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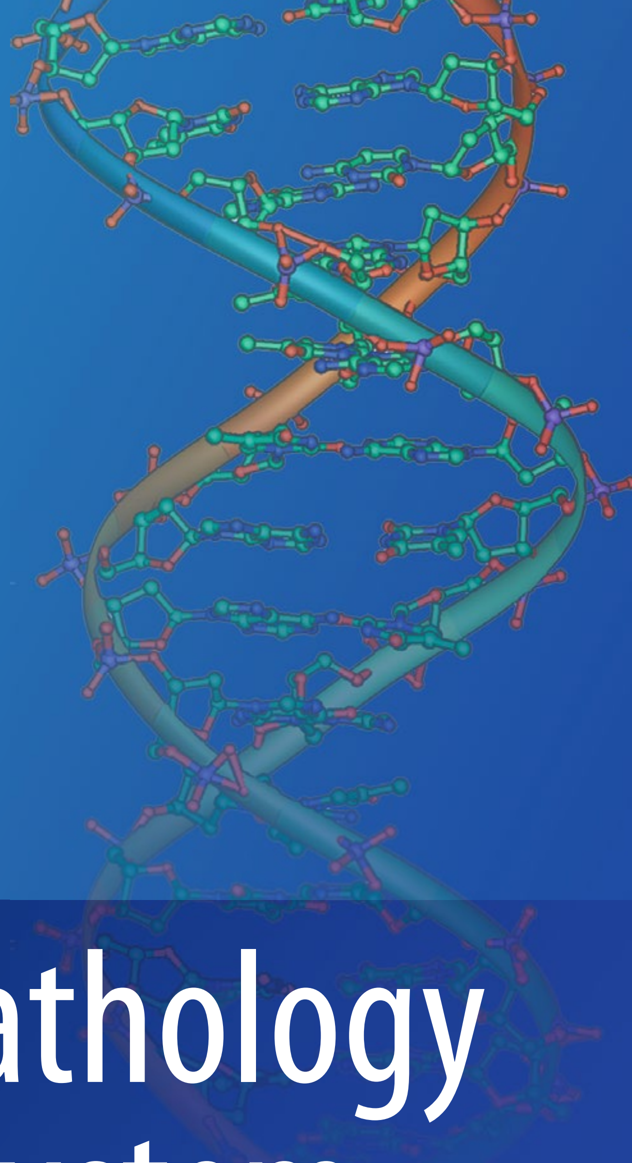

Matthias A. Karajannis
David Zagzag *Editors*

Molecular Pathology of Nervous System Tumors

Biological Stratification and Targeted Therapies

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Editors

Molecular Pathology of Nervous System Tumors

Biological Stratification and Targeted Therapies

Springer

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"To our patients, teachers, family, friends and colleagues."

Foreword

It is difficult to believe that in the space of my career so much progress has been made in the classification and treatment of CNS tumors. Beginning in an era when with diagnoses were determined almost solely by light microscopy of H&E-stained sections, and treatments were almost devoid of specificity, molecular features are increasingly instrumental to both diagnosis and treatment. Effective targeted approaches are now a reality for patients with some tumor types.

Prepared by an international panel of experts, this authoritative volume concisely and authoritatively delineates this current state of affairs. Obstacles to targeted treatments are freely acknowledged, but a refreshing vein of optimism pervades this book about a subject that in previous eras seemed to have so little promise. As such, this very readable work is highly recommended as an introduction to the new era of neuro-oncology with its potential for effective care of patients with tumors heretofore so difficult to control.

Peter C. Burger, M.D.

Preface

Recent advances in molecular biology and genetics have revolutionized our understanding of the biology that underlies the clinical diversity of nervous system tumors. The majority of these data have emerged from a number of large-scale genomic profiling projects and groups, and facilitated by the availability of ever more powerful next-generation sequencing and molecular profiling technologies. At the same time, functional studies have begun to characterize the biology of many of the molecular genetic alterations identified, including novel oncogenic driver mutations. In parallel, the advances in stem cell biology have provided valuable insight into the evolution and progression of brain tumors, including glioblastoma. Functional and preclinical studies are also aided by an increasing number and sophistication of genetically engineered mouse models that recapitulate the development of specific tumor subtypes.

Genomic profiling of disease entities that had been previously classified mainly through histomorphology and a limited set of immunohistochemical markers has revealed a diverse and complex biology of brain tumors. As a result, new molecular diagnostic tools are entering the field of neuropathology at a rapid pace. Newly defined sub-entities that are driven by divergent oncogenic pathways have been recognized to show distinctive clinical behavior and will likely require tailored risk-stratification and treatments. Molecular targeted therapies are increasingly entering clinical trials in neuro-oncology and hold promise for improving the outcome of patients with nervous system tumors, especially those that frequently recur despite aggressive multimodal therapy including surgery, radiotherapy, and chemotherapy. Molecular genetic testing that until very recently was limited to research labs is becoming increasingly available for routine clinical use.

This book is intended to be used as a comprehensive guide to the rapidly evolving field of molecular neuropathology of nervous system tumors, as well as the underlying biology and emerging molecular targeted therapies. We hope that it will serve as a useful resource for physicians as well as clinical and laboratory scientists involved with or interested in the up-to-date diagnosis and treatment of patients with brain tumors. Accordingly, the target audience includes neuropathologists, neuro-oncologists, neurosurgeons, radiation oncologists, neurologists, neuroradiologists, as well as residents and fellows, who diagnose and treat patients with nervous system tumors including tumors of the brain, spine, leptomeninges, and peripheral nerves.

Special emphasis was given to already established and emerging molecular diagnostic tests in neuropathology, as well as molecular targeted therapies. The book is organized by clinicopathologic disease entities, and each chapter has been prepared by a team of authors to cover a full spectrum of expertise including neuropathology, molecular biology, and clinical management, with a focus on practical diagnostic and clinical considerations.

New York, NY, USA

Matthias A. Karajannis, M.D., M.S.
David Zagzag, M.D., Ph.D.

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1 Hereditary Predisposition to Primary CNS Tumors

Uri Tabori, Matthias A. Karajannis, and John G. Pappas

Primary central nervous system (CNS) tumors remain among the most devastating cancers in adults and children. Although the majority of CNS tumors occur in adults, brain tumors are the most common solid tumor of childhood and the dominant cause of morbidity and mortality in pediatric oncology. Although the majority of brain tumors are thought to arise sporadically, recent advancements in our understanding of the molecular genetics of brain tumors have resulted in an increased awareness for germline predispositions to these cancers.

While most adult CNS cancers are sporadic, as many as 50 % of childhood brain tumors are caused by germline mutations [1, 2]. In some cases, a specific pathological entity only exists in the context of a condition resulting from these germline mutations [3, 4]. Since in many cases tumors are only a part of the clinical manifestations of the mutation, the term cancer predisposition syndrome is used to define these conditions.

Cancer predisposition syndromes are monogenic disorders. Discovery of each single gene causing each syndrome was initially pursued by linkage studies and positional cloning. Germline mutation in one of these syndromic genes is the first hit in the associated tumors as well as the etiology of the developmental abnormalities associated with features of the syndrome i.e., malformations, dysmorphic features. Somatic mutation in one of these genes can also be the first hit or subsequent hit in sporadic tumors. Contemporary research interrogates the genetic contribution to brain tumors by gene expression arrays, whole genome sequencing in tumor and non-tumor tissues in human as well as animal models. Different sequences of genetic events leading to tumorigenesis as well as the associated molecular pathways were elucidated by the study of hereditary syndromes [5].

It is of great importance for physicians to be aware of and recognize these conditions in order to be able to offer appropriate referrals to clinical geneticists or other specialists. Affected individuals and families require counseling and may benefit from following specific treatment and surveillance plans or protocols. More recently, molecular targeted therapies have begun to emerge for some conditions, and will likely become increasingly available.

A good example for the above is tuberous sclerosis complex (TSC). This genetic syndrome is associated with seizures, developmental delay, brain tumors (subependymal giant cell astrocytomas, SEGAs), and other tumors. However, understanding the genetic causes of the syndrome allowed for development of surveillance protocol and targeted therapy for the brain cancers affecting these patients with dramatic change in the clinical approach to patients with the syndrome.

As can be seen in Fig. 1.1, the majority of these mutations involve tumor suppressors and affect key signaling pathways of cancer. Interestingly, while most of these are autosomal dominant, autosomal recessive syndromes, such as those involving Fanconi anemia genes and the mismatch repair genes, predispose the individual to a different tumor spectrum as compared to heterozygous carriers.

Cancer predisposing syndromes can be grouped in a variety of ways. Some syndromes will have many clinical manifestations, among which cancer is just a rare feature, while others have cancer as the only clinical manifestation. Other ways to divide these conditions include by pathogenesis or by age of onset. However, we will present the syndromes grouped by the specific tumors they cause, since this will allow clinicians involved in the care of patients with brain tumors to consider the appropriate differential diagnoses based on the specific tumor histology.

This chapter will focus on the most common tumor predisposition syndromes and will elaborate on the genetic background, pathogenesis, and clinical approach to these disorders. Details on additional syndromes are presented in Table 1.1.

Syndromes Associated with Glioma

Gliomas are by far the most common group of brain tumors associated with cancer predisposition syndromes. These syndromes should always be considered if an index patient presents

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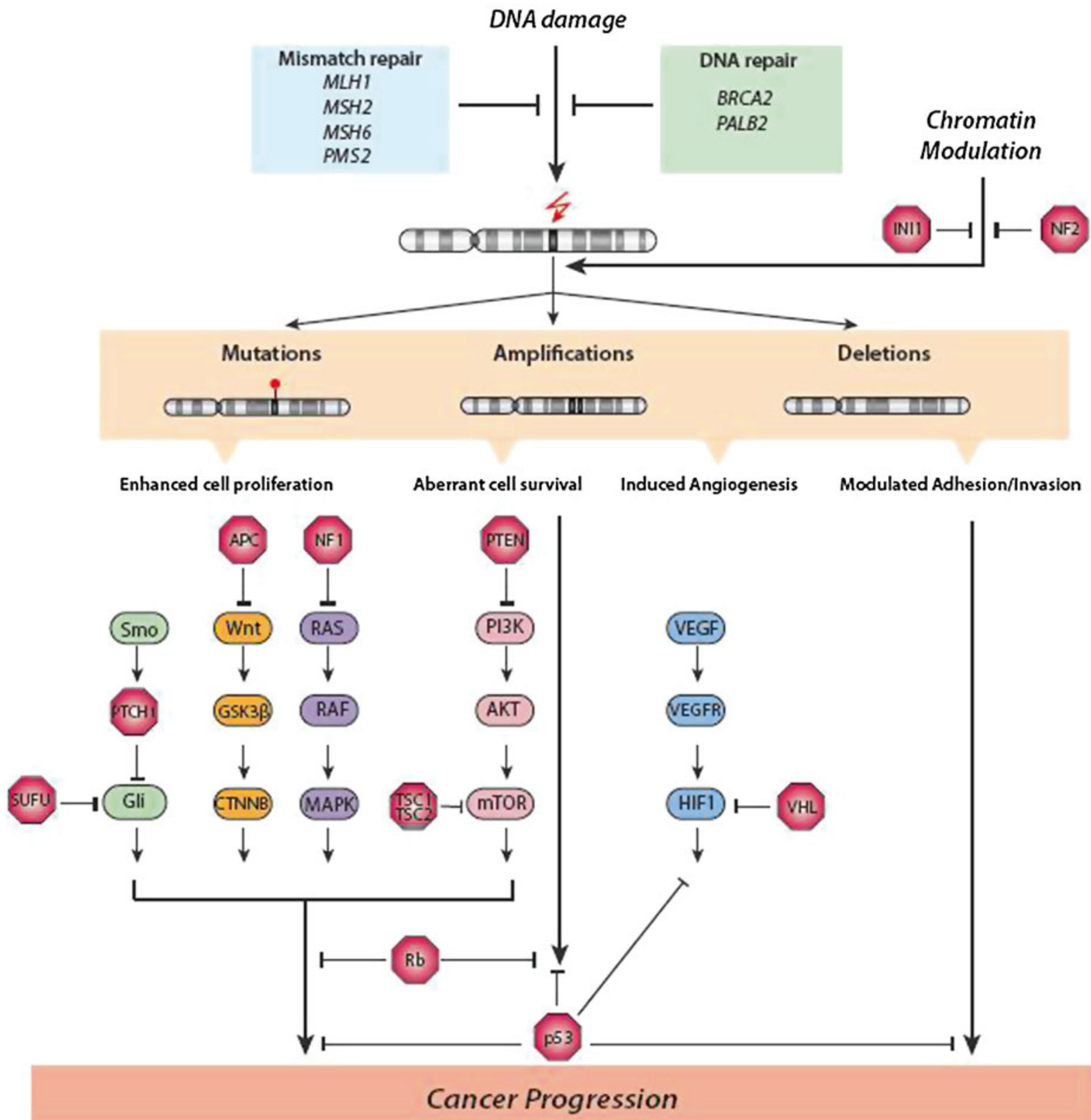

