrocytoma, clear cell ependymoma, and neuronal and glioneuronal tumors such as neurocytoma and dysembryoplastic neuroepithelial tumor (DNT). As discussed in more detail below, the presence of Rosenthal fibers suggests a “nondiffuse glioma,” but they can also occur in reactive lesions (“piloid gliosis”) and rare diffuse gliomas. Other histologic features that are helpful in this respect include the presence of a biphasic growth pattern (e.g., pilocytic astrocytomas), perivascular pseudorosettes and/or true rosettes (e.g., ependymal tumors), and the formation of specific glioneuronal elements (e.g., DNT). Ancillary immunohistochemistry frequently helps to reach the right diagnosis in such problematic cases. In some biopsy samples, reactive gliosis may be difficult to distinguish from a glioma (Rivera-Zengotita and Yachnis, 2012). An important clue is that GFAP-positive reactive astrocytes are often evenly spaced and have more regular, radially oriented cell processes than neoplastic astrocytes. Additionally, most diffuse lower grade (WHO grade II and III) gliomas are immunoreactive for IDH1 R132H-mutant protein and/or MAP2, both of which are negative in reactive astrocytes (Fig. 5.3D, E). A tumefactive multiple sclerosis lesion can histologically resemble high-grade glioma. Immunohistochemical demonstration of numerous CD68-positive macrophages, along with demyelination, helps to solve this differential diagnosis, the latter further supported by marked loss of myelin on a Luxol fast blue histochemical stain with relative preservation of axons on a neurofilament immunostain.

**OTHER ASTROCYTIC TUMORS**

Most other (i.e., “nondiffuse”) astrocytic tumors are slow-growing. Although some infiltration may be seen at their interface with adjacent brain, this is not nearly as extensive as encountered in diffuse gliomas and typically without the formation of secondary structures of Scherer. In contrast, anaplastic examples of “other astrocytic tumors” may show necrosis, MVP, and brisk mitotic activity, similar to that of glioblastoma, though the first two of these may also be seen in some of the low-grade forms. As such, they must be interpreted within the context of other diagnostic features.

**Pilocytic astrocytoma (WHO grade I) (Fig. 5.6)**

Pilocytic astrocytoma is the most common glial neoplasm in children and adolescents. This tumor is preferentially located in the cerebellum, optic pathways, hypothalamus/floor of the third-ventricle, brainstem, and spinal cord, but may originate from the basal ganglia or more laterally in the cerebral hemispheres as well.
Most pilocytic astrocytomas have a macroscopically discrete growth pattern, with or without cyst formation, and microscopically show a biphasic architecture with alternating loose and compact areas (Fig. 5.6A). Pilocytic astrocytomas in the optic pathways tend to show more diffuse infiltration in these structures and are sometimes associated with neurofibromatosis type 1 (NF1). Those originating in the brainstem frequently present as compact, “dorsal exophytic” lesions. The compact regions in pilocytic astrocytomas contain many closely apposed, delicate, and long, hair-like (“piloid”) processes, arranged in parallel or in a more irregular fashion. Such compact areas often contain multiple Rosenthal fibers, i.e., brightly eosinophilic, hyaline, corkscrew- or carrot-shaped structures in the tumor cell processes (Fig. 5.6B). The loose areas frequently show microcystic change and may contain globular, mulberry-shaped, eosinophilic granular bodies (Fig. 5.6C) (Collins et al., 2015). Both Rosenthal fibers and eosinophilic granular bodies are also often found in other, especially non-diffuse glial neoplasms, while Rosenthal fibers may occur in the context of reactive piloid gliosis, adjacent to slow-growing lesions. Not infrequently, pilocytic astrocytomas extensively invade in the leptomeninges, accompanied by variable desmoplastic reaction. Also, the tumors may show regressive changes such as vascular fibrosis or ectasia (sometimes mimicking a vascular malformation) and signs of old hemorrhage. Marked, or even glomeruloid, MVP is a frequent finding and does not indicate more aggressive behavior. Similarly, nuclear pleomorphism, infarct-like necrosis, and limited mitotic activity do not indicate malignancy (Giannini and Scheithauer, 1997). More widespread presence of “brisk mitotic activity” (defined as at least 4 per 10 consecutive high-power fields) is considered evidence of anaplastic pilocytic astrocytoma (Rodriguez et al., 2010).

