Molecular neuropathology of low-grade gliomas and its clinical impact

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With 5 Figures and 1 Table

Contents

Abstract

The term "low-grade glioma" refers to a heterogeneous group of slowly growing glial tumors corresponding histologically to World Health Organization (WHO) grade I or II. This group includes astrocytic, oligodendroglial, oligoastrocytic and ependymal tumor entities, most of which preferentially manifest in children and young adults. Depending on histological type and WHO grade, growth patterns of low-grade gliomas are quite variable, with some tumors diffusely infiltrating the surrounding central nervous system tissue and others showing well demarcated growth. Furthermore, some entities tend to recur and show spontaneous malignant progression while others remain stable for many years. This review provides a condensed overview concerning the molecular genetics of different glioma entities subsumed under the umbrella of low-grade glioma. For a better understanding the cardinal epidemiological, histological and immunohistochemical features of each entity are shortly outlined. Multiple cytogenetic, chromosomal and genetic alterations have been identified in low-grade gliomas to date, with distinct genetic patterns being associated with the individual tumor subtypes. Some of these molecular alterations may serve as a diagnostic adjunct for tumor classification in cases with ambiguous histological features. However, to date only few molecular changes have been associated with clinical outcome, such as the combined losses of chromosome arms 1p and 19q as a favorable prognostic marker in patients with oligodendroglial tumors.

Keywords: Astrocytoma; oligodendroglioma; ependymoma; 1p/19q; molecular genetics.

Abbreviations

WHO	World Health Organization
CBTRUS	Central Brain Tumor Registry of the United States
GFAP	glial fibrillary acid protein

MAP2	microtubule-associated protein 2
EGFR	epidermal growth factor receptor
LOH	loss of heterozygosity
PDGFRA	platelet-derived growth factor receptor alpha
PXA	pleomorphic xanthoastrocytoma
NF1	neurofibromatosis type 1
SEGA	subependymal giant cell astrocytoma
EMA	epithelial membrane antigen
CGH	comparative genomic hybridization

WHO classification and grading of low-grade gliomas

Gliomas are classified according to the WHO classification of tumors of the central nervous system [57]. In addition to tumor typing, the WHO classification includes a histological grading according to a four-tiered grading scale. WHO grade I lesions include tumors that have low proliferative potential and can be potentially cured following surgical resection alone. WHO grade II lesions are also characterized by a low proliferative activity, but are often infiltrative in nature and thus bear the tendency to recur. In addition, WHO grade II tumors often have the intrinsic ability to progress to higher grades of malignancy, with diffuse astrocytoma transforming to anaplastic astrocytoma and eventually secondary glioblastoma as a classic example. Nevertheless, owing to their shared low proliferative activity and the lack of histological signs of malignancy, WHO grade I and II gliomas are frequently subsumed under the category of "low-grade glioma". In contrast, glial tumors of WHO grades III and IV are referred to as "high-grade gliomas". It should be clear, however, that the dichotomy of "low-grade glioma" versus "high-grade gliomas", albeit common for clinical practice, is an oversimplification that carries potential pitfalls as heterogeneous tumor entities with different biological and clinical behavior are grouped together. For example, studies on "low-grade gliomas" often include both pilocytic and diffuse astrocytomas, which show quite distinct growth patterns and clinical outcome. Nevertheless, we stick here to the commonly used term of "low-grade glioma" and use it as a kind of umbrella to cover the different glioma entities and variants histologically corresponding to WHO grade I or II tumors. As stated previously, the low-grade glioma category includes a quite heterogeneous group of neoplasms that greatly differ in their clinical and histological appearances (Table 1). All these tumors are characterized by unique histological and immunohistochemical features. In addition, molecular studies during recent years have uncovered that they are also associated with distinct patterns of defined genetic changes, which may actually serve to supplement histopathological classification, especially in cases with borderline histological features.

Table 1. Classification of low-grade gliomas

Tumor type	WHO grade
Diffusely infiltrating astrocytic gliomas	
Diffuse astrocytoma	II
Fibrillary astrocytoma	II
Protoplasmic astrocytoma	II
Gemistocytic astrocytoma	II
Astrocytic gliomas with more circumscribed growth	
Pilocytic astrocytoma	I
Pilomyxoid astrocytoma	II
Pleomorphic xanthoastrocytoma	II
Subependymal giant cell astrocytoma	I
Oligodendrogliomas and mixed gliomas	
Oligodendroglioma	II
Oligoastrocytoma	II
Gliomas with ependymal differentiation	
Ependymoma	II
Myxopapillary ependymoma	I
Subependymoma	I
Other rare glial tumor types	
Chordoid glioma of the third ventricle	II
Astroblastoma	not determined
Angiocentric glioma	I

In this chapter we will provide an overview on the molecular aberrations typically associated with the individual entities of low-grade glioma. Each entity will be introduced with a brief paragraph on the characteristic epidemiological, histological and immunohistochemical features, followed by a review of the associated molecular characteristics.

Diffuse astrocytoma (WHO grade II)

Epidemiological, histological and immunohistochemical features

According to the Central Brain Tumor Registry of the United States (CBTRUS 2005), diffuse astrocytoma of WHO grade II has an annual incidence rate of 1.3/1 million population. Mean age of diagnosis is 46 years and the 5-year survival rate is about 45%. Histologically, the most common subtype is *fibrillary astrocytoma*, followed by *gemistocytic astrocytoma*, i.e. a diffuse astrocytoma consisting of more than 20% of gemistocytic astrocytes. Several reports indicate that

gemistocytic tumor cell differentiation is a prognostically unfavorable feature as these tumors tend to undergo more rapid malignant progression [72, 91]. A rare astrocytoma variant is *protoplasmatic astrocytoma*. In these cases, neoplastic astrocytes exhibit a small cell body with few, flaccid cell processes and only weak GFAP expression. Although diffuse astrocytomas are considered low-grade gliomas, they bear an inevitable tendency for recurrence and malignant progression to anaplastic astrocytoma and secondary glioblastoma.

Immunohistochemically, diffuse astrocytomas stain generally positive for glial fibrillary acid protein (GFAP), vimentin, protein S-100 and microtubule-associated protein 2 (MAP2). Nuclear accumulation of the tumor suppressor protein p53 is present in about 60% of diffuse astrocytomas, while immunoreactivitity for the epidermal growth factor receptor (EGFR) is rather a feature of high-grade gliomas. Labeling indices for the proliferation-associated antigen Ki-67 (MIB-1), while exhibiting considerable inter- and intratumoral variability, usually do not surpass a value of 5% positive tumor cells.