FIG. 1.1. Genes involved in predisposition to brain tumors.

with glioma. Each syndrome has unique features regarding clinical manifestations and personal or family history.

Neurofibromatosis Type I (NF1, von Recklinghausen's Disease)

NF1 is by far the most common CNS tumor predisposition syndrome. It is an autosomal dominant condition with a worldwide incidence of 1 per 2,500–3,000 individuals [6]. Importantly, this is a multisystem condition and diagnosis is generally made based on clinical criteria [6–8]. The following criteria are sensitive and specific in adults but affected children may not fulfil the criteria and genetic testing may aid the diagnosis [8]:

1. Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
2. Two or more neurofibromas of any type or one plexiform neurofibroma
3. Freckling in the axillary or inguinal regions
4. Optic glioma

TABLE 1.1 Genetic syndromes not described in this chapter and associated with brain tumors.

Syndrome	Inheritance	Genetic abnormality	Cardinal features	Brain tumors	References
Aicardi syndrome	X-linked dominant lethal in males	Unknown	callosal agenesis, infantile spasms, chorioretinal lacunae	Choroid plexus papilloma	Frye RE, Polling JS, Ma LC. Choroid plexus papilloma expansion over 7 years in Aicardi syndrome. J Child Neurol. 2007 Apr;22(4):484-7. PubMed PMID: 17621535; PubMed Central PMCID: PMC2536525.
Distal 22q11.2 deletion syndrome	Autosomal dominant microdeletion usually sporadic	3.4 Mb deletion of chromosome 22q11.2, distal to the common DiGeorge syndrome (DGS) region encompassing the *INI1/ SMARCB1*	asymmetric face with preauricular sinuses and skin tags, cognitive disability	teratoid/rhabdoid tumor	Beddow RA, Smith M, Kidd A, Corbett R, Hunter AG. Diagnosis of distal 22q11.2 deletion syndrome in a patient with a teratoid/rhabdoid tumour. Eur J Med Genet. 2011 May-Jun;54(3):295-8. doi: 10.1016/j.ejmg.2010.12.007. Epub 2010 Dec 25. PubMed PMID: 21187175 Lafay-Cousin L, Payne E, Strother D, Chernos J, Chan M, Bernier FP. Goldenhar phenotype in a child with distal 22q11.2 deletion and intracranial atypical teratoid rhabdoid tumor. Am J Med Genet A. 2009 Dec;149A(12):2855-9. doi: 10.1002/ajmg.a.33119. PubMed PMID: 19938088 Jackson EM, Shaikh TH, Gururangan S, Jones MC, Malkin D, Nikkel SM, Zuppan CW, Wainwright LM, Zhang F, Biegel JA. High-density single nucleotide polymorphism array analysis in patients with germline deletions of 22q11.2 and malignant rhabdoid tumor. Hum Genet. 2007 Sep;122(2):117-27. Epub 2007 May 31. PubMed PMID: 17541642
Encephalocraniocutaneous lipomatosis	Isolated cases	unknown	Eye choristoma, nonscarring alopecia, nevus psiloliparus, subcutaneous fatty masses, nodular skin tags, and aplastic scalp defects, intracranial and intraspinal lipomas, congenital abnormalities of the meninges, seizures, developmental delays	low-grade glioma	Valera ET, Brassesco MS, Scrideli CA, de Castro Barros MV, Santos AC, Oliveira RS, Machado HR, Tone LG. Are patients with encephalocraniocutaneous lipomatosis at increased risk of developing low-grade gliomas? Childs Nerv Syst. 2012 Jan;28(1):19-22. doi: 10.1007/s00381-011-1601-z. Epub 2011 Oct 8. PubMed PMID: 21983849
Schimmelpenning-Feuerstein-Mims syndrome	Somatic mosaicism	*HRAS, KRAS*	sebaceous nevi, often on the face, associated with variable ipsilateral abnormalities of the central nervous system, ocular anomalies, and skeletal defects, seizures, developmental delays	Optic pathway glioma	Pavlidis E, Cantalupo G, Boria S, Cossu G, Pisani F. Hemimegalencephalic variant of epidermal nevus syndrome: case report and literature review. Eur J Paediatr Neurol. 2012 Jul;16(4):332-42. doi: 10.1016/j.ejpn.2011.12.004. Epub 2011 Dec 24. PubMed PMID: 22200538

(continued)

Table 1.1 (continued)

Syndrome	Inheritance	Genetic abnormality	Cardinal features	Brain tumors	References
Hypomelanosis of Ito	Somatic mosaicism	Chromsomal mosaicism	Skin macular hypopigmented whorls, streaks, and patches, developmental delays, seizures	choroid plexus papilloma	Morigaki R, Pooh KH, Shouno K, Taniguchi H, Endo S, Nakagawa Y. Choroid plexus papilloma in a girl with hypomelanosis of Ito. J Neurosurg Pediatr. 2012 Sep;10(3):182-5. doi: 10.3171/2012.5.PEDS11556. Epub 2012 Jul 13. Erratum in: J Neurosurg Pediatr. 2013 Jan;11(1):103. PubMed PMID: 22793165
Maffucci syndrome	Somatic mosaicism	*IDH1* or *IDH2*	multiple central cartilaginous tumors accompanied by soft tissue hemangiomas	Glioma, Pituitary adenoma, meningioma	Moriya K, Kaneko MK, Liu X, Hosaka M, Fujishima F, Sakuma J, Ogasawara S, Watanabe M, Sasahara Y, Kure S, Kato Y. IDH2 and TP53 mutations are correlated with gliomagenesis in a patient with Maffucci syndrome. Cancer Sci. 2014 Mar;105(3):359-62. doi: 10.1111/cas.12337. PubMed PMID: 24344754
Pai syndrome	Autosomal dominant	unknown	Median cleft lip, corpus callosum lipoma, skin polyps	Midline central nervous system lipomas	Mishima K, Mori Y, Minami K, Sakuda M, Sugahara T. A case of Pai syndrome. Plast Reconstr Surg. 1999 Jan;103(1):166-70. Review. PubMed PMID: 9915178.
Mupliple endicrine neoplasia type 1	Autosomal dominant	*MEN1*	tumors of parathyroids, pancreatic islets, duodenal endocrine cells, and the anterior pituitary	Meningioma and spinal cord ependymoma	Rogers L, Barani I, Chamberlain M, Kaley TJ, McDermott M, Raizer J, Schiff D, Weber DC, Wen PY, Vogelbaum MA. Meningiomas: knowledge base, treatment outcomes, and uncertainties. A RANO review. J Neurosurg. 2014 Oct 24:1-20. [Epub ahead of print] PubMed PMID: 25343186
Carney complex type 1	Autosomal dominant	*PRKAR1A*	cardiac, endocrine, cutaneous, and neural myxomatous tumors, as well as a variety of pigmented lesions of the skin and mucosae	Pituitary adenoma, schwannoma	Gadelha MR, Trivellin G, Hernández Ramírez LC, Korbonits M. Genetics of pituitary adenomas. Front Horm Res. 2013;41:111-40. doi: 10.1159/000345673. Epub 2013 Mar 19. Review. PubMed PMID: 23652674
Oral-facial-digital syndrome type VI	Autosomal recessive	*C5orf42*, *TMEM216*	Cerebellar malformations (molar tooth sign, tongue hamartoma, additional tongue frenula, upper lip notch, mesoaxial polydactyly of hands or feet	hypothalamic hamartoma	Lopez E, Thauvin-Robinet C, Reversade B, Khartoufi NE, Devisme L, Holder M, Ansart-Franquet H, Avila M, Lacombe D, Kleinfinger P, Kaori I, Takanashi J, Le Merrer M, Martinovic J, Noël C, Shboul M, Ho L, Güven Y, Razavi F, Burglen L, Gigot N, Darmency-Stamboul V, Thevenon J, Aral B, Kayserili H, Huet F, Lyonnet S, Le Caignec C, Franco B, Rivière JB, Faivre L, Attié-Bitach T. C5orf42 is the major gene responsible for OFD syndrome type VI. Hum Genet. 2014 Mar;133(3):367-77. doi: 10.1007/s00439-013-1385-1. Epub 2013 Nov 1. PubMed PMID: 24178751