The astrocytic tumor cells, especially in compact areas, generally show strong staining for GFAP (and for other...
glial markers such as S100 and OLIG2). The Rosenthal fibers only stain peripherally with GFAP but are strongly positive for α-B crystallin. Not infrequently, synaptophysin positivity is found in pilocytic astrocytomas. Especially in less compact, more cellular areas the tumor cells may have an oligodendroglial phenotype (Fig. 5.6D), eliciting a differential diagnosis of oligodendroglioma and (clear cell) ependymoma. Furthermore, as pilocytic astrocytomas often show some infiltration in the surrounding brain tissue, discrimination from a diffuse glioma can be difficult. Pilomyxoid astrocytoma is a variant of pilocytic astrocytoma characterized by bipolar, monomorphic tumor cells in a prominent myxoid background, often with perivascular clustering of tumor cells (Fig. 5.6E, F). Rosenthal fibers and eosinophilic granular bodies are typically lacking. Pilomyxoid astrocytoma preferentially occurs in the region of the hypothalamus/optic chiasm in infants and young children and has been reported to have a somewhat more aggressive behavior. However, unequivocal discrimination of this variant from pilocytic astrocytomas is difficult, particularly since some tumors show mixed features of pilomyxoid and pilocytic astrocytoma, while some pilomyxoid astrocytomas “mature” into a tumor resembling classic pilocytic astrocytoma as the child gets older (Johnson et al., 2010; Colin et al., 2013).

Subependymal giant cell astrocytoma (SEGA) (WHO grade I) (Fig. 5.7)

Almost all SEGAs occur in the context of the tuberous sclerosis complex and arise from the walls of the lateral ventricles near the foramen of Monro. Most of these tumors are diagnosed in the first two decades of life. Histologically, the neoplasm consists of large, plump cells with a wide phenotypic variability (Fig. 5.7A, B).

The tumor cells may resemble gemistocytic astrocytes or have a spindle cell phenotype. The cells may be arranged in bundles, sheets, nests, and sometimes pseudopalisading clusters around blood vessels. A subset of the tumor cells may resemble ganglion cells with a vesicular nucleus and prominent nucleolus. Multinucleate tumor cells, nuclear pleomorphism, hyalinization of the wall of blood vessels, lymphocytic infiltrates, and calcification are frequently present. Also, some mitotic activity, MVP, and necrosis may be seen, but do not readily indicate more aggressive behavior (Hirose et al., 1995; Sharma et al., 2004a, b).

Immunohistochemically, the tumor cells in SEGA generally show variable GFAP staining and uniform S100 staining. Additionally, staining for neuronal markers (synaptophysin, Neu-N, neurofilament) is variably present, indicating that SEGA is a glioneuronal rather than pure astrocytic neoplasm and that the term subependymal giant cell tumor may be more appropriate.

Pleomorphic xanthoastrocytoma (PXA) (WHO grade II) (Fig. 5.8, left half)