Molecular genetics

The most common genetic alteration in diffuse astrocytomas is mutation of the *TP53* tumor suppressor gene at 17p13.1 in approximately 60% of cases [36] (Fig. 1). In the gemistocytic variant *TP53* mutations are found in up to 80% of the cases [105]. Not only are *TP53* mutations already present in the first biopsy, but their frequency does not increase in recurrences, suggesting that those mutations are among the earliest events in astrocytoma development. This hypothesis is supported by the fact that brain tumors in patients harboring a *TP53* germline mutation predominantly correspond to astrocytic tumors (usually anaplastic astrocytoma or glioblastoma). In line with Knudson's double hit hypothesis, *TP53* mutations in diffuse astrocytomas are commonly associated with loss of heterozygosity (LOH) at polymorphic loci on 17p resulting in complete loss of wild-type p53 in the tumor cells. Diffuse astrocytomas with-

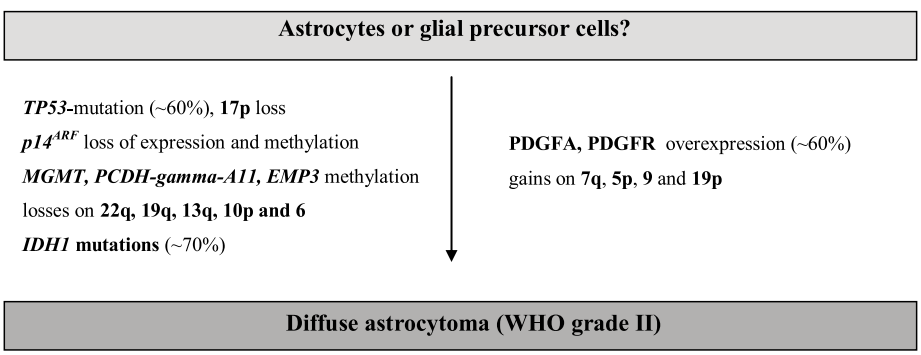

Fig. 1. Schematic representation of the molecular pathogenesis of diffuse astrocytoma

out *TP53* alterations frequently exhibit promoter methylation and loss of expression of the $p14^{ARF}$ gene at 9p21, the gene product of which regulates MDM2-mediated degradation of p53 [106]. Other frequent, just recently identified molecular alterations in diffuse astrocytomas are codon 132 mutations of the isocitrate dehydrogenase 1 (*IDH1*) gene, originally described preferentially in glioblastomas [71] and later shown to occur in also about 70% of diffuse astrocytomas [3]. Genes that have been reported to be epigenetically silenced in more than 50% of diffuse astrocytomas include the *MGMT* gene at 10q26 [106], the protocadherin-gamma subfamily A11 (*PCDH-gamma-A11*) gene at 5q31 [103], and the *EMP3* gene at 19q13 [50]. Interestingly, *MGMT* hypermethylation was found to be associated with *TP53* mutation but is mutually exclusive to $p14^{ARF}$ hypermethylation [106].

Another common alteration in diffuse astrocytomas is overexpression of the platelet-derived growth factor receptor alpha (PDGFRA) and its ligand PDGFalpha, thereby enabling an autocrine growth stimulation of the tumor cells [31]. *PDGFRA* amplification, however, is restricted to a small subset of high-grade gliomas, in particular glioblastomas [23].

Karyotyping and comparative genomic hybridization analyses revealed trisomy 7 or gains of chromosome 7q as a common genomic imbalance, which is detectable in up to 50% of diffuse astrocytomas. Further chromosomal aberrations comprise losses on 22q, 19q, 13q, 10p, 6 and the sex chromosomes as well as gains on 5p, 9 and 19p [79]. In contrast to oligodendrogliomas (see below), combined losses on 1p and 19q are rare in diffuse astrocytomas.

Pleomorphic xantoastrocytoma (WHO grade II)

Epidemiological, histological and immunohistochemical features

Pleomorphic xanthoastrocytoma (PXA) accounts for less than 1% of all astrocytic neoplasms. The tumor usually manifests within the first two decades of life; however, older patients are also affected on occasion. PXA is typically located superficially in the cerebral hemispheres, most often the temporal lobe, with frequent involvement of the leptomeninges. Therefore, patients often present with a long-standing history of seizures.

Histologically, PXA is a relatively compact and well circumscribed tumor growing in the cerebral cortex and invading the meninges. It is composed of pleomorphic astrocytic tumor cells, including bipolar spindle cells growing in fascicles, epitheloid cells, as well as multinucleated giant cells, with variable subsets of the neoplastic cells displaying cytoplasmatic lipidization. Further characteristic features include a pericellular or perilobular reticulin network, eosinophilic protein droplets and prominent lymphocytic infiltrates. The vast majority of PXAs correspond to WHO grade II. The rare cases that exhibit

anaplastic changes, such as increased mitotic count and necroses, are referred to as *pleomorphic xanthoastrocytoma with anaplastic features* [26].

Immunohistochemically, PXAs stain positively for GFAP and protein S-100. Nuclear immunoreactivity for the p53 tumor suppressor protein and expression of the epidermal growth factor receptor (EGFR) is usually absent. Instead, expression of the CD34 antigen is often found not only in vascular endothelial cells but also in tumor cells [80]. The Ki-67 (MIB-1) labeling index usually does not exceed 5% (with exception of the rare PXA with anaplastic features).

Molecular genetics

Loss on chromosome 9 is the most common genomic imbalance in pleomorphic xanthoastrocytoma, which is detectable by CGH analysis in 50% of cases [108]. Other losses affect chromosomes 17 (10%), 8, 18 and 22 (4% each). Chromosomal gains could be identified on chromosomes X (16%), 7, 9q, 20 (8% each), 4, 5 and 19 (4% each) [108]. *TP53* mutations are seen in a small fraction of tumors (<10 of cases), while 1p/19q losses as well as amplification of *EGFR*, *CDK4* and *MDM2* are absent [25, 44]. In contrast, homozygous deletion of the tumor suppressor genes *CDKN2A*, $p14^{ARF}$ and *CDKN2B* on 9p21.3 is common in PXA [108]. Interestingly, transcript levels of the *TSC1* gene on 9q were found to be consistently low in PXA; however, the causative mechanism still remains unclear, as there was no evidence for *TSC1* mutations or promoter methylation [108].

Pilocytic astrocytoma (WHO grade I)

Epidemiological, histological and immunohistochemical features

Pilocytic astrocytomas comprise approximately 5–6% of all gliomas. They are the most frequent primary brain tumors in children and most commonly develop during the first two decades of life. The majority of pilocytic astrocytomas develop in the cerebellum. Other typical sites include midline structures, such as the optic nerve and optic chiasm, hypothalamus, thalamus, basal ganglia, and brain stem, but also the cerebral hemispheres or the spinal cord may be affected.