Pallister-Hall syndrome	Autosomal dominant usually sporadic	*GLI3*	central polydactyly, anorectal malformations	hypothalamic hamartoma	Kang S, Graham JM Jr, Olney AH, Biesecker LG. GLI3 frameshift mutations cause autosomal dominant Pallister-Hall syndrome. Nat Genet. 1997 Mar;15(3):266-8. PubMed PMID: 9054938
Proteus syndrome	Somatic mosaicism	*AKT1*	disproportionate, asymmetric, and distorting overgrowth, bone abnormalities; characteristic cerebriform connective tissue nevus; epidermal nevi; vascular malformations of the capillary, venous, or lymphatic types; dysregulated adipose tissue; bullous lung alterations; intellectual disability; seizures; brain malformations	Meningioma	Cohen MM Jr. Proteus syndrome review: molecular, clinical, and pathologic features. Clin Genet. 2014 Feb;85(2):111-9. doi: 10.1111/cge.12266. Epub 2013 Oct 23. PubMed PMID: 23992099
Rubinstein-Taybi syndrome	Autosomal dominant usually sporadic	*CREBBP*	mental retardation, postnatal growth deficiency, microcephaly, broad thumbs and halluces, dysmorphic facial features	Meduloblastoma, meningioma	Roelfsema JH, Peters DJ. Rubinstein-Taybi syndrome: clinical and molecular overview. Expert Rev Mol Med. 2007 Aug 20;9(23):1-16. Review. PubMed PMID: 17942008
Short-rib thoracic dysplasia 12	Autosomal recessive	unknown	constricted thoracic cage, short ribs, shortened tubular bones, 'trident' appearance of the acetabular roof	Hypothalamic hamartoma	den Hollander NS, van der Harten HJ, Laudy JA, van de Weg P, Wladimiroff JW. Early transvaginal ultrasonographic diagnosis of Beemer-Langer dysplasia: a report of two cases. Ultrasound Obstet Gynecol. 1998 Apr;11(4):298-302. PubMed PMID: 9618859
Wiskott-Aldrich syndrome	X-linked recessive	*WAS*	Immunodeficiency, thrombocytopenia, eczema, and recurrent infections	Primary reticulum cell sarcoma of the brain	Model LM. Primary reticulum cell sarcoma of the brain in Wiskott-Aldrich syndrome. Report of a case. Arch Neurol. 1977 Oct;34(10):633-5. PubMed PMID: 334130
Xeroderma pigmentosum	Autosomal recessive	*XPA*, *ERCC1*, *ERCC3* (XP-B), *XPC*, *ERCC2* (XP-D), *DDB2* (XP-E), *ERCC4* (XP-F), *ERCC5* (XP-G), *POLH* (XPV)	severe sunburn with blistering, persistent erythema on minimal sun exposure, marked freckle-like pigmentation of the face, (photophobia, keratitis, atrophy of the skin of the eyelids, increased risk of cutaneous neoplasms	astrocytoma, medulloblastoma, schwannomas	Rapin I, Lindenbaum Y, Dickson DW, Kraemer KH, Robbins JH. Cockayne syndrome and xeroderma pigmentosum. Neurology. 2000 Nov 28;55(10):1442-9. Review. PubMed PMID: 11185579

5. Two or more Lisch nodules (iris hamartomas)
6. A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis
7. A first-degree relative (parent, sib, or offspring) with NF1 as defined by the above criteria

Individuals with NF1 can have significant morbidity unrelated to cancer predisposition [9]. The nervous system is commonly affected in NF1, and most cancers are of nervous system origin including gliomas, benign neurofibromas, and malignant nerve sheath tumors (MPNSTs). However, other cancers including chronic myelomonocytic leukemia, breast cancer, certain endocrine tumors, rhabdomyosarcoma, and neuroblastoma are reported with this condition [10].

Molecular Pathogenesis

NF1 results in loss of function of the tumor suppressor protein Neurofibromin. This large protein is a key negative regulator of the RAS pathway by catalyzing the hydrolysis of active guanosine triphosphate-bound RAS to inactive guanosine diphosphate-bound RAS [11]. Dysfunctional neurofibromin results in constitutive activation of downstream oncogenic pathways including MAPK and mTOR. Mutations or deletions in the *NF1*, gene can be identified in more than 95 % of individuals with NF1 [12]. However, since the gene is very large and difficult to analyze, diagnosis and management can be made based on clinical criteria. RAS/MAPK pathway activation is seen in almost all pediatric low-grade astrocytomas [13] and in 88 % of adult malignant gliomas [14]. Actual somatic mutations in *NF1* occur in 20 % of adult gliomas.

Mouse models of gliomas frequently alter the RAS/MAPK pathway. However, additional alterations in major tumor suppressor pathways such as TP53, RB, and PTEN are required to generate tumors [15].

Indeed, NF1 deficient mice do not have tumors but hyperplasia mimicking the optic pathway gliomas (OPG) commonly seen in these individuals [16]. Taken together, the benign nature of tumors seen in the CNS in patients with NF1 support the concept of oncogene induced senescence as a mechanism to explain the spontaneous growth arrest of these tumors when the RAS pathway is constitutively active [17].

Gliomas

The most common CNS tumor in NF1 are optic pathway gliomas (OPG) affecting up to 15 % of individuals with the syndrome. Conversely, up to a third of children with OPG have germline mutations in *NF1*. Bilateral optic nerve gliomas exist almost exclusively in children with NF1 (Fig. 1.2).

NF1 related OPG typically have an indolent course with spontaneous growth arrest. Indeed, the vast majority of these OPG will not progress after initial diagnosis. Up to 15 % of these tumors, however, do progress, resulting in visual loss or other symptoms and requiring intervention. High-grade gliomas are relatively uncommon, but have been reported and should be considered in patients whose tumors arise in an uncharacteristic location or demonstrate particularly aggressive behavior [18, 19].

Patients with NF1 often exhibit multiple lesions, mainly in the basal ganglia and brainstem which are difficult to assess. These include T2 bright lesions on MRI, without significant mass effect, termed FLAIR (fluid attenuated inversion recovery) associated sub-cortical intensities or FASCI (Fig. 1.2). These lesions tend to disappear spontaneously after initial growth and rarely cause symptoms. Differentiating between FASCI and low-grade gliomas in NF1 patients may be challenging.

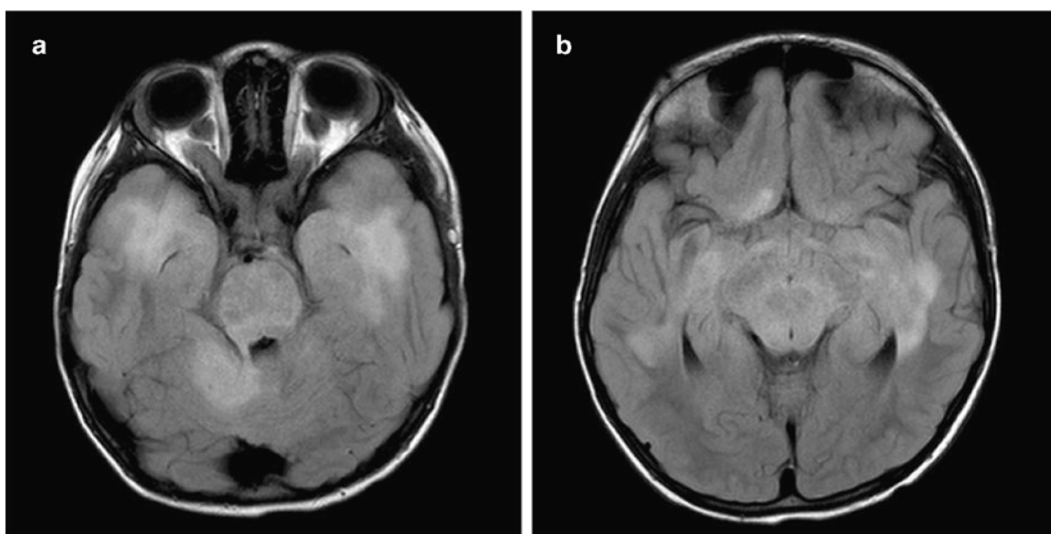

Fig. 1.2. Pathognomonic MRI findings in NF1. Bilateral optic nerve glioma (**a**) and FASCI (**b**) are almost exclusively seen only in children with NF1.

Clinical Implications

Since NF1 is a multisystem condition, careful monitoring is recommended in multidisciplinary clinics [6]. Due to the marked variability in clinical manifestations, including tumor occurrence, strategies for surveillance and follow-up must be tailored to each individual patient. Optic gliomas affecting both optic nerves and/or coexistence of FASCI should raise a suspicion of NF1 even in the absence of typical neurocutaneous findings such as café-au-lait macules, and genetic counseling is recommended.

Unfortunately, surveillance neuroimaging in asymptomatic children with NF1 has not been shown to reduce the incidence of visual loss in this population, and frequent neuro-ophthalmologic examination remains standard of care [20]. For FASCI and other atypical brain lesions, close monitoring is recommended and treatment should be reserved for patients with progressive clinical symptoms and in some cases, radiological progression.

Individuals with NF1 are particularly sensitive to the damaging effects of ionizing irradiation, leading both to an increased incidence of irradiation-induced cancers [21], as well as to cerebrovascular damage (Moyamoya syndrome) [22, 23]. Cranial irradiation in NF1 patients with OPG in particular and brain tumors in general, should be avoided as long as reasonable alternative treatment options exist.

Molecular Targeted Therapies

Inhibitors targeting the RAS and mTOR pathways are of great interest for the potential treatment of NF1 and NF1-related tumors, and are being investigated as novel therapeutic approaches in preclinical and clinical studies. Furthermore, targeting the microenvironment believed to be necessary for NF1 tumor growth may allow for additional NF1 specific therapies for these patients [24, 25]. For example, inhibition of c-KIT has shown encouraging efficacy for peripheral NF1 related neurofibromas in subsets of patients [26, 27], although it remains to be seen whether c-KIT represents a molecular target of value in NF1 related CNS tumors.

Li–Fraumeni Syndrome

The Li–Fraumeni syndrome (LFS) is the prototype cancer predisposition syndrome, causing cancer in multiple sites at different ages. LFS is an autosomal dominant condition affecting 1 in 5,000–10,000. Individuals with the disorder have a lifetime risk of 85–100 % of developing cancer. Originally described by Frederick Pei Li and Joseph F. Fraumeni, Jr. in 1969 [28] as a familial breast, soft tissue sarcoma and brain tumor predisposition syndrome, it is now known that these individuals have a risk of developing cancer in many additional organs, including rare tumors such as adrenocortical carcinomas, as well as hematologic malignancies [29].

According to Li et al. 1988 [30], who described the syndrome, the diagnosis is clinically established in families with a proband with a sarcoma diagnosed before age 45 years and a first-degree relative with any cancer before age 45 years and a first- or second-degree relative with any cancer before age 45 years or a sarcoma at any age. The association of LFS with germline mutations in the *TP53* prompted the formation of criteria to enhance the yield of *TP53* clinical genetic testing. The following criteria were published by Chompret et al. [31] and revised and evaluated by Gonzalez et al. [32], Tinat et al. [33], and Ruijs et al. [34] According to these studies the risk of a *TP53* mutation exceeds 20 % in any individual with:

1. A tumor belonging to the LFS tumor spectrum (e.g., soft tissue sarcoma, osteosarcoma, brain tumor, premenopausal breast cancer, adrenocortical carcinoma, leukemia, lung bronchoalveolar cancer) before age 46 years and at least one first- or second-degree relative with a LFS tumor (except breast cancer if the proband has breast cancer) before age 56 years or with multiple tumors; or
2. Multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum and the first of which occurred before age 46 years; or
3. Adrenocortical carcinoma or choroid plexus tumor, regardless of family history.

Molecular Pathogenesis

In 1990 the association between LFS and germline mutations in the tumor suppressor gene *TP53* was made [35]. *TP53* is located at chromosome 17p13.1 and has been called the "gatekeeper of the genome," since it represents one of the key proteins that maintain genome integrity after DNA damage, hypoxia, and other stressors. *TP53* activation results in cell cycle arrest, senescence, and apoptosis. *TP53* is also involved in key metabolic pathways in the cell including cell metabolism and mitochondrial function [36]. *TP53* represents one of the most commonly mutated tumor suppressors known. Molecular genetic evidence of TP53 pathway disruption can be found in more than 50 % of tumors from adult cancer patients, as well as in 80 % of adult [13] and 50 % of pediatric high-grade gliomas [37].