PXA is a rare tumor, most often diagnosed in children and young adults, occasionally in the context of neurofibromatosis type I. PXAs are typically superficially located cerebral tumors (temporal lobe most common), often, but not invariably, presenting with a cystic component and extensive leptomeningeal involvement. Histologically, PXAs are characterized by large, pleomorphic and partly multinucleated, astrocytic tumor cells with variably lipidized (“xanthomatous”) cytoplasm (Fig. 5.8A). These cells are often closely packed, causing an epithelioid appearance, and may have prominent nucleoli and intranuclear cytoplasmic vacuoles. In other areas, the tumor can have spindled morphology.
reminiscent of a mesenchymal tumor. A dense reticulin network (Fig. 5.8C), multiple eosinophilic granular bodies, and dispersed or perivascular aggregates of lymphocytes are often seen. Necrosis and MVP may occur, but mitotic activity is typically low (Giannini and Scheithauer, 1997; Ida et al., 2015). The staining of tumor cells for GFAP corroborates their astrocytic nature. In addition, the cells may express neuronal markers (synaptophysin, neurofilament, class III β-tubulin, MAP2), as well as CD34 (Fig. 5.8G) (Giannini et al., 2002). Over half also express the BRAF V600E-mutant protein (Fig. 5.8H). The differential diagnosis with ganglioglioma, diffuse astrocytoma, and pilocytic astrocytoma may be difficult. Occasionally, “composite tumors” with areas of PXA and ganglioglioma occur (Perry et al., 1997).

**Anaplastic PXA (WHO grade III)** (Fig. 5.8, right half)

The clinical, histologic, and immunohistochemical characteristics of this tumor resemble PXA grade II, but in
addition anaplastic PXA shows brisk mitotic activity (defined in the WHO 2016 classification as 5 or more mitoses per 10 consecutive high-power fields), either focally or diffusely. Typically foci of anaplastic transformation become less pleomorphic and often resemble diffuse fibrillary astrocytoma (Fig. 5.8B). MVP and necrosis are frequently present, the latter of which may be pseudopalisading (Tekkok and Sav, 2004; Ida et al., 2015). Anaplastic foci often lose the reticulin-rich background of lower-grade PXAs (Fig. 5.8D) and show an increased Ki-67 LI (Fig. 5.8F). As such, the presence of a lower-grade PXA component or a prior history of such at the same location is needed to reliably distinguish anaplastic PXA from other high-grade astrocytomas.

**EPENDYMALTUMORS (FIG. 5.9)**

These tumors are considered to originate from radial glia, subependymal glial cells, ependymal cells, or precursors thereof. Histologically, the majority of these tumors are low-grade (WHO grade II) or anaplastic (WHO grade III) “classic” ependymoma, with a smaller subset diagnosed as subependymoma or myxopapillary ependymoma (both WHO grade I). Indeed, most of the tumors in this group ultrastructurally show ependymal characteristics such as formation of cilia and microvilli at the luminal surface of the tumor cells, presence of intracytoplasmic microlumina, and junctional complexes at the lateral cellular surfaces. These structures explain the EMA staining of the luminal surface of ependymal rosettes and the dot- or ring-like intracytoplasmic staining of nonrosetted cells. Ependymal tumors are variably GFAP-positive, typically show only limited, if any, Olig-2 and cytokeratin expression, and are negative for neuronal markers except in very rare examples (Rodriguez et al., 2007). Recent studies suggest that a more robust and clinically relevant classification of ependymal tumors can be achieved by combining histology with information about tumor location (supratentorial, posterior fossa, spinal canal) and molecular characteristics (Pajtler et al., 2015).

**Subependymoma (WHO grade I)**

Subependymomas are diagnosed most often in middle-aged and older patients, occasionally in familial setting. They are slow-growing, intraventricular or spinal, sharply demarcated neoplasms, histologically showing clusters of monomorphic nuclei in abundant, paucicellular, fibrillar matrix with variable microcystic change (Fig. 5.9A) (Prayson and Suh, 1999). Nuclear pleomorphism may be present but does not carry prognostic significance. The tumor shows variable GFAP staining. Ependymal rosettes and perivascular pseudorosettes are often not prominent, and EMA staining may be absent.