Pilocytic astrocytomas belong to the group of astrocytic tumors that exhibit a more circumscribed growth pattern and thus can be cured following surgical resection alone. Histologically, they are tumors of low to moderate cellularity characterized by a biphasic growth pattern with areas of compacted bipolar (piloid) cells and loose-textured microcystic areas with multipolar cells. Rosenthal fibers and eosinophilic granular bodies are a common though not

specific diagnostic feature. The majority of pilocytic astrocytomas correspond to WHO grade I. Rare cases with anaplastic features have been described.

Recently, the pilomyxoid astrocytoma has been recognized as a histologically and clinically distinct variant of pilocytic astrocytoma with a less favorable prognosis [98]. Local recurrences as well as cerebrospinal spread occur more often in pilomyxoid tumors than in pilocytic astrocytomas. The WHO classification thus assigns these tumors to WHO grade II. Histologically, pilomyxoid astrocytomas are characterized by a monomorphic population of bipolar neoplastic astrocytes in a myxoid matrix. Pseudorosette-like angiocentric architectures are typical. Rosenthal fibers are often missing. Immunohistochemically, both pilocytic and pilomyxoid astrocytomas stain positively for GFAP, S-100 and vimentin. Ki-67 labeling indices are of minor diagnostic importance as they considerably overlap between both tumors and may vary substantially.

Molecular genetics

In comparison to diffuse astrocytic gliomas, far less is known about the chromosomal and genetic aberrations in pilocytic astrocytomas (Fig. 2). Molecular cytogenetic investigation of 48 pilocytic astrocytomas using comparative genomic hybridization revealed chromosomal imbalances only in a small subgroup of 7 neoplasms [88]. Gain of 9q34.1-qter in three cases was the most common abnormality. Another study reports on recurrent trisomies of chromosomes 5 and chromosome 7 in a series of 53 pilocytic astrocytomas [74].

Pilocytic astrocytomas are the most common gliomas in patients with neurofibromatosis type 1 (NF1). In this setting, pilocytic astrocytomas are typically located in the optic nerve, often bilaterally, and carry allelic losses at the *NF1* tumor suppressor gene locus at 17q11.2 [45]. Sporadic pilocytic astrocytomas, in contrast, rarely demonstrate allelic loss at the *NF1* locus [45]. Also, neither *NF1* mutations nor loss of *NF1* mRNA expression were found in sporadic pilocytic astrocytomas [110]. Pilocytic astrocytomas do not

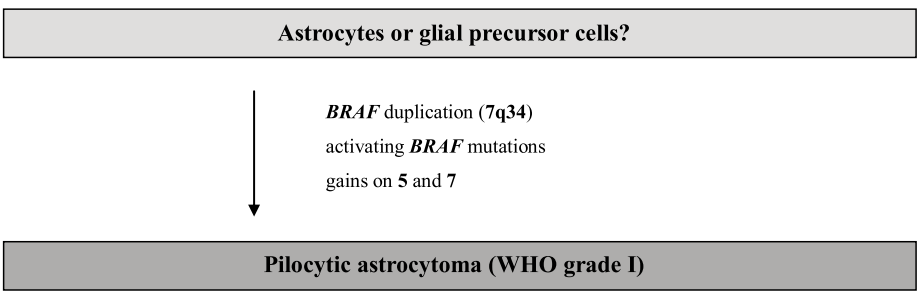

Fig. 2. Schematic representation of the molecular pathogenesis of pilocytic astrocytomas

share the high rate of allelic losses on 17p and mutations in the *TP53* tumor suppressor genes that are observed in diffuse astrocytomas [45, 52, 68]. However, circumscribed duplication of the *BRAF* gene at 7q34 resulting in increased BRAF expression has been identified as a common aberration in pilocytic astrocytomas [74]. A small subset of tumors alternatively carries activating *BRAF* mutations, thus implicating this gene as an important proto-oncogene in these tumors. Microarray-based expression profiling of pilocytic astrocytomas did not discriminate clinically aggressive or recurrent tumors from more indolent cases [93]. Similarly, expression profiles did not significantly differ between sporadic and NF1-associated pilocytic astrocytomas. However, supratentorial versus infratentorial tumors showed distinct gene expression signatures, suggesting that pilocytic astrocytomas, similar to findings in ependymomas, are characterized by lineage-specific molecular signatures that reflect the brain region in which they originate [93].

Subependymal giant cell astrocytoma (WHO grade I)

Epidemiological, histological and immunohistochemical features

Subependymal giant cell astrocytoma (SEGA) is closely associated with tuberous sclerosis, with an estimated 6% to 16% of tuberous sclerosis patients developing one or more of these tumors. As an intraventricular lesion, most often located in the region of the foramen of Monro, the tumor commonly manifests with symptoms of obstructive hydrocephalus and increased intracranial pressure. All SEGA patients should be clinically checked for the presence of other manifestations of tuberous sclerosis.

Histologically, the circumscribed, moderately cellular tumor is composed of large pleomorphic cells with abundant glassy eosinophilic cytoplasm, round ganglioid nuclei and distinct nucleoli. Intermixed smaller spindle cells as well as calcifications may be encountered. Mitoses are usually absent or rare. Immunohistochemically, SEGAs show variable expression of GFAP and S-100. In addition, immunoreactivity for neuronal markers such as synaptophysin or neurofilaments may be detectable. The Ki-67 (MIB-1) labeling index usually does not exceed 5%.

Molecular genetics

Biallelic inactivation of either the *TSC1* or the *TSC2* tumor suppressor gene is typical for these tumors [13]. Since the corresponding gene products have an inhibitory function on the mTOR pathway, their mutational inactivation leads to aberrant activation of mTOR signaling, which in turn represents an interesting novel target for specific pharmacologic inhibition. A comparative geno-

mic hybridization study on subependymal giant cell astrocytomas indicated that chromosomal imbalances are rare or absent [84].

Oligodendroglioma (WHO grade II)

Epidemiological, histological and immunohistochemical features

Oligodendroglioma accounts for approximately 2.5% of all primary brain tumors and 5–6% of all gliomas. Estimated annual incidence rates range from 0.27 to 0.35 per 100,000 persons. Oligodendrogliomas can develop at any age, but the majority of tumors arise in adults with an incidence peak between 40 and 45 years of age. In children younger than 14 years of age, oligodendroglial tumors account for only 2% of all brain tumors (CBTRUS 2005) [53, 70].