Development of faithful preclinical models of LFS has been hindered by the fact that TP53 alteration in animal models generally fails to recapitulate the tumor phenotypes of the human disorder, and brain tumors are not a part of the phenotype even in some of the newer models [38]. Nevertheless, many glioma and medulloblastoma mouse models utilize TP53 alterations in conjunction with other cancer genes to mimic the human disease [39].

Three types of brain tumors are associated with LFS: high-grade gliomas, choroid plexus carcinoma, and medulloblastoma. Choroid plexus tumors affect LFS carriers in the first decade of life and medulloblastomas usually in the second, while malignant gliomas can occur throughout childhood, but more commonly in young adults.

Gliomas have been recognized as part of LFS from the earliest reports [28]. Although TP53 expression is associated with worse outcome in childhood glioblastoma [40], currently, no data exist regarding the significance of germline *TP53* mutations in pediatric high-grade gliomas. The surveillance protocol [41] developed by our group uncovered several low-grade gliomas suggesting that some of LFS associated glioblastomas arise as secondary glioblastomas and may benefit from early intervention.

Choroid plexus carcinomas are one of the most common presentations of LFS in young children and were recently added to the criteria for the diagnosis of the syndrome [33]. Furthermore, a significant number of patients with choroid plexus carcinoma will harbor germline *TP53* mutations. Somatic mutations in *TP53* are observed in up to 50 % of choroid plexus carcinomas, and this confers a poorer chance of survival for these patients [2]. This phenomenon may be caused by increased resistance of *TP53* mutant tumors to radiation and chemotherapy [42].

Medulloblastomas harbor somatic *TP53* mutations in 5–10 %, and appear strictly confined to the WNT (wingless-related integration site) and SHH (sonic hedgehog) subgroups of tumors [43]. Remarkably, *TP53* mutations do not alter the excellent survival of patients with WNT medulloblastomas, while *TP53* mutant SHH medulloblastomas are commonly seen in the second decade of life and have unfavorable outcome. Interestingly, these are commonly individuals with LFS. SHH medulloblastomas from LFS patients have a unique molecular genetic profile, suggesting chromothripsis ("chromosome shattering") as the initiating event [44].

Clinical Implications

Current recommendation is to screen for germline *TP53* mutations, i.e., LFS, in all individuals presenting either with a high-grade glioma and a family history of LFS tumors, or patients diagnosed with a choroid plexus carcinoma or medulloblastoma harboring somatic *TP53* mutations [33]. Cancer surveillance protocols developed specifically for individuals with LFS have revealed a high rate of early tumor detection [45]. Recently, a striking survival benefit for children has been observed using these protocols, mainly due to improved early detection of brain tumors (Fig. 1.3). Although no molecular targeted therapy for *TP53* mutated tumors is currently available, detection of a germline *TP53* mutation has significant prognostic and therapeutic implications for the patient. Both children and adults with LFS have been considered to be at an increased risk for developing radiation therapy-induced secondary malignant tumors [46, 47], as well as secondary myelodysplastic syndrome following specific chemotherapies [48].

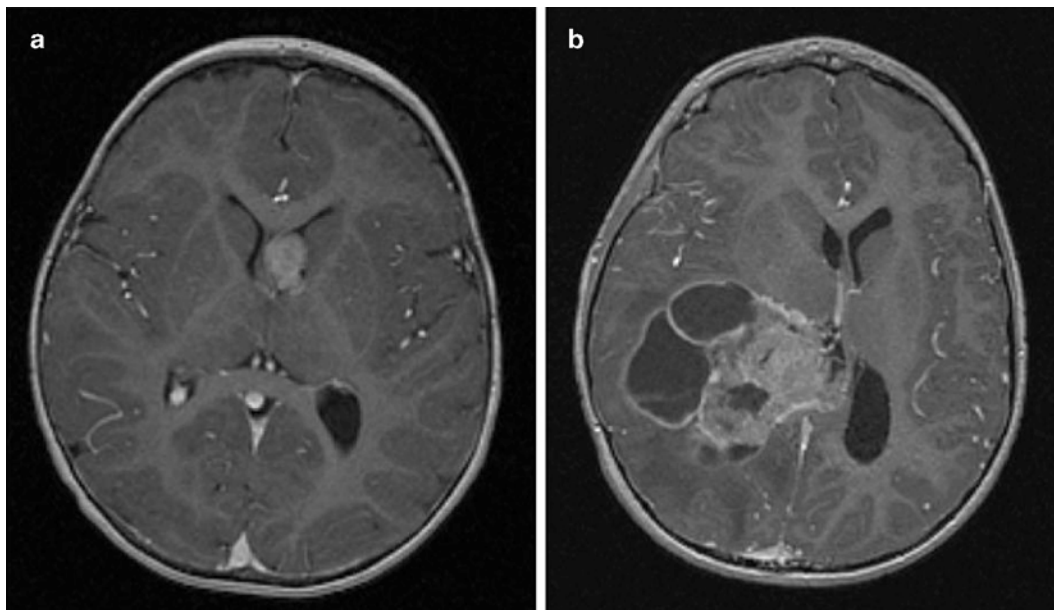

Fig. 1.3. Early detection of CPC after implementation of the surveillance protocol for LFS. Early detection of asymptomatic choroid plexus carcinoma in an LFS patient undergoing a surveillance protocol (**a**). A tumor from a patient with sporadic symptomatic choroid plexus carcinoma (**b**). The LFS patient underwent complete tumor resection followed by chemotherapy and is alive 7 years later. The patient with sporadic tumor did not survive despite radiation therapy and multiple courses of chemotherapy.

Constitutional Mismatch Repair Deficiency Syndrome

Constitutional Mismatch Repair Deficiency Syndrome (CMMR-D) is a rare familial cancer predisposition syndrome that has a unique clinical phenotype. This syndrome frequently presents with cafe-au-lait macules like NF1 [49, 50], resulting in occasional misdiagnosis and inappropriate management. CMMR-D is due to germline biallelic (homozygous or compound heterozygous) mutations in one of the mismatch repair (MMR) genes. Germline monoallelic mutations in MMR genes cause hereditary nonpolyposis colon cancer, Lynch syndrome and brain tumor-polyposis syndrome type 1 (BTPS1 or Turcot type 1) [51, 52]. The brain tumor in BTPS1 is glioblastoma multiforme.

Individuals with CMMR-D are predisposed to different and more aggressive cancers than Lynch syndrome. Children with CMMR-D are usually affected within the first two decades of life and present with hematological malignancies (most commonly T-cell lymphomas), malignant brain tumors, and gastrointestinal cancers.

Molecular Pathogenesis

Germline mutations in *MLH1*, *MSH2*, *MSH6*, and *PMS2* have been reported in association with CMMR-D. These mismatch repair genes are critical in repairing single base pair mismatches and misalignments [49]. In the absence of such genes, high mutation rates are observed, including in cancers which are described as "mutator phenotype" [14]. CMMR-D is inherited in an autosomal recessive fashion and is found mostly in consanguineous families. Some patients may have NF1 in addition to CMMR-D, which is thought to be caused by "secondary" early or germline mutations in the *NF1* gene as a part of the mutator phenotype [53].

Interestingly, mouse models of mismatch repair deficiency recapitulate cancers of the gastrointestinal tract and lymphomas, but fail to develop brain tumors [54, 55].

Brain Tumors

Malignant gliomas are the most common type of tumor observed in individuals with CMMR-D, usually presenting in the second decade of life. Some patients are initially diagnosed with low-grade gliomas, but these tend to transform to high-grade tumors. Medulloblastomas and PNETs (primitive neuroectodermal tumors) are also seen, but may have glial markers suggesting an earlier cell of origin. Some of these patients with high-grade gliomas have been reported as long-term survivors, possibly suggesting a somewhat more favorable prognosis compared to other adults and children with high-grade gliomas [56, 57].

Clinical Implications

Diagnosis of Lynch-related cancers can be made by evidence of microsatellite instability [58]. However, this method has not been shown to be sensitive in CMMR-D, especially in brain tumors and lymphomas. Immunostain of tumor tissue for the MMR proteins is almost universally negative in CMMR-D cancers, and has the unique diagnostic feature of negative stain in the corresponding normal tissue.

Any patient with gliomas, T-cell lymphoma and either café-au-lait macules, consanguinity or a family history of colon cancer should be screened for any of the four mismatch repair genes. Similarly, high index of suspicion should be raised for "NF1" patients with malignant gliomas and consanguinity.

Individuals with Lynch syndrome benefit from a strict surveillance protocol (www.NCCN.org) and from preventive colectomy. Therefore, early and accurate diagnosis may benefit parents and other family members. Since the risk of gliomas and lymphoma is extremely high for biallelic MMR patients, following a surveillance protocol may be beneficial for children with CMMR-D [59]. There are several reports of MMR tumors responding to specific agents including retinoic acid [60], which may be exploited for the treatment of these tumors.

Melanoma Astrocytoma Syndrome

In the mid-1990s, a syndrome of melanoma and other skin lesions associated with CNS malignancies was first described [61]. Since then, several other reports have delineated the association between familial melanoma and glioma. A common locus on the short arm of chromosome 9 was uncovered, and germline mutations were described in CDKN2A which codes for two proteins: p16(INK4) and p14(ARF) [62, 63]. Additional mutations in another gene in that location, *PTPRD*, were reported [64]. ARF and INK4A are major tumor suppressors in the TP53 and RB1 pathways respectively, and are altered in the majority of sporadic gliomas [14]. Further data, however, will be needed before specific screening and surveillance recommendations can be developed.

Syndromes Associated with Medulloblastoma

Medulloblastoma is the most common malignant brain tumor in children, but is also seen in adults. Several syndromes were first described to be associated with childhood medulloblastoma, but further data supports involvement of some of these syndromes in adult tumors as well [43]. We summarize here the most common syndromes, while others are described in Table 1.1.

Gorlin Syndrome (Basal Cell Nevus Syndrome)

Basal cell nevus syndrome (BCNS) is an autosomal dominant condition associated with multiple developmental anomalies and predisposition to benign and malignant tumors. The hallmark of BCNS is development of basal cell carcinomas and medulloblastomas. The association of multiple nevoid basal cell "epithelioma," jaw cysts and bifid ribs was first reported in 1960 [65].

Evans et al. [66] and Kimonis et al. [67] have published criteria for clinical diagnosis. Two major criteria and one minor or one major and three minor criteria are diagnostic of Gorlin syndrome. Some criteria require X-rays. Exposure to X-rays increases the risk for basal cell carcinoma and should be avoided.

Major Criteria

1. Falx calcification ascertained by AP skull X-rays.
2. Jaw keratocysts seen as translucencies on orthopantogram X-rays.
3. Two or more palmar/plantar pits.
4. Basal cell carcinoma before age 30 or multiple after age 30.
5. A first-degree relative with Gorlin syndrome.

Minor Criteria

1. Childhood medulloblastoma (primitive neuroectodermal tumor [PNET]).
2. Lympho-mesenteric or pleural cysts.
3. Macrocephaly (OFC >97th centile).
4. Cleft lip/palate.
5. Vertebral/rib anomalies (bifid vertebra), bifid/splayed/extra ribs ascertained by X-rays.
6. Preaxial or postaxial polydactyly.
7. Ovarian/cardiac fibromas.
8. Ocular anomalies (cataract, developmental defects, and pigmentary changes of the retinal epithelium).