**Myxopapillary ependymoma (WHO grade I)**

This entity almost exclusively occurs in the lumbosacral region of the spinal cord. Histologically, the tumor is characterized by a variably papillary architecture with central, often markedly hyalinized, fibrovascular cores (Fig. 5.9B). These cores are surrounded by radially arranged, elongated to cuboidal tumor cells with a basophilic myxoid or mucoid matrix accumulated between the ependymal cells and perivascular tumor processes (Prayson, 1997). Occasionally, a more solid growth pattern predominates, making the differential diagnosis with classic ependymoma more difficult. Of interest, most myxopapillary ependymomas lack the luminal and intracytoplasmic dot-like EMA staining typically encountered in classic ependymoma (Cho et al., 2009). Positive GFAP staining is helpful to recognize these tumors as glial (rather than, e.g., metastatic (papillary) carcinoma, chondroma, chondrosarcoma, paraganglioma). Especially after incomplete resection, the clinical behavior of myxopapillary ependymoma may be more aggressive than its WHO grade I designation suggests (Stephen et al., 2012).

**Ependymoma (WHO grade II)**

Low-grade ependymomas may originate in the supratentorial compartment and posterior fossa, typically (but not always) along the ventricular system, as well as in or adjacent to the spinal cord. Posterior fossa ependymomas are most frequent in children, whereas most spinal ependymomas occur in adults. Patients with neurofibromatosis type 2 often have multiple tumors in the spinal cord (Tarapore et al., 2013). Classic ependymomas are circumscribed neoplasms with variable cellularity, low proliferative activity, and a high nuclear-to-cytoplasmic ratio with bland nuclei and speckled chromatin. An important diagnostic clue is the formation of perivascular pseudorosettes, i.e., perivascular nuclear-free zones caused by radially arranged, GFAP-positive fibrillar tumor cell processes (Fig. 5.9D, E). In a minority of these neoplasms, ependymal (“true”) rosettes are present, consisting of radially arranged tumor cells around a small, round lumen. Even less frequent is the formation of larger canals surrounded by such tumor cells (Fig. 5.9C). The luminal sides of the cells in these rosettes and canals as well as the intracytoplasmic lumina are EMA-positive. Variants of ependymoma included in the WHO 2016 classification are: papillary ependymoma, showing fronds of tumor cells surrounding glovascular cores, the tumor cell processes abutting the vessels in a pseudorosette-like manner (Fig. 5.9G); clear cell ependymoma, most often occurring as anaplastic supratentorial masses with tumor cells showing clear, perinuclear haloes (Fig. 5.9H),
Fig. 5.9. See legend on next page.
thereby not infrequently raising the differential diagnosis of oligodendroglioma, pilocytic astrocytoma, neurocytoma, clear cell carcinoma and/or hemangioblastoma; tanycytic ependymoma, mostly occurring in the spinal cord and characterized by bundles of elongated spindled cells, often with less easily discernible pseudorosette formation (Fig. 5.9I), thus often mimicking pilocytic astrocytoma or schwannoma. Occasionally, ependymomas may show neuronal differentiation (Prayson, 1999; Rodriguez et al., 2007; Godfraind, 2009).

**Anaplastic ependymoma (WHO grade III)**

At present, anaplastic ependymoma is defined as an ependymoma showing a high nuclear-to-cyttoplasmic ratio and a high mitotic count (Fig. 5.9H), although definitions often vary among investigators. Nodules of high cellular density and areas of necrosis may occur in low-grade ependymoma, but pseudopalisading necrosis and MVP (Fig. 5.9I) argue for increased malignancy grade. The presence of areas of very high cell density in these tumors may raise the differential diagnosis of an embryonal tumor such as medulloblastoma, especially in cases where pseudorosettes are difficult to identify. Anaplastic ependymomas mainly occur intracranially and may be of the papillary, clear cell, or (more rarely) tanycytic variant. Importantly, the association between the histologic grade (II versus III) and clinical behavior is weaker than with the diffuse gliomas (Godfraind, 2009; Ellison et al., 2011). This can at least partly be explained by intratumoral heterogeneity for the histologic features used for grading and the fact that they leave room for subjective interpretation. More importantly, however, their typically solid growth patterns make them more amenable to resection than diffuse gliomas and, as such, extent of resection is a more powerful prognostic variable than tumor grade.