Oligodendrogliomas are monomorphous, moderately cellular, slowly growing, but diffusely infiltrating gliomas corresponding to WHO grade II. Histologically, the isomorphic tumor cells often display a characteristic "honeycomb" or "fried-egg" appearance on routine formalin-fixed paraffin sections, with uniform round to slightly oval nuclei and perinuclear halos due to cellular swelling and retraction of cytoplasmic processes. Microcalcifications, mucoid/cystic degeneration as well as a delicate, branching, so-called "chicken wire" vascular pattern are additional characteristic features in this entity. Prominent microvascular proliferation, necrosis and significantly increased mitotic or proliferative activity are absent in low-grade oligodendrogliomas. The infiltration patterns of oligodendrogliomas parallel those of other diffuse gliomas with more common involvement of cortical structures.

To date, the classification of oligodendrogliomas is still mainly based on the recognition of the histomorphological features described above, due to the fact that no specific oligodendroglial tumor markers have yet been identified. Oligodendrocyte lineage-specific transcription factors, such as OLIG-1 and OLIG-2, which originally appeared to be promising diagnostic markers for oligodendrogliomas [58, 61], have all been shown to be detectable not only in oligodendrogliomas but also in the vast majority of other gliomas [56, 85]. Oligodendrogliomas in general exhibit invariable and strong expression of the microtubule-associated protein 2 (MAP2) [5] as well as frequent overexpression of the epidermal growth factor receptor (EGFR). Immunoreactivity for protein S-100 is also common. In contrast to astrocytomas, GFAP immunoreactivity is either absent or scarce in oligodendrogliomas and generally restricted to special cell types, refered to as gliofibrillary oligodendrocytes and minigemistocytes. The tumors usually lack nuclear p53 staining, which may be due to the fact that *TP53* mutations and p53 protein accumulation are mutually exclusive to 1p/19q deletions (see below).

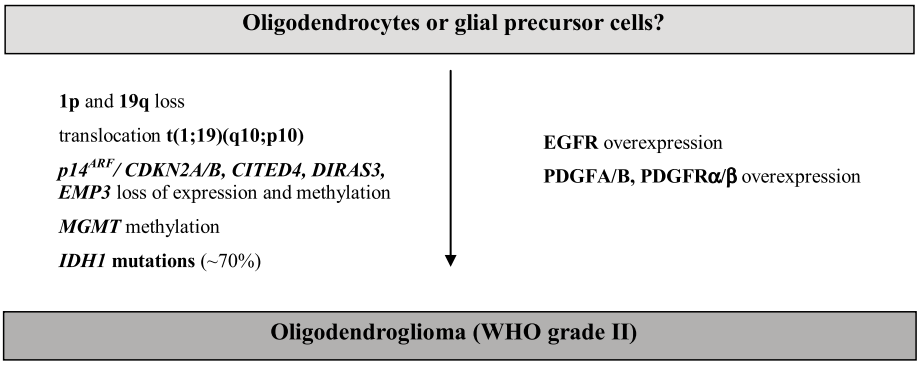

Fig. 3. Schematic representation of the molecular pathogenesis of oligodendrogliomas

Molecular genetics

The most common genetic alterations in oligodendrogliomas are combined deletions of chromosomal arms 1p and 19q as well as *IDH1* mutations. While oligodendrogliomas share the high frequency of *IDH1* mutations (~70%) with astrocytic gliomas [3], combined 1p/19q deletions are considered the hallmark genetic aberration in oligodendrogliomas and are found in up to 80% of the cases [83] (Fig. 3). Two recent studies reported that an unbalanced t(1;19)(q10;p10) translocation, with the chromosomal breakpoints located close to the centromers of both chromosomes, serves as the cytogenetic mechanism responsible for the frequent co-deletions of both chromosome arms in oligodendrogliomas [28, 39]. It is still unclear, however, which genes are the relevant targets on 1p and 19q. A number of candidate tumor suppressor genes from different regions on 1p have been proposed, including *TP73* (1p36.3), the calmodulin-binding transcription activator 1 gene (*CAMTA1*, 1p36), the DNA fragmentation factor subunit *β* gene (*DFFB*, 1p36), *SHREW1* (1p36.32), *CITED4* (1p34.2), *RAD54* (1p32), *CDKN2C* (1p32), and *DIRAS3* (1p31) [4, 16, 62, 63, 81, 86, 96]. In addition, the *NOTCH2* gene, which maps closest to the breakpoint region on 1p13-p11, has been reported as a putative oligodendroglioma-associated tumor suppressor gene, with intragenic homozygous deletions found in two tumors [7]. Candidate tumor suppressor genes on 19q include the *p190RhoGAP* gene at 19q13.3 [111], the myelin-related epithelial membrane protein gene 3 (*EMP3*) at 19q13.3 [1], *ZNF342*, a zinc-finger transcription factor gene at 19q13 [35], and the maternally imprinted *PEG3* gene at 19q13.4 [100]. However, none of these genes has been definitely proven to be directly involved in the tumorigenesis of oligodendrogliomas. Thus, the question remains as to whether two or more specific "1p/19q genes" exist in oligodendrogliomas.

Clinically, combined deletion on 1p and 19q has become of paramount importance as it was shown to be a powerful molecular marker for response

to chemotherapy and prolonged survival, in particular in patients with high-grade oligodendroglial tumors [10, 11, 102]. The prognostic role of 1p/19q deletion in low-grade oligodendroglioma patients is less clear. Several retrospective studies on small numbers of patients independently reported that 1p deletion or 1p/19q co-deletion were also associated with a trend towards longer survival [22, 89, 94]. More recent studies including larger numbers of low-grade oligodendroglioma patients indeed indicated a prognostic significance [43, 49]. In addition, clinical trials on low-grade oligodendroglioma patients treated with temozolomide revealed that 1p loss was associated with objective response to treatment [33, 55]. On the other hand, a study on low-grade oligodendroglioma patients treated by surgical resection alone did not reveal significantly longer survival of patients with 1p/19q deletion, suggesting that the prognostic significance of this genetic feature is linked to cytotoxic treatments, such as radio- and chemotherapy [109]. Concerning tumor location, it has been reported that allelic losses on 1p are common in oligodendroglial tumors of the frontal, parietal and occipital lobes but rare in tumors of the temporal lobe, insula and diencephalon [113].