Molecular Pathogenesis

The gene responsible for BCNS is *PTCH1* which is located on chromosome 9q22.3 [68]. PTCH1 is a protein that is a major suppressor of the sonic hedgehog (SHH) pathway by direct inhibition of SMO. Disruption of *PTCH1* leads to constitutive activation of the pathway and induction of *GLI* target genes and cell proliferation and survival. *SHH* is involved in neural development and midline segregation, which can explain some of the syndromatic manifestations of BCNS. Germline mutations in *SUFU*, which is a direct inhibitor of *GLI* have been reported in familial and sporadic medulloblastoma [69] in up to 50 % of desmoplastic tumors [70].

Most mouse models of medulloblastoma utilize alterations in the SHH pathway [71]. While alteration of PTCH1 only results in a tumor incidence of 10–20 %, its combination with other alterations results in very efficient formation of aggressive tumors.

Sequence analysis of *PTCH1* yields a mutation in 50–80 % of patients with Gorlin syndrome [72, 73]. Partial and whole-gene deletions are found in 6–21 % of patients [74]. In patients with mental retardation chromosome analysis and chromosomal microarray may reveal the 9q22.3 microdeletion. The 9q22.3 microdeletion syndrome is associated with cognitive impairment, metopic synostosis, obstructive hydrocephalus, macrosomia and seizures, in addition to the features of Gorlin syndrome [75].

Medulloblastoma

The first report of a brain tumor, medulloblastoma, in association with this hereditable syndrome [76] was published in 1963. The development of medulloblastoma in the setting of Gorlin syndrome occurs earlier compared to sporadic tumors. Most patients are younger than 3 years of age, and almost all tumors have a specific pathological subtype termed desmoplastic variant [77]. More specifically, desmoplasia with extensive nodularity (MBEN) is almost pathognomonic for the syndrome in young children [78, 79]. Of note, desmoplastic medulloblastomas in young children usually have a favorable outcome even without radiation therapy [80].

Meningioma

Several reports have documented the development of intracranial meningiomas in patients with Gorlin syndrome with or without prior craniospinal irradiation [81], although strength of association remains unknown.

Clinical Implications

Individuals with the clinical manifestations of BCNS, a family history of basal cell carcinomas, or other manifestations of the syndrome should be screened for germline mutations in *PTCH1 and SUFU*.

Patients with medulloblastoma and any of the above should also be screened, since radiation therapy is associated with an increased risk of developing basal cell carcinomas within the irradiated fields [82] in almost all patients. Furthermore, since the rate of germline mutations in the SHH pathway is extremely high in young children with desmoplastic medulloblastoma (Fig. 1.4) [70], children less than 3 years of age with desmoplastic tumors should be screened even without clinical manifestations of the syndrome. The current consensus recommends yearly brain MRI scans for all patients with BCNS until the age of 8 years [83].

The recent development of novel SHH pathway inhibitors [84] may lead to future targeted therapies for individuals with both Gorlin and SUFU syndromes, possibly including primary tumor prevention strategies.

Brain Tumor-Polyposis Syndrome 2 (BTPS 2 or Turcot Type 2; Familial Adenomatosis Coli)

Familial adenomatous polyposis (FAP) is an autosomal dominant cancer predisposition syndrome. Although the hallmark

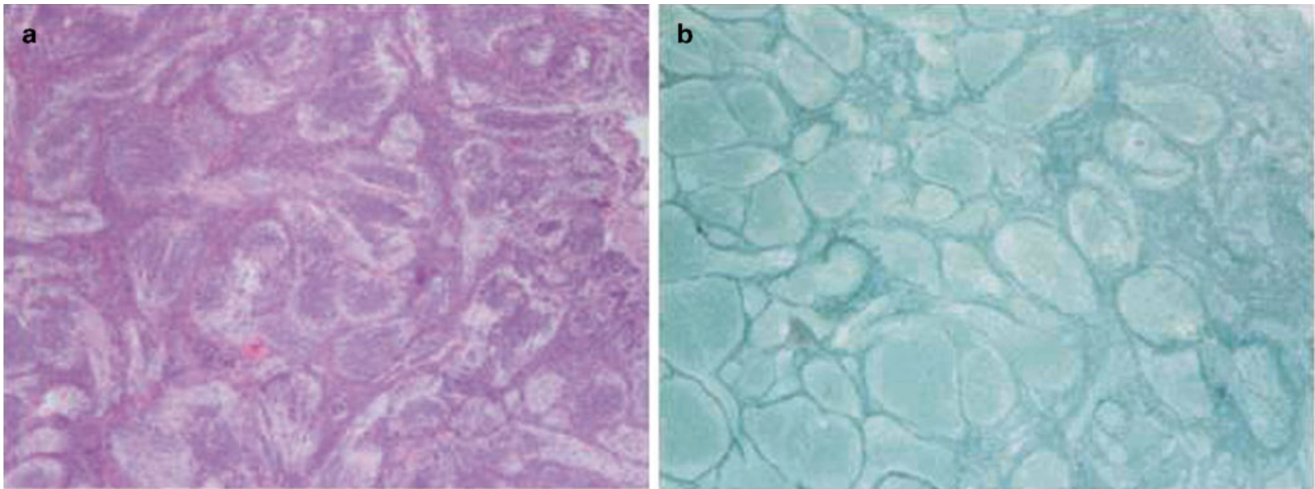

Fig. 1.4 Desmoplastic medulloblastoma. (**a**) Pale areas surrounded by densely packed hyperchromatic cells. (**b**) Same tumor reveals fine reticular areas with islands lacking reticulin

of this syndrome is the development of multiple gastrointestinal tract polyps and subsequent cancers, children with FAP are also at risk of developing medulloblastoma, hepatoblastoma, and aggressive fibromatoses.

The association between colorectal carcinoma and medulloblastoma was first reported in 1949 [85] and medulloblastoma is the only tumor observed in children with this syndrome. These patients and/or their family members display numerous (>100) small colonic polyps with later onset of malignant transformation to adenocarcinoma later in life. The pattern of inheritance here, distinct from BPTS type 1 (see glioma syndromes), is autosomal dominant and has been shown to be due to a heterozygous germline mutation in the adenomatous polyposis coli (*APC*) gene. Although the risk of developing medulloblastoma in patients with FAP is estimated 92-fold that of the general population [86], it is still a rare phenomenon among carriers.

Molecular Pathogenesis

FAP is caused by germline mutations in the gene *adenomatosis polyposis coli* (*APC*) [87]. *APC* is located on chromosome 5q21-22 and is a major regulator of the WNT pathway, which plays a paramount role in controlling embryonic development, stem cell viability and proliferation. Hyperactivation of the WNT pathway is reported in 5–10 % of medulloblastomas, usually as a result of mutations in *CTNNB1* [88, 89]. *APC* mutations are rare in sporadic medulloblastoma. Interestingly, no current mouse models exist for APC-driven medulloblastoma.

Clinical Implications

Since medulloblastoma is rare in FAP, carriers are not routinely screened for these tumors. However, a patient with medulloblastoma and FAP should undergo GI cancer surveillance, since the development of medulloblastoma in PBTS 2 may precede the development of colonic adenocarcinoma. Indeed, patients are reported with simultaneous diagnoses of medulloblastoma and colonic adenocarcinoma. It is important to note that *APC* mutated medulloblastomas are distinct from most *WNT* activated medulloblastomas. WNT tumors will have *CTNNB1* mutations that can be diagnosed by nuclear staining of the gene product. Although WNT pathway activation generally confers favorable survival in sporadic medulloblastomas, the prognosis of *APC* mutated tumors is still uncertain, and FAP patients therefore should not be treated with less aggressive protocols.

Fanconi Anemia Cancer Predisposition Disorders

Fanconi anemia is a cancer predisposition syndrome with bone marrow failure and characteristic malformations. The malformations are seen in about 60 % of affected individuals and include low birth weight, short stature, pigmentary abnormalities of the skin, abnormal thumbs, and hypoplastic radii [90]. There is genetic variability in Fanconi anemia (FA): patients may have mutations in one out of 15 known genes that cause the syndrome. One of the genes, FANCB, is associated with X-linked recessive inheritance and the rest with autosomal recessive [90].

The following features of FA suggest the clinical diagnosis and genetic testing [91]:

1. Characteristic congenital malformations as well as growth and developmental delays
2. Bone marrow failure in childhood which is usually progressive
3. Aplastic anemia in adults

4. Unexpected bone marrow failure after chemotherapy or radiation
5. Myelodysplastic syndrome or acute myelogenous leukemia
6. Solid tumors in young age including squamous cell carcinomas of the head and neck, esophagus, and vulva

If Fanconi anemia (FA) is clinically suspected the next step is chromosome breakage studies using diepoxybutane or mitomycin C as clastogenic agent [92]. If increased chromosome breakage is ascertained then DNA sequencing and deletion/duplication tests are available for all the known genes: *FNCA, FANCB, FANCC, BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, BRIP1, FANCL, FANCM, PALB2, RAD51C*, and *SLX4* [93]. If the patient is Ashkenazi Jewish with no history of carrier testing in the patient or the parents then testing for the *FANCC* mutation (c.456+4A>C) should be the first step [94].

Bone marrow failure states and/or myeloid dysplasias or leukemias (median age for onset is 14 years) develop in most individuals with FA [91]. In addition, a variety of solid tumors have long been recognized to develop with increasing frequency, particularly liver adenomas (in association with prior androgenic steroid use for the bone marrow failure) [90], and gastrointestinal and gynecological carcinomas, with a median age at diagnosis of about 29 years [95]. The median age for onset of the leukemias is 14 years. It has been estimated that, by theoretically removing the competing risks of marrow failure and leukemias, individuals with FA have an estimated cumulative probability of developing a solid tumor of 76 % by the age of 45 years [96].

Although brain tumors in FA patients were reported in the past [97, 98], the involvement of medulloblastoma [99] and glioma [100] in specific germline mutations has not been suggested until recently.

Molecular Pathogenesis

FA is a genetically heterogeneous disorder associated with either biallelic mutations in any of the known 14 autosomal genes or a mutation in an X-linked gene [101]. Individuals in the FA complementation group *FANCD1* are estimated to represent no more than 3 % of all individuals with FA, and it is this group in whom biallelic mutations with *BRCA2* are found. *BRCA2* mutations are well known to be associated with familial predisposition to breast and ovarian cancer. Brain tumors have also been reported in such families [102]. These individuals may present a more severe phenotype with early onset of cancer. In particular, the cumulative probability of developing a brain tumor (almost always medulloblastoma) could be high as 85 % in the first decade [99]. This knowledge has prompted a search for other genes in the pathway and familial childhood cancers. Recently, germline mutations in *PALB2*, another gene in the Fanconi pathway, were reported to be associated with medulloblastomas and other pediatric cancers [103].

Mouse models of FA usually fail to produce the cancers and other organ damage seen in humans [104]. However, an increased incidence of tumors has been observed in *Fancd2* deficient mice [105]. This gene interacts with *BRCA2* and *PALB2*.

Clinical Implications

The rare individuals who develop medulloblastoma in the setting of FA do so at a very early age, often before a diagnosis of FA has been made. Individuals with FA undergoing treatment for cancer are known to be highly sensitive to both irradiation and chemotherapy, with increased susceptibility for treatment-associated toxicities, especially from alkylator-based chemotherapy [106]. Thus, any early onset pediatric brain tumor with cutaneous, skeletal, or neurological abnormalities consistent with a diagnosis of FA or in case of severe unexpected toxicity from chemotherapy, genetic counseling is recommended.