**Other gliomas (Fig. 5.10)**

In the WHO 2016 classification, the group of “other gliomas” encompasses chordoid gliomas of the third ventricle, angiocentric glioma, and astroblastoma. The tumor cells in these neoplasms show glial and even ependymal differentiation, but they show unique features that separate them from the conventional ependymomas. These “other gliomas” are rare, generally slow-growing and (relatively) circumscribed macroscopically. However, occasional examples show anaplastic change and/or more aggressive behavior.

**Chordoid glioma of the third ventricle (WHO grade II)**

This entity occurs in (the anterior portion of) the third ventricle by definition and is typically encountered in adults. Histologically, the tumor generally consists of cords and clusters of epithelioid tumor cells (“chordoid” morphology) with low mitotic activity (Fig. 5.10A). In between these cells, mucinous stroma and a lymphoplasmacytic infiltrate with multiple Russell bodies are often present. The surrounding brain parenchyma may show reactive piloid gliosis with Rosenthal fibers. Less commonly, the epithelioid tumor cells may show a more solid, papillary, alveolar, or pseudoglandular growth pattern, and collections of fusiform tumor cells may be seen. Extensive fibrosis may be present as well. Immunohistochemically, the tumor cells are positive for GFAP, vimentin, and CD34, variably positive for cytokeratin and EMA, but negative for neuronal markers. EMA staining and the presence of microvilli, hemidesmosome-like structures and (abnormal) cilia at the ultrastructural level indicate ependymal differentiation, while the consistent nuclear TTF1 staining in these tumors may suggest that the neoplasm originates from the organum vasculosum of the lamina terminalis (Brat et al., 1998; Bielle et al., 2015).

**Fig. 5.9.** Ependymal tumors. Examples of World Health Organization grades I (A, B), II (C–G, I), and III (H, J–L). (A) Subependymoma is characterized by a vaguely nodular growth pattern, a densely fibrillar eosinophilic matrix, and clustering of rounded to oval tumor nuclei. (B) Myxopapillary ependymomas show central hyalinized blood vessels (arrow) surrounded by delicate tumor processes intermixed with a rich basophilic mucoid stroma and a concentric layer of tumor nuclei. (C) In very well-differentiated ependymomas, one may find ependymal canals (arrows) and true ependymal rosettes (arrowheads). (D) However, most ependymomas are characterized by perivascular pseudorosettes (arrows) with nuclear-free fibrillar zones surrounding blood vessels. (E) These pseudorosettes contain glial fibrillary acidic protein immunopositive cyttoplasmic processes (arrows) radiating towards central blood vessels. (F) Most ependymomas display dot-like epithelial membrane antigen immunoreactivity. (G) Pseudorosettes are also present in subependymomas. (H) Clear cell ependymomas have clear cytoplasm mimicking oligodendrogliaoma and often show anaplastic features, such as frequent mitoses (arrows). (I) Tanycytic ependymomas feature long thin cytoplasmic processes and less conspicuous pseudorosettes, mimicking schwannoma or pilocytic astrocytoma. (J) Anaplastic ependymomas often feature microvascular proliferation (arrows) and hypercellularity. A solid growth pattern with lack of centrally entrapped neurofilament-positive axons distinguishes anaplastic ependymoma from high-grade diffuse gliomas (K), while the higher-grade designation is supported by a high Ki-67 labeling index (L).
Angiocentric glioma (WHO grade I)