While 1p/19q deletions are by far the most frequent alterations in oligodendrogliomas, several other genetic and epigenetic alterations have been described. Cytogenetic alterations that are less frequent than 1p/19q losses but occur at more than random frequency in oligodendrogliomas are gains on chromosomes 7 and 19p as well as losses on chromosomes 4, 6, 9p, 10q, 11p, 14, 18q, and 22q [41, 81]. Interestingly, several of these chromosomal imbalances have been suggested as being linked to poor outcome, including gain of 7p and 8q as well as losses on 9p and 18q [99]. Allelic losses on 17p and *TP53* gene mutations are rare in low-grade oligodendrogliomas and mutually exclusive to 1p/19q losses. Nevertheless, inactivation of the p53 pathway in 1p/19q-deleted tumors may be mediated by alterations of other members of the p53 pathway, such as epigenetic silencing of the $p14^{ARF}$ gene. $p14^{ARF}$ encodes a negative regulator of p53 activity that binds to Mdm2 and in this way inhibits the Mdm2-mediated degradation of p53 [107, 112]. Additional hypermethylated genes in subsets of oligodendrogliomas include the tumor suppressors *CDKN2A*, *CDKN2B* and *RB1*, as well as *DAPK1* (death-associated protein kinase 1), *ESR1* (estrogen receptor 1), *THBS* (thrombospondin 1) and *TIMP3* (tissue inhibitor of metalloproteinase 3) [2, 17, 112]. Frequent promoter hypermethylation and reduced expression of the *MGMT* gene in oligodendrogliomas and consecutive impairment of MGMT-mediated DNA-repair might in part contribute to the chemosensitivity of these neoplasms [64, 66].

Finally, low-grade oligodendrogliomas frequently demonstrate increased expression of growth factor receptors, such as EGFR, PDGFR and VEGF [15, 81]. While the mechanisms causing upregulation of EGFR expression in

these tumors are widely unknown, and *EGFR* amplification is restricted to rare cases of anaplastic tumors [24, 34, 37], PDGFR and its ligand PDGF are frequently co-expressed in oligodendroglial tumors, suggesting auto- and/or paracrine growth stimulatory activities of this signaling pathway [15].

Oligoastrocytoma (WHO grade II)

Epidemiological, histological and immunohistochemical features

Oligoastrocytoma is defined as a diffusely infiltrating glioma composed of a conspicuous mixture of two distinct neoplastic cell types morphologically resembling the tumor cells in oligodendroglioma and diffuse astrocytoma of WHO grade II. The oligodendroglial and astroglial components may either be diffusely intermingled or separated into distinct, biphasic areas. Oligoastrocytomas are diffusely infiltrating gliomas of moderate cellularity and low mitotic activity.

The role of a quantitative assessment of the astrocytic and oligodendroglial tumor components in oligoastrocytomas is disputed. In particular, the WHO classification does not define a diagnostic threshold that is minimally required for the minor tumor component. Owing to these loosely defined classification criteria, the fraction of oligoastrocytomas among all low-grade diffuse gliomas varies considerably between different studies, with values ranging from 13% up to 27% in larger series. Estimated annual incidence rates range between 0.10 and 0.16 per 100,000 population (CBTRUS 2005) [49, 70, 101].

Molecular genetics

In contrast to the phenotypic heterogeneity at the histological level, molecular analysis of microdissected oligodendroglial and astrocytic tumor parts in individual tumors revealed uniform genetic changes, indicating a monoclonal origin of both components [48]. Oligoastrocytomas share the high frequency of *IDH1* point mutations with diffuse astrocytomas and oligodendrogliomas [3]. Approximately 30–50% of the oligoastrocytomas show allelic losses on 1p and 19q [20, 70, 81]. Loss of heterozygosity on 17p and/or *TP53* mutation have been detected in approximately 30–40% of the cases, with the *TP53* mutation being mutually exclusive to 1p/19q deletion [60, 67, 70, 112]. Histologically, oligoastrocytomas with 1p/19q loss are frequently oligodendroglioma-predominant, whereas oligoastrocytomas with *TP53* mutations are more often astrocytoma-predominant [60]. Furthermore, oligoastrocytomas of the temporal lobe demonstrate less frequent 1p and 19q losses when compared to oligoastrocytomas in other tumor locations [67]. On the contrary,

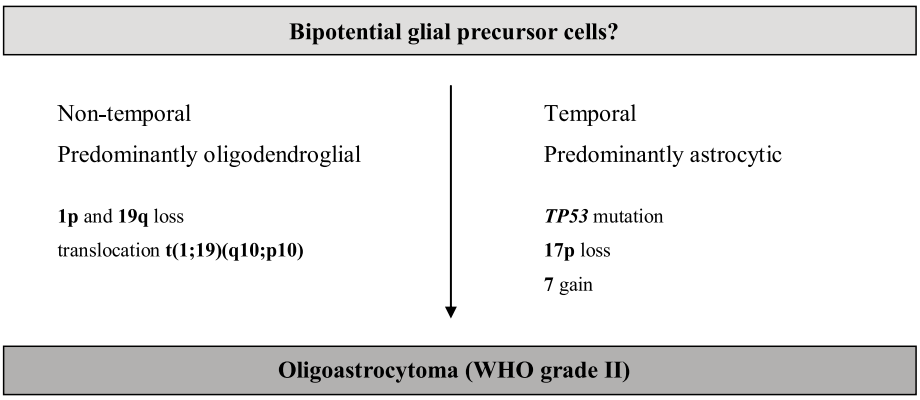

Fig. 4. Schematic representation of the molecular pathogenesis of oligoastrocytomas

TP53 mutations were significantly more common in temporal oligoastrocytomas than in oligoastrocytomas affecting other cerebral lobes (Fig. 4).

To date, no specific genetic abnormalities have been identified that genetically may separate oligoastrocytomas from oligodendrogliomas on the one side and diffuse astrocytomas on the other. Thus, in times of modern tumor biology and evolving molecular diagnostics, the nosological position of oligoastrocytomas is debatable. In support of this notion, oligoastrocytomas with 1p and 19q deletion behave clinically like oligodendrogliomas, while oligoastrocytomas without these deletions show a clinical course similar to diffuse astrocytomas [20]. Thus, the combination of morphological and molecular parameters may likely reshape the definition of oligoastrocytoma in the future.

Ependymoma (WHO grade II)

Epidemiological, histological and immunohistochemical features

Ependymal tumors account for approximately 2.3% of all primary brain tumors and 5.6% of all gliomas. The adjusted annual incidence rate is 0.33 per 100,000 population (CBTRUS 2005). There are two distinct incidence peaks: one in adults between 35 and 45 years of age, and one in children below 14 years of age.

Histologically, ependymomas are moderately cellular, slowly growing tumors that typically originate from the walls of the cerebral ventricles or the spinal cord. The tumor cells are uniform in shape and size, and usually have monomorphic round or oval nuclei with abundant, clumped chromatin. Hallmark histological features are perivascular pseudorosettes and true ependymal rosettes. In contrast to the diffusely infiltrating astrocytic and oligoden-

droglial tumors, ependymomas are characterized by a usually sharp interface with the surrounding CNS parenchyma.