The concept of synthetic lethality is being exploited in the use of PARP inhibitors in BRCA1/2 deficient breast, ovarian, and pancreatic cancers [107, 108], which could be of value in FA as well. To date, however, no data exist on the use of PARP inhibitors in the treatment of FA-related medulloblastoma.

Meningioma

Meningiomas are slow growing CNS lesions accounting for roughly a third of CNS tumors in adults [109]. While most of these tumors are sporadic or occur as a result of prior radiation therapy, familial cases of meningiomas are well reported. The most common genetic syndrome associated with meningiomas is neurofibromatosis type 2 (NF2). This syndrome has additional clinical features, and will be discussed in the section of tumor specific syndromes. However, several kindreds with familial meningioma lack linkage to the NF2 locus [110] are diagnosed with other known syndromes such as Li–Fraumeni, Cowden, Gorlin, and multiple endocrine neoplasia (MEN) [111]. Recently, genetic analysis of familial meningiomas uncovered involvement of the SWI/SNF family members *SMARCB1* and *SMARCE1* in several families diagnosed with schwannomatosis [112] with meningiomatosis [113]. Furthermore, as described above, patients with Gorlin syndrome including *SUFU* mutations [114], carry an increased risk of meningiomas.

Indeed, several reports implicate the SHH pathway in a subset of sporadic meningiomas of non-NF2 origin, offering a potential molecular target for the treatment of these tumors [115, 116].

Tumor Specific Syndromes

These tumors are unique to a specific cancer predisposition syndrome. Whenever that specific pathology is recognized, a high index of suspicion for the corresponding cancer syndrome should exist, regardless of family of personal history.

Although we will elaborate on several specific syndromes, other cancers are highly suggestive of predisposition to cancer, and germline analysis is recommended. A good example is choroid plexus carcinoma [2], which is strongly associated with LFS.

Subependymal Giant Cell Astrocytoma

SEGA is seen almost exclusively in the context of patients with tuberous sclerosis (TS) complex and conversely, SEGA is the only CNS tumor seen in TS. TS is an autosomal dominant multisystem condition affecting both children and adults [3]. Tumors outside the CNS arising in these patients are generally slow growing and include cardiac rhabdomyoma, renal angiomyolipoma, and pulmonary lymphangioleiomyomatosis. Although these lesions are histologically benign, they can cause significant organ dysfunction resulting in morbidity and in some cases mortality. Additionally, individuals with TS can occasionally develop malignant renal cell carcinomas.

Molecular Pathogenesis

Linkage analysis enabled the discovery of two genes responsible for the TS syndrome. These are *TSC1*, also known as *Hamartin*, located on chromosome 9q34 [117] and *TSC2* or *Tuberin* on chromosome 16p13. These genes exert their tumor suppressor activity by inhibition of *RHEB*, which is the major activator of the mammalian target of rapamycin (mTOR) complex. The AKT/mTOR pathway is a key driver of tumorigenesis [118] in TS patients and an important therapeutic target.

SEGAs develop in 5–15 % of patients with TS complex, usually in the first two decades of life. Rarely, SEGAs may occur in patients without any other evidence of TS, typically in older adults. SEGAs are intraventricularly located tumors, usually in close proximity to the foramen of Monroe, are histologically benign (WHO grade I), but can nevertheless lead to significant morbidity and mortality due to development of hydrocephalus from obstruction of cerebrospinal fluid (CSF) flow at the foramen, as well as due to subependymal invasion into eloquent brain parenchyma.

Clinical Implications

In TS patients, brain MRI scans should be obtained at least annually during childhood and adolescence, when the risk for SEGA development is greatest [119].

TS represents a prototype disease in which biological discoveries have led to the successful development of effective targeted therapies, with profound consequences on clinical management. First-generation mTOR inhibitors (termed rapamycin analogs or rapalogs, including rapamycin) are mTOR complex 1 (mTORC1) specific inhibitors, acting downstream of TSC 1 and 2. As predicted by preclinical data, clinical trials using rapalogs have revealed striking tumor regression of virtually all SEGAs in treated TS patients [120–122] (Fig. 1.5), as well as improvement in pulmonary function for patients with lymphangioleyomyomatosis [123]. Additional evidence suggests that rapalogs can improve other aspects of the syndrome including neurological symptoms including seizures [124, 125]. As a consequence, everolimus was granted United States Food and Drug Administration (FDA) approval for the treatment of pediatric and adult TS patients with SEGA. In addition, prevention strategies and protocols for long-term therapy with rapalogs are currently being developed for these patients [126].

Atypical Teratoid Rhabdoid Tumor

This deadly pediatric embryonal tumor exists solely in the context of the *Rhabdoid Tumor Predisposition Syndrome* (*RTPS*).

Malignant rhabdoid tumor of the kidney was initially reported in 1978, but the association with second primary "embryonal" tumors in the brain was not recognized until much later [127]. Historically, these tumors were classified as medulloblastoma or PNET based on histologic resemblance, but recognized as a distinct clinico-pathologic entity in 1995 and termed CNS atypical teratoid/rhabdoid tumor (AT/RT) of infancy and childhood [128]. They were recognized to be highly lethal tumors, with virtually all children dying of progressive tumor within 6–12 months of diagnosis [128].

Cytogenetically, AT/RT commonly harbor monosomy of chromosome 22. In 1999, both germline (constitutional) and acquired mutations on chromosome 22q11.2 in children with CNS AT/RT were reported [129], and shortly thereafter the term "rhabdoid predisposition syndrome" was coined to define the newly recognized heritable syndrome predisposing to both renal or extra-renal malignant rhabdoid tumors and malignant brain tumors [130]. By 2008, the entity of "rhabdoid predisposition syndrome" was sufficiently well documented to merit inclusion within the "World Health Organization (WHO) Classification of Tumours of the CNS (4th Edition)" [131].

Molecular Pathogenesis

The *SMARCB1* gene, also known as *INI1/hSNF5*, was cloned in 1998 [132] and is located on chromosome 22q11. Heterozygous germline loss-of-function mutations in the gene were first described in 1999 [130]. This facilitated the definition and permitted assessment of the risk of germline mutations in individuals with AT/RT. Germline mutations occur in up to 35 % of AT/RT patients, are more common in younger patients, and can be invariably found in patients presenting with both CNS and extra-cranial tumors. The biologic function of *SMARCB1* remains poorly understood. However, it is thought to be involved in nucleosome modification [133] and disruption of the gene results in spindle checkpoint defects and a high rate of chromosomal instability [134].

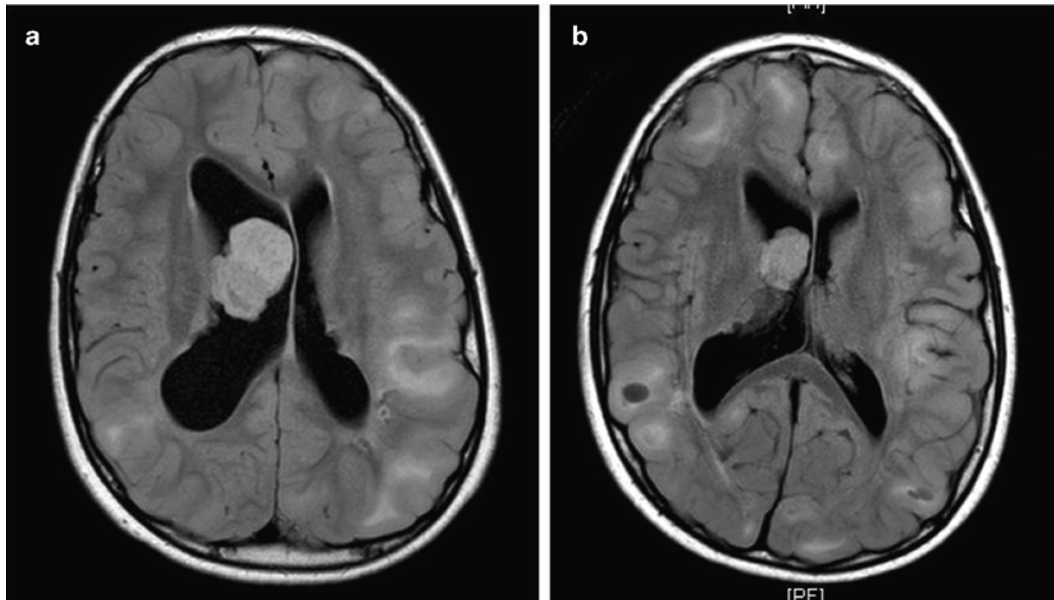

Fig. 1.5 Response of SEGA to oral treatment with sirolimus. Right ventricular SEGA causing mild hydrocephalus (**a**). The same lesion (**b**) after 3 months of oral therapy with sirolimus

Recent observation that loss of *SMARCB1* leads to activation of the *SHH* pathway is intriguing [135]. Mouse models using haploinsufficiency in Smarcb1 result in soft tissue sarcomas mimicking rhabdoid tumors but do not recapitulate AT/RT [136].

Clinical Implications

Patients with RTPS may present with synchronous or metachronous tumors. Known RTPS patients should undergo periodic surveillance imaging of both the abdomen (ultrasound or MRI) and the brain (MRI).

Until recently, CNS AT/RT was considered almost uniformly incurable and rapidly lethal. In recent years, however, the prognosis for young children with CNS AT/RT appears to have improved through better molecular diagnosis of the tumor and implementation of aggressive surgical resection of primary tumors followed by intensive chemotherapy [137–139]. It remains unclear whether the improved prognosis in older children with CNS AT/RT primarily reflects their ability to better tolerate radiation therapy, or rather is linked to differences in biology compared to the same tumors arising in infants. The outcome for children with CNS AT/RT specifically in the setting of the RTPS has not yet been reported.

The spectrum of tumors with *SMARCB1* somatic mutations is growing and includes other soft tissue sarcomas as well as schwannomas [140, 141]. Germline *SMSRCB1* mutations are the cause of familial schwannomatosis in about 40 % of the families with the condition [141, 142] and about 80 % of the remaining families with familial schwannomatosis have a mutation in *LZTR1* [143]. Because very few patients with recognized RTPS are long-term survivors, the cancer spectrum and lifetime risk of other malignancies in carriers remain unknown.

Hemangioblastoma

This tumor is the hallmark of the von Hippel–Lindau (VHL) syndrome, and patients diagnosed with hemangioblastoma require a genetic workup.

VHL syndrome is a tumor predisposition syndrome characterized by a variety of CNS and extraneural tumors. Von Hippel originally described retinal tumors as well as vascular tumors in the viscera, and the connection with the often fatal cystic vascular tumors of the cerebellum was first recognized by Lindau in 1926 [144].

The VHL syndrome is inherited as an autosomal dominant disorder with very high penetrance of over 90 % by age 65 years. The most common manifestation of the disease is CNS and retinal hemangioblastomas, which occur in 70 % and 60 % of patients, respectively [145]. The reduced life expectancy, however, is primarily linked to renal cell carcinomas that occur in up to 20 % of individuals.