This neoplasm is generally superficially located in the cerebral hemisphere of children or young adults and causes epilepsy. Histologically, the tumor is characterized by perivascular (“angiocentric”) cuffs of monomorphous, bipolar or, less frequently, epithelioid cells with a parallel or radial orientation to vessel walls (Fig. 5.10B,C). This latter arrangement resembles pseudorosette formation, as is also seen in ependymal tumors and astroblastoma. Furthermore, the tumor cells may show subpial aggregation, form solid clusters, and/or show somewhat more diffuse infiltration in the brain parenchyma. Immunohistochemically, the tumor cells are GFAP-positive and negative for neuronal markers, while EMA-positive surface and dot-like (microlumen-type) positivity is often found (Fig. 5.10D), consistent with ependymal differentiation (Lellouch-Tubiana et al., 2005; Shakur et al., 2009). Angiocentric gliomas may rarely be associated with cortical dysplasia (Marburger and Prayson, 2011). Increased proliferative activity and unusual histologic features (e.g., a ganglioglioma- or astroblastoma-like component) are not clearly associated with more aggressive behavior (Li et al., 2012; Ni et al., 2015). The exact relationship between angiocentric gliomas and lesions reported as cortical ependymomas remains to be elucidated (Lehman et al., 2003; Van Gompel et al., 2011).

Astroblastoma

Astroblastoma typically occurs in the cerebral hemispheres of children and young adults. Histologically, epithelioid tumor cells with ample, eosinophilic, variably GFAP-positive cytoplasm characterize the neoplasm (Fig. 5.10E, F). The tumor cells are typically radially oriented around blood vessels with broad or slightly tapering processes towards vessel walls (“astroblastic pseudorosettes”). These vessel walls often show marked
fibrous thickening, sometimes with few neoplastic cells remaining in between. In other areas the tumor cells may be detached from the vessel walls and have a more cuboidal morphology and papillary architecture. Although the tumor cells may show some dot-like EMA staining, immunohistochemical and ultrastructural evidence for ependymal differentiation is found only focally in most examples. The clinical behavior of this rare tumor is variable, and the WHO grade is not entirely clear given the rarity of this subtype (Brat et al., 2000). Cases histologically showing increased mitotic activity, anaplastic nuclear features, MVP, and/or pseudopalisading necrosis tend to behave more aggressively (Salvati et al., 2009). Of note, focal astroblastoma-like histology may occasionally occur in other astrocytic and ependymal neoplasms.

**MIXED NEURONAL-GLIAL TUMORS**

**FIG. 5.11**

The neoplasms in this group are rare and characterized by proliferation of neoplastic cells showing a mixture of neuronal and glial (most often astrocytic) differentiation. Discrimination of mixed neuronal-glial tumors from diffuse gliomas with entrapped neurons may sometimes be difficult. Furthermore, even though gangliocytomas, dysembryoplastic neuroepithelial tumors (DNTs) and neurocytomas may display some glial differentiation, these tumors are generally not considered glial neoplasms and will not be further discussed here. For the diagnosis of mixed neuronal-glial tumors, immunohistochemistry using antibodies against neuronal (e.g., synaptophysin, Neu-N, MAP2, neurofilament) and glial (e.g., GFAP, OLIG2) markers is helpful or often essential. Most neuronal-glial tumors are well-delineated and slow-growing lesions (WHO grade I), but rare examples show anaplastic signs (especially increased mitotic activity and/or pseudopalisading necrosis) associated with more aggressive behavior.

**Ganglioglioma (WHO grade I)**

Gangliogliomas occur in different age groups and throughout the CNS with a marked predilection for the temporal lobe. The relative contribution of neoplastic ganglion cells and glial cells and their morphologic phenotype is quite variable, both within and between tumors. The neoplastic ganglion cells generally show dysmorphic features (e.g., binucleate forms, abnormally large size, abnormal position of Nissl substance) and abnormal orientation (Fig. 5.11A–C). The neoplastic glial cells are considered to be the proliferative component and may have an astrocytic (either resembling fibrillary or pilocytic type) or oligodendrogial phenotype, although the latter is quite rare. As in other “nondiffuse gliomas,” Rosenthal fibers and eosinophilic granular bodies are often encountered. Furthermore, calcifications and perivascular lymphocytic infiltrates may be present, while typically mitotic activity is low and necrosis is absent (Luyken et al., 2004). In contrast to normal brain and diffuse gliomas, the vast majority of gangliogliomas show CD34 staining of not only endothelial cells, but also distinct cells with branching processes (Fig. 5.11D), either within the tumor or adjacent cortex (Blumcke et al., 1999; Blumcke and Wiestler, 2002). As with PXAs and DNTs, staining for BRAF V600E-mutant protein is also common, with the strongest expression typically seen in the ganglion cell component (Koelsche et al., 2013; Prabowo et al., 2014).