Immunohistochemically, the majority of ependymomas stains with antibodies against the epithelial membrane antigen (EMA). Characteristic is a dot- or ring-like staining pattern or a linear labeling of luminal surfaces [30]. Expression of glial markers, such as GFAP, vimentin and protein S-100 is also commonly observed. The Ki-67 (MIB-1) index is generally low (< 5%).

Molecular genetics

Recent studies using comparative genomic hybridization (CGH) analysis reported on distinct patterns of chromosomal aberrations being linked to certain clinical and pathological features of ependymomas, such as patient age, tumor location, and histological subtype or WHO grade [12, 18, 40, 65, 95]. Overall, the most common copy number changes were losses of chromosomes 6q, 10, 13, 14, and 22q, as well as gains of chromosomes 1q, 7, 9, 12q, 15q, and 18. While spinal intramedullary ependymomas preferentially demonstrated losses of chromosomes 22q and 14q as well as gains on chromosomes 7q, 9p and 16, intracranial ependymomas frequently carried gains of 1q and losses on 6q (Fig. 5). Losses on 22q and gains of chromosome 4 were more common in adult tumors [65]. Gains on 1q correlated with the presence of structural chromosomal aberrations, pediatric age, high-grade histology and aggressive clinical behavior [12, 18, 65]. The distinct genetic profiles associated with tumor location were also reflected in regionally different mRNA expression signatures. Supratentorial ependymomas, for example, expressed elevated levels of members of the EPHB-EPHRIN and NOTCH families, whereas spinal ependymomas showed up-regulated expression of multiple HOX gene family members [95].

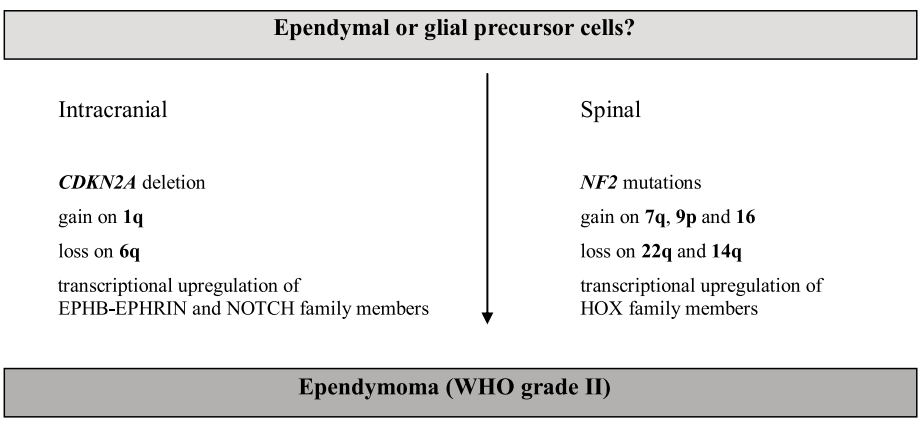

Fig. 5. Schematic representation of the molecular pathogenesis of ependymal gliomas

Molecular genetic studies on selected candidate genes revealed frequent *NF2* gene mutations in intramedullary spinal ependymomas, while deletions of the *CDKN2A* gene were frequent in intracranial supratentorial ependymomas but rare in ependymomas from other locations [95]. Mutations in the *TP53*, *PTEN* and *INI1* tumor suppressor genes are rare or absent in ependymomas [19, 47, 69]. Epigenetic silencing by aberrant promoter methylation has been described in ependymomas, affecting the known tumor suppressor genes *RASSF1*, *CDKN2A*, *CDKN2B*, $p14^{ARF}$ and *TP73*, as well as other genes potentially involved in the tumorigenesis of ependymomas, such as *CASP1*, *MGMT*, *TIMP3* and *THBS1* [29, 87]. As in other gliomas, growth factor receptors, such as EGFR and the related ERBB2 and ERBB4 receptors, are commonly upregulated in ependymal tumors and have been linked to faster tumor growth and shorter survival [27, 65]. Amplification of the respective genes, however, does not occur in low-grade ependymomas [65].

Myxopapillary ependymoma (WHO grade I)

Epidemiological, histological and immunohistochemical features

Myxopapillary ependymoma constitutes a distinct type of a benign ependymal neoplasm that typically arises in the conus-cauda-filum terminale region of the spinal cord. Corresponding to WHO grade I, this slowly growing tumor follows a favorable benign course. Anaplastic variants are virtually unknown. The frequency of myxopapillary ependymomas among all ependymomas is about 10% [51, 92]. In the conus-cauda region myxopapillary ependymomas are the most common intramedullary neoplasms with incidence rates of about 0.05 to 0.08 per 100,000 persons per year (CBTRUS 2005).

Histologically, myxopapillary ependymomas are characterized by tumor cells forming papillary structures around vascularized mucoid stroma cores. An alcian-blue positive, myxoid and often microcystic matrix is abundant between tumor cells and blood vessels. Immunohistochemistry is positive for GFAP, S-100 and vimentin. Punctate or ring-like EMA-staining may also be observed. Mitotic activity and MIB-1 labeling indices are generally low.

Molecular genetics

In spite of their benign clinical behavior, myxopapillary ependymomas are often aneuploid or tetraploid and carry numerous chromosomal imbalances as determined by CGH analysis. In fact, the average number of chromosomal aberrations per tumor is considerably higher than that in ependymomas

and anaplastic ependymomas [90]. The most common imbalances are concurrent gains of chromosomes 9 and 18 [59]. Additional recurrent alterations include gains of chromosomes 3, 4, 7, 8, 11, 13, 17q, 20 and X, as well as losses of chromosomes 10 and 22. Also, cDNA profiles with high expression levels of HOXB5, PLA2G5 and ITH2 in myxopapillary ependymomas clearly differed from those in intracranial ependymomas [46], thus indicating that myxopapillary ependymomas are molecularly distinct from other ependymal tumors.

Subependymoma (WHO grade I)

Epidemiological, histological and immunohistochemical features

Subependymoma is a slowly growing, benign glioma corresponding to WHO grade I. It is typically attached to a ventricular wall, and arises most frequently in the fourth ventricle, followed by the lateral ventricles. As subependymomas often remain asymptomatic, precise estimation of their incidence is difficult. In a retrospective series of 298 ependymal tumors, subependymoma accounted for 8.3% of the cases [92].

Histologically, subependymomas are paucicellular lesions composed of clusters of uniform, cytologically bland cells embedded in a densely fibrillar glial matrix. Many tumors exhibit microcystic degeneration. Formation of perivascular pseudorosettes is indistinct and true ependymal rosettes are usually absent. Immunohistochemically, subependymomas are positive for GFAP, protein S-100 and vimentin. Focal dot-like EMA staining may be seen. MIB-1 positivity is low, with reported mean labeling indices of less than 1% [76].