The clinical diagnosis of VHL is considered in an individual without family history of VHL when two of the following are present [145–147]:

- Two or more hemangioblastomas of the retina, spine, or brain or a single hemangioblastoma in association with a visceral manifestation (e.g., multiple kidney or pancreatic cysts)
- Renal cell carcinoma

- Adrenal or extra-adrenal pheochromocytomas
- Less commonly, endolymphatic sac tumors, papillary cystadenomas of the epididymis or broad ligament, or neuroendocrine tumors of the pancreas

The clinical diagnosis of VHL is considered in an individual with a positive family history of VHL when one of the following is present:

- Retinal angioma
- Spinal or cerebellar hemangioblastoma
- Adrenal or extra-adrenal pheochromocytoma
- Renal cell carcinoma
- Multiple renal and pancreatic cysts

Clinical genetic testing for VHL is available with 72 % of the mutations being sequence variants and 28 % partial or whole-gene deletions [148–150]. Atypical presentation can be due to somatic mosaicism [151]. There is no genetic heterogeneity associated with the VHL phenotype.

Molecular Pathogenesis

The *VHL* gene is located on the short arm of chromosome 3 (3p25-26) and was first identified as the VHL tumor suppressor gene in 1993 [152]. VHL interacts with other proteins and forms a substrate recognition unit for ubiquitin ligase, which targets the hypoxia-inducible factor (HIF) genes 1 and 2 for degradation. Under normal circumstances, hypoxia results in HIF proteins to activate multiple metabolic and oncogenic pathways in the cell, including increased levels of VEGF, PDGF, erythropoietin and TGF. Abnormal VHL protein results in constitutive activation of HIF and other factors, leading to reduced apoptosis, increased proliferation, and increased angiogenesis [153], resulting in tumor formation [154].

Interestingly, mouse models using different alterations of *Vhl* resulted in erythrocytosis (polycythemia) and renal abnormalities [155], but did not produce a cancer phenotype or CNS lesions [156].

CNS Hemangioblastomas

These tumors arise, in order of diminishing frequency, in the cerebellum (44–72 % of all patients with VHL syndrome), the retina (25–60 %), intramedullary spinal cord (13–50 %), brainstem (10–25 %), supratentorial compartment (<1 %), and lumbosacral nerve roots (<1 %) [145]. CNS hemangioblastomas arising as single tumors outside of the posterior fossa are rarely sporadic, and multiple hemangioblastomas are virtually pathognomonic for the presence of a *VHL* germline mutation. The mean age of diagnosis of CNS hemangioblastomas is between 30 and 35 years. While CNS hemangioblastomas are considered “benign” tumors, these tumors were associated with significant morbidity and mortality prior to the recognition of their association with VHL and the establishment of screening guidelines for early detection.

Clinical Implications

All patients diagnosed with hemangioblastoma should be screened for germline mutations in *VHL*. De novo mutations are common and are detectable in up to 20 % of patients. Since mosaic mutations are reported, multiple hemangioblastomas, several tumors or family history of tumors compatible with the VHL spectrum can establish the diagnosis of VHL syndrome even in the absence of a detectable mutation in blood leukocytes. A surveillance protocol has been developed and it is aimed at not only improving survival, but also reducing morbidity from earlier interventions for VHL tumors [157, 158]. The suggested surveillance includes:

Starting at age 1 year: annual evaluation for neurologic symptoms, vision problems, and hearing disturbance; annual blood pressure monitoring; annual ophthalmology evaluation.

Starting at age 5 years: annual blood or urinary fractionated metanephrines; audiology assessment every 2–3 years; thin-slice MRI with contrast of the internal auditory canal in those with repeated ear infections. Starting at age 16 years: annual abdominal ultrasound and every other year MRI scan of the abdomen; MRI of the brain and total spine every 2 years.

Development of New Medications

The association of VHL and renal cell carcinoma has led the development of compounds which target VEGF receptor signaling, as VEGF is significantly overexpressed as a result of HIF activation in sporadic renal cell carcinoma. These “anti-angiogenic” drugs and others have been used in patients with hemangioblastomas with some success [159–161]. It is hoped that drugs that provide satisfactory long-term tumor control or are suitable for prevention will be developed for VHL patients in the future.

Cerebellar Dysplastic Gangliocytoma (Lhermitte–Duclos Disease)

This brain lesion is pathognomonic for Cowden syndrome or multiple hamartoma syndrome, “Cowden’s disease.” Cowden syndrome was first described in 1963 and named after the first reported patient, Rachel Cowden, who had multiple mucocutaneous hamartomatous abnormalities [162]. About 90 % of patients who develop Cowden Syndrome develop clinical manifestations before 20 years of age, although may not be diagnosed until the third decade of life. Women have between a 25 and 50 % lifetime risk of developing breast cancer as well as an increased risk of developing endometrial cancer, and both men and women have a 10 % lifetime risk of developing epithelial thyroid cancer. About 50 % of cases of Cowden syndrome are considered to be inherited.

Brain Tumors

The recognition that cerebellar dysplastic gangliocytoma (Lhermitte–Duclos Disease) might be a manifestation of

Cowden syndrome was first reported in 1991 [163]. While more commonly seen in adults [164] about 5–10 % occur during childhood.

Molecular Pathogenesis

Cowden syndrome is a member of the PTEN Hamartoma Tumor Syndrome (PHTS). This entity encompasses four major, clinically distinct syndromes associated with germline mutations in the tumor suppressor *PTEN*. These allelic disorders, Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome, and Proteus-like syndrome are associated with dysregulated cellular proliferation leading to the formation of hamartomas [165, 166]. Thus far, an increased risk of malignancy has only been documented in Cowden syndrome.

PTEN is located on chromosome 10q23 and is a phosphatase that competes with PI3K, a major protein kinase which acts by reducing PI3P levels in cells. Reduction of 3-phosphoinositides decreases activity of kinases downstream of PI3K such as Akt and mTOR, and is responsible for its tumor suppressor activity. The PI3K/Akt/mTOR pathway is a major oncogenic signaling pathway, which regulates cell metabolism, survival, proliferation, migration, and angiogenesis [167]. Although *PTEN* alterations are a major component of adult gliomagenesis [13], individuals with germline *PTEN* mutations do not have increased susceptibility for these tumors. Cowden syndrome has been associated with a germline mutation of the *PTEN* gene in about 80 % of cases, with an additional 10 % harboring mutations in the *PTEN* promoter region.

Clinical Implications

Overall, the incidence of cerebellar dysplastic gangliocytoma has been estimated to be 15 % among patients with Cowden syndrome undergoing magnetic resonance imaging surveillance scans, with additional patients revealing meningiomas (5 %) and other vascular malformations in 30 % [168]. Therefore, it is recommended that patients with Cowden syndrome undergo annual surveillance screening with brain MRI. Surveillance guidelines for individuals with Cowden syndrome are available (www.NCCN.org) and should be utilized for affected family members [167]. Recent observations of reduction in hamartomas for patients with PHTS after treatment with rapamycin [169], and the finding of excess levels of *mTOR* pathway expression in Lhermitte–Duclos disease (LDD) tumor tissue [170] suggest that medical prevention or treatment of LDD and other neoplasms in individuals with Cowden syndrome is feasible.

Vestibular Schwannomas (Acoustic Neuromas)

Bilateral vestibular schwannomas (VS), arising at the vestibular branch of the eighth cranial nerve, are pathognomonic for the tumor predisposition syndrome neurofibromatosis type 2 (NF2, Fig. 1.6). For historical reasons, NF2 has been grouped together with NF1 as phakomatoses (or "neurocutaneous syndromes"), but differs from neurofibromatosis type 1 with respect to the underlying genetic defect, disease biology, clinical manifestations, and tumor spectrum.

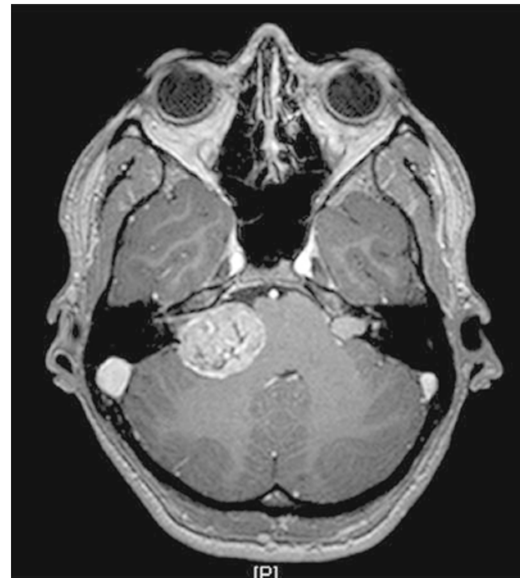

Fig. 1.6 Bilateral acoustic neuroma in NF2

Neurofibromatosis type II (NF2) is an autosomal dominant tumor predisposition syndrome characterized by the development of bilateral VS and schwannomas of other cranial, spinal, and peripheral nerves. Individuals with NF2 are also predisposed to developing intracranial, spinal, and optic nerve sheath meningiomas, as well as low-grade ependymomas of the CNS [171, 172].

The cardinal feature is bilateral vestibular schwannoma presenting clinically with any or the combination of hearing loss, tinnitus, and imbalance. One of the modified NIH criteria [173] is sufficient for the diagnosis:

1. Bilateral vestibular schwannomas
2. A first-degree relative with NF2 and (a) or (b)
   (a) Unilateral vestibular schwannoma
   (b) Any two of: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
3. Unilateral vestibular schwannoma and any two of: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
4. Multiple meningiomas and (a) or (b)
   (a) Unilateral vestibular schwannoma
   (b) Any two of: schwannoma, glioma, neurofibroma, cataract

Most affected have bilateral vestibular schwannomas by age 30. About 50 % of individuals have an affected parent. About 20–30 % of simplex cases (only case affected in a family) are mosaic for an *NF2* mutation. NF2 is due to all type of mutations in the *NF2* gene including all size deletions

and chromosome abnormalities. The mutation detection rate approaches 72 % in simplex cases and exceeds 92 % for familial cases [66, 174–176].

Molecular Pathogenesis

The gene responsible for NF2 was discovered in 1993 as *Neurofibromin 2* or *Merlin* (HGNC Approved Gene Symbol: *NF2*), located on chromosome 22q12.2 [177, 178]. Despite intensive efforts, the physiologic role of Merlin, as well as its function as a tumor suppressor remains incompletely understood [179]. There is a high rate of mosaicism in individuals with a de novo mutation, termed "founders." Therefore the chance of transmission, which is autosomal dominant, may be less than 50 % in such individuals.

Intracranial Tumors

The hallmark of NF2 is the development of bilateral VS, with a lifetime penetrance of over 95 % in NF2 individuals. Although historically considered to be a syndrome mainly presenting in young adulthood, up to 20 % of patients will present prior to 15 years of age [180]. Alternatively, NF2 patients may first present with non-vestibular schwannomas (33 %), meningiomas (31 %), or spinal tumors (11.5 %). Over time, the majority if NF2 patients will develop bilateral VS, as well an increasing tumor burden including other intracranial schwannomas, as well meningiomas and ependymomas. Progressive VS result in neurological complications including hearing loss, facial weakness, and brainstem compression. Depending on location, other intracranial tumors may cause cranial nerve dysfunction, brain compression, and/or seizures.