**Anaplastic ganglioglioma (WHO grade III)**

Anaplastic transformation is rare in gangliogliomas, but occurs most often in the glial component; criteria include brisk mitotic activity, often combined with increased cellularity, nuclear pleomorphism and, in some cases, even florid MVP and/pseudopalisading necrosis (Fig. 5.11E) (Majores et al., 2008). These tumors are designated as anaplastic (WHO grade III) gangliogliomas.

**Desmoplastic infantile astrocytoma (DIA) and ganglioglioma (DIG) (WHO grade I)**

DIAs and DIGs typically are large, partly cystic tumors, often with focal dural attachment and calcifications in the cerebral hemisphere of infants. As the name implies, DIAs and DIGs are characterized by the presence of a reticulin-rich desmoplastic stroma which often imparts the impression of a mesenchymal neoplasm, particularly since the neoplastic glial and neuronal cells (in DIG only) are often small and inconspicuous compared to conventional gangliogliomas (Fig. 5.11G, H). Some examples feature a component of smaller, less-differentiated neuroepithelial cells resembling an embryonal tumor. This latter component may be dominant and look worrisome, especially when mitotic activity is increased. However, even in such cases the clinical behavior is generally benign (Louis et al., 1992; VandenBerg, 1993; Tamburrini et al., 2003).

**Papillary glioneuronal tumor (WHO grade I)**

Papillary glioneuronal tumors occur mostly in young adults, and have a predilection for the cerebral hemispheres, in particular the temporal lobe. Histologically, these neoplasms typically show flat to cuboidal astrocytic cells, lining hyalinized blood vessels and arranged in pseudopapillary structures, with intervening sheets of neurocytes, more mature ganglion cells, and sometimes minigemistocytes (Fig. 5.11J). Rosenthal fibers,
Fig. 5.11. See legend on next page.
Rosette-forming glioneuronal tumor (WHO grade I)

These neoplasms mostly occur in the midline, especially in the fourth ventricle or adjacent structures of adults, although an increasing range of locations are being reported (Hsu et al., 2012). Histologically, the tumor is characterized by a low to moderate cellularity with the neuronal component manifesting as neurocytic rosettes and rarely ganglion cells, while the glial component resembles pilocytic astrocytoma and oligodendroglioma. Rounded glioneuronal cells with clear haloes form neurocytic rosettes and perivascular pseudo-rosettes in a mucinous, microcystic background, with synaptophysin mainly staining only the central neuropil of these (pseudo)rosettes (Fig. 5.11K). The astrocytic component is GFAP-positive and may harbor Rosenthal fibers, eosinophilic granular bodies, and calcifications. MVP and thrombosis may be seen, but mitotic activity is usually low and necrosis absent (Komori et al., 2002; Jacques et al., 2006).

Diffuse leptomeningeal glioneuronal tumor

This rare tumor was only recently described, occurs predominantly in children, and has previously been reported as disseminated oligodendroglioma-like leptomeningeal neoplasm or similar names. As this former name indicates, the tumor is characterized by extensive spread of oligodendrocyte-like cells in the leptomeninges, often without a clear mass in the brain or spinal cord and sometimes with a neoplastic neuronal component (Fig. 5.11L). The oligodendroglia-like tumor cells generally show staining for S100 and OLIG2, while staining for GFAP and synaptophysin is variable. Most examples lack histologic features of anaplasia and show slow clinical progression, but a definitive WHO grade has not yet been assigned (Rodriguez et al., 2012). Interestingly, the molecular aberrations found in diffuse leptomeningeal glioneuronal tumor overlap with those of pilocytic astrocytoma (KIAA1549-BRAF gene fusion) and adult oligodendrogiomas (complete lp/19 co-deletion, although solitary lp loss is more common) (Schniederjan et al., 2013; Rodriguez et al., 2015).