Molecular genetics

Molecular genetic data on subependymomas are scarce. Cytogenetic investigation of two tumors revealed no structural or numerical abnormalities [14]. Individual cases studied for allelic losses on chromosome arms 10q and 22q, as well as for mutations in the *hSNF5/INI1*, *NF2* and *PTEN* genes did not show any aberrations [14].

Other rare low-grade gliomas

Chordoid glioma of the third ventricle (WHO grade II)

Chordoid glioma of the third ventricle is a rare, slowly growing, non-invasive glioma located in the anterior parts of the third ventricle and histologically

corresponds to WHO grade II. Chordoid gliomas preferentially manifest in adults with a wide age range from 25 to 75 years [9, 78, 82]. The histogenesis of third ventricular chordoid gliomas is unknown, with proposed origins either from specialized ependymal cells in the subcommissural organ or from so-called tanycytes.

Histologically, chordoid gliomas are solid tumors of moderate cellularity characterized by clusters, ribbons and cords of epitheloid tumor cells with prominent eosinophilic cytoplasm and relatively uniform nuclei that are embedded in an alcianophilic, mucinous and sometimes vacuolated matrix. The tumors are sharply demarcated from the surrounding brain tissue, mitotic activity is low and histological signs of anaplasia are absent. Immunohistochemically, chordoid gliomas strongly express GFAP, vimentin and CD34. EGF receptors and merlin/schwannomin may also be expressed [82]. The MIB-1 labeling index is generally low.

To date, only a few cases have been subjected to molecular analyses. A comparative genomic hybridization study of four tumors did not identify any chromosomal imbalances [82]. Hallmark alterations of other common central nervous system tumors like deletions on chromosome 22 in meningiomas or genetic alterations of the *TP53, CDKN2A, EGFR, CDK4* and *MDM2* genes were all absent, reinforcing the notion that chordoid gliomas of the third ventricle must be regarded as a distinct glioma entity.

Astroblastoma

Astroblastoma is a rare glial neoplasm that mainly affects children, adolescents and young adults. In a study of 20 patients, the average age at diagnosis was 14 years (range: 3–46 years; [8]. Individual cases of congenital astroblastoma as well as astroblastomas in patients over 50 years of age have also been reported [32, 75, 97].

Astroblastoma is a usually well-circumscribed glioma. Its histological hallmark is the formation of distinctive perivascular pseudorosettes (so-called "astroblastic pseudorosettes"). These pseudorosettes are characterized by a single layer of epitheloid tumor cells sending broad, non-tapering processes towards a central blood vessel. Vascular thickening and hyalinization are further characteristic histological features. Astroblastoma is not assigned to a distinct WHO grade because exact grading is still an undefined issue. However, histological subdivision into low-grade (well-differentiated) and high-grade (anaplastic) lesions has been suggested and is of prognostic significance [6, 8, 97]. Astroblastomas show immunoreactivity for GFAP, S-100 protein, vimentin and Leu-7/HNK-1 [6, 8, 38]. A mean MIB-1 index of 3.2% has been reported in well-differentiated tumors as compared to 15.5% in anaplastic variants [8].

Knowledge about the chromosomal and genetic alterations in astroblastomas is limited and restricted to small series of tumors or single reported cases. Cytogenetic analysis of an astroblastoma from a 15-year-old girl showed an abnormal hypodiploid karyotype with 45 chromosomes, monosomies of chromosomes 10, 21, and 22 and two marker chromosomes [38]. Studies employing comparative genomic hybridization analysis revealed gains of chromosome arm 20q and chromosome 19 as the most frequent genomic alterations [8]. Recurrent losses were found on 9q, 10 and the X-chromosome. These results suggest a distinct pattern of genetic aberrations in astroblastomas as compared to other glioma entities.

Angiocentric glioma (WHO grade I)

Angiocentric glioma is a rare, stable or slowly growing cerebral tumor that histologically corresponds to WHO grade I and is often associated with chronic epilepsy. Angiocentric glioma has only recently been defined as a distinct entity and was newly introduced in the 2007 WHO classification [57]. To date, less than 30 patients have been reported, with a mean age at diagnosis of 17 years (range 2.3–70 years) [54, 77, 104].

Histologically, the tumor is characterized by remarkably monomorphic, bipolar spindle cells with an angiocentric growth pattern. The cells form mono- or multi-layered sleeves that extend lengthwise along vascular axes or may appear as radial pseudorosettes of ependymomatous nature. Immunohistochemically, tumor cells label for GFAP, S-100 and vimentin and also exhibit frequent dot-like cytoplasmatic labelling for EMA, as commonly observed in ependymomas. MIB-1 labelling indexes are usually below 5%.

Due to the limited number of reported cases, little is known about the underlying cytogenetic, chromosomal or genetic alterations. Chromosomal comparative genomic hybridization (CGH) revealed a loss of the chromosomal region 6q24-q25 in one out of eight cases. High-resolution array CGH suggested the *PTPRJ* (protein-tyrosine phosphatise receptor type J) gene as a possible target of copy number gain at 11p11.2 in one out of three cases investigated [77].

Clinical significance of molecular genetic alterations

The classification of low-grade gliomas is still mainly based on histological findings. Immunohistochemistry serves as a valuable adjunct, especially in cases with inconclusive or borderline histological features. As outlined above, a considerable amount of knowledge concerning the molecular alterations involved in the initiation or progression of distinct glioma entities has

been accumulated over the past years. Several of the identified molecular alterations have been investigated in regard to diagnostic and/or prognostic implications, but so far only few aberrations qualified as clinically relevant markers.

In this regard, combined deletion of chromosome arm 1p and 19q is undisputably the most important alteration. Initially reported by Cairncross and colleagues in 1998 [11], several retrospective studies have confirmed combined deletions of 1p and 19q as an independent marker of favorable response to radio- and chemotherapy as well as longer survival [21, 22, 34, 94]. The considerable prognostic value of 1p/19q deletion in patients with anaplastic oligodendroglial tumors has been corroborated in two recently published prospective and randomized phase III trials involving 368 patients and 289 patients, respectively [10, 102]. As a consequence of the major prognostic significance of the 1p/19q status in patients with anaplastic gliomas treated with radio- and/or chemotherapy, ongoing prospective trials are no longer stratifying anaplastic glioma patients according to histological type but according to the 1p/19q deletion status. Thus, it is likely that molecular testing for 1p/19q deletion will become a routine adjunct to histology in the diagnostic assessment of anaplastic gliomas.