WHO’S NEXT: COMBINATION OF HISTOLOGIC AND MOLECULAR INFORMATION

While histologic classification of glial neoplasms is a time-tested approach with important prognostic and therapeutic implications, unequivocal typing and grading of many of these tumors remained challenging. The identification of molecular biomarkers for different subsets of glial tumors enabled introduction of molecular characteristics in their definition and diagnosis. In 2014, a group of expert neuropathologists published the International Society of Neuropathology–Haarlem consensus guidelines, with suggestions as to how molecular information could be incorporated in the routine classification of CNS tumors (Louis et al., 2014). These guidelines laid the groundwork for an update of the WHO CNS tumor classification that indeed integrates histologic and molecular findings for a substantial number of glial neoplasms (Table 5.1 and Fig. 5.4). Additionally, in these guidelines a layered diagnostic approach was proposed that retains the option of a pure histologic diagnosis for centers/countries where molecular diagnostics is not available. In those cases, the abbreviation “NOS” (not otherwise specified) can be added to the
Fig. 5.12. See legend on next page.
histology-based diagnosis in order to send the message that molecular proof for that particular diagnosis was not obtained.

It is now clear that integration of molecular information in the pathologic diagnosis of gliomas is a paradigm shift that substantially improves the reproducibility of this diagnosis and of clinicopathologic predictions, especially in situations where histologic criteria are not precise enough for recognition of clinically relevant subgroups and where the tissue specimens are small or lack cardinal morphologic features of a specific entity (Sahm et al., 2014; Reuss et al., 2015a; Weller et al., 2015). At the same time, such an integrated approach brings new challenges as well. For instance, criteria for grading within molecularly defined categories of diffuse gliomas will require modification. In fact, parts of the histologic basis for typing and grading of gliomas are likely to become less relevant or may even be overruled by certain molecular findings (Reuss et al., 2015b). Meanwhile, it is important to realize that the molecular tests used for detection of particular aberrations may differ between centers and may yield some false-positive or false-negative results (Preusser et al., 2011; Wesseling et al., 2015).

In some situations the “molecular fingerprint” of glial and neuronal-glia neoplasms can be translated back to histology by utilizing immunohistochemical surrogate stains. Examples of how these are now commonly applied in diffuse glioma diagnoses (including those with mixed oligodendroglial and astrocytic features on histology) are shown in Figure 5.12. For some time already, widespread and strong nuclear staining for p53 protein in (glial) neoplasms has been used to suggest the presence of a TP53 mutation. As over half of the WHO grade II diffuse astrocytomas harbor this mutation, immunohistochemistry for p53 may be of help in solving the differential diagnosis of diffuse astrocytoma versus reactive astrocytosis or oligodendroglioma (Camelo-Piragua et al., 2011). More recently, highly specific antibodies targeting mutant IDH1 R132H, BRAF V600E, and H3 K27M protein have become commercially available (Capper et al., 2009; Routhier et al., 2013; Bechet et al., 2014). Staining of tumor cells for these antibodies reflects the presence of the corresponding mutation in these genes. Similarly, absence of ATRX expression in tumor cell nuclei correlates well (albeit not perfectly) with ATRX mutation (Wiestler et al., 2013). Obviously, such relatively simple, immunohistologic tests are much more widely available for the global community and may help to assess an integrated morphologic and molecular diagnosis in a substantial number of patients without performing molecular testing in a strict sense (Reuss et al., 2015c). Meanwhile, new discoveries in glioma biology and genetics will continue to have an impact on diagnostic strategies. A multidisciplinary team effort is key for optimal, coordinated translation of such new insights into improved management of the patients suffering from these tumors (Riemenschneider et al., 2013; Louis et al., 2014).

References


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