As outlined above (see paragraph on oligodendroglioma), the role of 1p/19q testing in patients with low-grade oligodendroglial tumors is less clear. This is due to the fact that the clinical implications of 1p/19q loss in WHO grade II oligodendrogliomas are still based on only a few retrospective studies, most of which suggested that low-grade oligodendrogliomas with 1p/19q loss are also associated with longer survival times and greater likelihood of response to chemotherapy at the time of recurrence. Thus, 1p/19q testing in WHO grade II oligodendrogliomas is being requested by more and more clinicians and patients. For example, the demonstration of 1p/19q loss may provide additional reassurance that a particular tumor can be closely followed, rather than aggressively treated up-front with either chemotherapy or radiation. In addition, knowledge of the 1p/19q status may be helpful to make therapeutic decisions at tumor progression.

The diagnostic significance of the 1p/19q status is still debated. Although 1p/19q deletion is closely associated with oligodendrogliomas showing classic histological features, 1p/19q loss should not be used as a decisive diagnostic criterion for the diagnosis of oligodendroglioma. In other words, 1p/19q testing is not be recommended to "rule in" or "rule out" a diagnosis of oligodendroglioma [81]. In line with this statement, the definition of oligodendroglioma in the latest WHO classification recognizes the frequent presence of 1p/19q deletions in these tumors but does not require this genetic alteration as an obligatory feature for making the diagnosis of oligodendroglioma. In fact, there are rare cases of histologically classic oligodendroglioma that lack detectable

1p/19q deletions, possibly due to small alterations that escape current detection methods.

For as much is known about 1p/19q as a prognostic marker, so little is known about the molecular mechanisms underlying its prognostic significance. As outlined above, the relevant target genes on 1p and 19q are still unidentified. Thus, it is unclear whether alterations in one or more genes on these chromosome arms, or rather completely different molecular changes may account for the clinically less aggressive behavior of 1p/19q-deleted tumors. It might also be conceivable that 1p/19q status is simply a surrogate marker for genetic or epigenetic alterations that influence treatment response and survival and are located on other chromosome arms. The observation that *MGMT* promoter hypermethylation is common in oligodendrogliomas with losses on 1p and 19q may point to at least one possible mechanism contributing to the chemosensitivity of these tumors [66]. Furthermore, one may speculate that not primarily the presence of 1p/19q loss but rather the absence of other prognostically unfavorable genetic alterations in 1p/19q-deleted tumors, e.g. losses of chromosome arms 9p, 10 and 18q or gains of chromosomes 7, 8q, 19q and 20, are responsible for the distinct clinical behavior [99]. All these issues remain to be investigated in future molecular and translational studies.

In contrast to oligodendrogliomas, mutations of the *TP53* gene and loss of heterozygosity are hallmark genetic changes in diffuse astrocytomas. Thus, the demonstration of a *TP53* mutation or the immunohistochemical detection of a nuclear accumulation of the p53 protein argues in favor of a diffuse astrocytoma as compared to oligodendroglioma. However, the sensitivity of *TP53* mutations and nuclear p53 accumulation as diagnostic markers for diffuse astrocytomas is not very high, as indicated by the fact that approximately 40% of diffuse astrocytoma lack these aberrations. In terms of prognosis, it has been suggested that diffuse astrocytomas with *TP53* mutation progress more frequently and earlier than diffuse astrocytomas without mutation. On univariate analysis, *TP53* mutation was a significant predictor of shorter time to progression [73]. However, this effect was largely due to a higher frequency of *TP53* mutation in gemistocytic astrocytomas, which tend to undergo malignant progression more rapidly than fibrillary astrocytomas. Thus, only the gemistocytic subtype but not *TP53* mutation remained as an unfavorable prognostic marker on multivariate analysis [73].

A diagnostic issue that may prospectively be facilitated by help of molecular markers is the differential diagnosis between diffuse and pilocytic astrocytomas. In addition to frequent *TP53* mutations, a recent integrated genomic analysis identified the isocitrate dehydrogenase 1 (*IDH1*) gene as frequently mutated in diffusely infiltrating astrocytic gliomas. In the initial study on 22 glioblastoma patients, mutations in the active site of *IDH1* occurred in a large fraction of young patients and were associated with an increased overall survival in

secondary glioblastoma patients [71]. A follow-up study analyzed *IDH1* codon 132 mutations in a larger series of 685 brain tumors comprising all major glioma subtypes and reported *IDH1* mutation frequencies of up to 70% in diffuse astrocytomas, while virtually no mutations were detected in pilocytic astrocytomas [3]. Pilocytic astrocytomas, in contrast, have been recently indicated to be molecularly characterized by gene duplication/fusion or mutation of the *BRAF* gene on 7q34. These *BRAF* gene alterations occur in about 60–80% of pilocytic astrocytomas but are infrequent in diffusely infiltrating low-grade astrocytomas [42, 74].

BRAF gene aberrations in pilocytic astrocytomas may not only be of diagnostic but also of potential clinical relevance with respect to a targeted therapy [74]. Tumors with duplications or activating mutations of the *BRAF* oncogene showed significantly increased mRNA levels of *BRAF* and its downstream target, *CCND1*, as compared to tumors without these molecular alterations. In subsequent functional analyses both the stable silencing of *BRAF* through shRNA lentiviral transduction and pharmacological inhibition of MEK1/2, the immediate downstream phosphorylation target of *BRAF*, blocked the proliferation and arrested the growth of cultured tumor cells derived from low-grade gliomas [74]. These findings suggest that pharmacological inhibition of the MAPK pathway may serve as a novel potential treatment option in pilocytic astrocytoma patients.

Conclusions

Low-grade gliomas are classified into distinct entities and variants on the basis of histological and immunohistochemical features as defined in the 2007 WHO classification of tumors of the central nervous system. Molecular studies during recent years have provided fresh insights into the pathogenesis of different low-grade glioma subtypes. The increasing knowledge about key molecular alterations may be helpful in the prognostic assessment of certain glioma entities, as exemplified by the 1p/19q deletion status in oligodendrogliomas and oligoastrocytomas. The impact of genetic alterations as diagnostic markers to facilitate the differential diagnosis between different types of gliomas is still limited but may become of future relevance. As such the differential diagnosis between pilocytic and diffuse astrocytomas can be facilitated using molecular analyses for *BRAF* alterations and *IDH1* mutations, respectively. A better understanding of aberrant molecular pathways in the tumors may guide the way towards innovative targeted therapies, which need to be evaluated further in preclinical and early clinical studies. Taken together, we are optimistic that intensified research efforts involving modern genome- and proteome-wide profiling techniques will reveal powerful diagnostic, prognostic and predictive biomarkers as well as novel targets for individualized and pathogenesis-based therapies.

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