Spinal Tumors

The incidence of spinal tumors in patients with NF2 may reach 89 % [181]. About one-third of spinal tumors in association with NF2 are intramedullary ependymomas. Of the extra-medullary tumors, schwannomas are most common, followed by meningiomas, with neurofibromas being very uncommon. These tumors are usually multifocal, and often asymptomatic. Progressive growth, however, can lead to pain, cord compression, myelopathy, and/or neurologic impairment.

Clinical Implications

A consensus meeting has produced surveillance guidelines for individuals with NF2 [182]. Asymptomatic children carrying the mutation should be followed expectantly and screened with MRI surveillance and audiograms beginning at age 10 years. Like other patients with rare diseases, NF2 are best managed by a multidisciplinary team with disease-specific expertise. Although surgery and supportive therapy have been the mainstay of treatment for NF2 patients, bevacizumab has recently emerged as a therapeutic option that can lead to tumor shrinkage and hearing improvement in a subset of NF2 patients with VS [182]. Other targeted therapies are under development and evaluation consensus recommendations for current treatments and accelerating clinical trials for patients with neurofibromatosis type 2 [183].

Implications of Molecular Tumor Testing on Genetic Counseling

Most patients are referred to genetic counseling due to combination of family history of cancer or other diseases and findings on clinical examination. This approach may change in the near future due to implementation of pathological and genetic tests as routine for tumor diagnosis. These may suggest cancer predisposition in the absence of the above clinical findings. Several examples are worth mentioning. A child diagnosed with desmoplastic medulloblastoma less than 3 years old has 50 % chance of having BCNS (Gorlin syndrome). ATRT and choroid plexus carcinomas mutated for *SMARCB1* and *TP53*, respectively, carry very high rate of germline mutations too. Furthermore, children older than 5 years with *TP53* mutant SHH medulloblastoma are most probably LFS patients. Since these molecular tests are routinely used and will be a part of all modern clinical trials, the indications for genetic counseling may change and the spectrum of tumors and clinical manifestations of some of these syndromes may change as a result.

Summary

This chapter does not aim at summarizing all clinical and molecular aspects of predisposition syndromes affecting brain tumors patients. Further information is available in the references provided. Furthermore, the relatively common syndromes were elaborated above while other less common syndromes are mentioned in Table 1.1. Nevertheless, the burden of cancer predisposition on neuro-oncology is significant and knowledge of the diagnosis, management, and appropriate treatment will impact the patient and family members. Recognizing hereditary conditions that predispose to brain tumors is important for providing the appropriate treatment and surveillance. Surveillance protocols have shown survival benefit and novel therapies exist for some specific genetic alterations.

In the research arena, detailed phenotyping and genotyping informs molecular pathophysiology with ensuing discovery of new genetic tests and new treatments. Individuals with germline mutations in cancer predisposing genes may benefit from early detection and personalized therapies for their cancer which will eventually offer improved morbidity and mortality.

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2 Brain Tumor Stem Cells

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Primary brain tumors represent a challenging biological and clinical entity. The limited therapeutic options and high rates of morbidity and mortality associated with them highlight the need for a better understanding of their molecular and pathophysiological complexity [1]. In recent years, it has become clear that such tumors are highly heterogeneous, not just histologically but at the molecular level as well [2–5]. This heterogeneity raises the possibility that within each tumor exist different cell types, each with distinct roles in maintaining tumor heterogeneity and bearing unique combinations of signaling pathways and molecular markers.

One important question related to tumor heterogeneity is: Are the different cell types equally important for tumor growth or not? Over the past decade, accumulating evidence supports the theory that brain tumors are governed by a cellular hierarchy dominated by brain tumor stem cells (BTSCs). Indeed, small subpopulations of cells within such tumors possess stem-like properties and the enhanced ability to regenerate tumors in laboratory animals [6–13].

Cancer Stem Cell Definition

Although initially defined in liquid tumors, i.e., leukemias, some of the most compelling evidence for cancer stem cells in solid tumors originates in brain malignancies, and especially gliomas [14, 15]. BTSCs are very well defined by several functional criteria, which are borrowed from developmental biology. To be considered a stem cell, whether normal or cancerous, a cell should be able to *self-renew*, which refers to the limitless ability to proliferate and maintain the undifferentiated phenotype; and *differentiate* into different lineages, a property termed *multipotency*. Stem cells have the ability to undergo asymmetric division giving rise to a stem cell (self-renewal) and a lineage-restricted progenitor cell, which is limited in its differentiation potential and generates terminally differentiated mature cells after proliferating (Fig. 2.1).

It is hypothesized that variations in self-renewal and proliferative abilities generate a cellular hierarchy within brain tumors, with BTSCs at the apex of this hierarchy [6]. In addition to these obligate properties, BTSCs should be able to *initiate tumors* and phenocopy the original tumor when injected into animal models [7, 16]. The presence of BTSCs in brain tumors raises another important question: Do brain tumors arise from the oncogenic transformation of normal neural stem cells (NSCs) residing within the brain? Or can differentiated brain cells undergo mutations that lead to their dedifferentiation and tumorigenesis?

This chapter focuses on two aspects of stem cell biology in brain tumors. The first part will cover the role of NSCs and progenitor cells as candidates for the cell of origin in brain tumors. The second part will discuss molecular characteristics of BTSCs and their therapeutic implications. We believe that understanding cancer stem cell biology and its therapeutic implications will be crucial for developing fundamentally new, and hopefully more effective, treatments.

Brain Tumor Initiation

Mechanisms of brain tumor initiation are unclear; mouse models have revealed that a number of mutations are capable of initiating tumors and that the cell of origin may differ amongst different genetic subclasses of brain tumors or even within a given tumor type. Also, the question of how tumor cells acquire a BTSC phenotype during or after tumor initiation remains to be answered. Two dominant hypotheses have emerged to account for the presence of BTSCs within gliomas: (1) Brain tumors arise from the transformation of endogenous NSCs or progenitor cells that acquire aberrant self-renewal and differentiation properties; and (2) differentiated brain cells undergo oncogenic transformation, dedifferentiate and acquire stem cell characteristics. This section will provide background on neurogenesis and normal NSC biology, which will lay the foundation for understanding gliomagenesis and the molecular characteristics of BTSCs.

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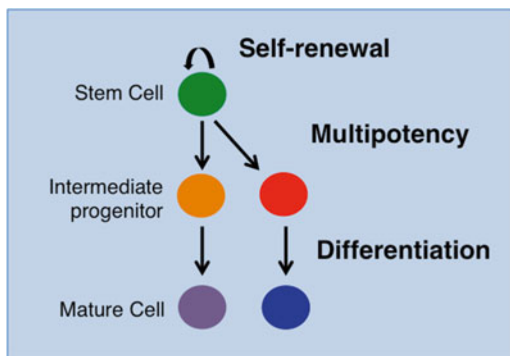

Fig. 2.1. Properties of stem cells. Stem cells have the ability to self-renew and differentiate to a mature cell through an intermediate progenitor. The ability to give rise to cells from different lineages is termed multipotency.

Neurogenesis in the Adult Brain

The cell of origin in brain tumors, including gliomas, is still a matter of debate [17]. However, there has been intense speculation that brain tumors may arise from neurogenic niches in the brain [18–27]. Since the discovery of adult neurogenesis, new insights have emerged about the mechanisms by which the brain maintains a small population of NSCs that can replenish both neurons and glia [28, 29]. Given the intrinsic ability of both BTSCs and NSCs to self-renew and differentiate, it is important to consider how cancer stem cells' ability to regulate their own function in the tumor deviates (or remains the same) from that of normal NSCs. NSCs in the adult brain actively generate both neurons and glia that contribute to the brain's cellular and functional homeostasis, as well as plasticity, remodeling, and response to injury [30–32]. In contrast to post-mitotic neurons, it is the cell types undergoing mitoses that are thought to harbor the greatest potential to give rise to brain tumors. Such mitotically active cells are found in neurogenic niches. Cells in the neurogenic niche perturbed by mutagenic events can theoretically serve as starting points for brain tumor formation and may harbor intrinsically higher oncogenic potential than other dormant neural cell types. Understanding neurogenesis and gliogenesis will allow us to explore concepts relevant to the cell-of-origin question in brain tumors and the regulation of BTSCs during tumorigenesis.

NSCs have been identified in at least two regions of the adult brain: the subventricular zone (SVZ) of the lateral ventricles, and the subgranular zone (SGZ) of the hippocampus [28, 33–35]. NSCs may also exist within the subcortical white matter [36]. During fetal life, radial glia, which are derived from neuroepithelium, are responsible for neurogenesis. Radial glia participate in neural organization; immature neurons that arise from radial glia move along their transcortical extensions for migratory guidance to their respective areas in the cortex, contributing to its stratified organization during the late embryonic stages [37]. With the transition to postnatal life, radial glia differentiate into many different cell types, including neurons, astrocytes, oligodendrocytes, ependymal cells, and the SVZ NSCs, which contribute to adult neurogenesis in the mammalian brain in postnatal life [28, 32, 34, 38, 39]. The adult SVZ NSCs, or type B cells, line the subependymal zone of the lateral ventricles. B cells give rise to intermediate progenitors termed transit-amplifying cells (or type C cells), which proliferate and have the ability to form immature neurons termed neuroblasts (type A cells). Neuroblasts migrate through the rostral migratory stream (RMS) to the olfactory bulb in rodents [40] and, additionally, through the medial prefrontal migratory stream in humans [41], eventually becoming mature post-mitotic neurons. Type B cells additionally give rise to oligodendrocyte precursor cells (OPCs) and astroglia depending on growth or inhibitory signals ([31, 42], see reviews [29, 43]).

In the hippocampal formation, the dentate gyrus SGZ houses radial astrocytes, which serve as a source of neurogenesis in the region [28, 33]. Radial astrocytes (type 1 cells) form intermediate progenitor cells (type 2 cells), which become immature granule cells (type 3 cells) and subsequently mature granule neurons [44–47]. The SGZ and SVZ house the two identified groups of stem cells responsible for formation of new neuronal and glial cell types in adult mammals. The majority of what we have learned about neurogenesis has emerged from studies conducted using rodent models. Some of these findings have been validated in postmortem human brain samples [30, 35, 41], although such studies are limited in number. The discovery of active neurogenesis in the adult human brain has numerous implications for the pathobiology of brain tumor formation, neurodegenerative disorders, and response to injury.

Gliomagenesis

In the very beginning of brain tumorigenesis, the cellular composition is thought to differ drastically from that of a mature tumor. Given the known heterogeneity of fully developed tumors and the overwhelming possibility that the tumor began from a single cell type, an open question remains: How does this heterogeneity arise? To address this question, we will focus on gliomas, whose developmental biology is perhaps better understood than any other brain tumor.

We know that BTSCs from mature gliomas can phenocopy the tumors they were derived from in animal models. The BTSCs found in gliomas must have arisen from a tumor that lacked cells with stem cell properties or a tumor that arose from an endogenous NSC (Fig. 2.2). The cell of origin is defined as the cell type that has accumulated the correct combination of mutations that induces proliferation and eventually tumor formation (oncogenic transformation). Although not mutually exclusive, the cell of origin differs from the cell type that acquires mutations, which is referred to as the cell of mutation. This is because the cell of mutation, after acquiring the first oncogenic hit, may differentiate

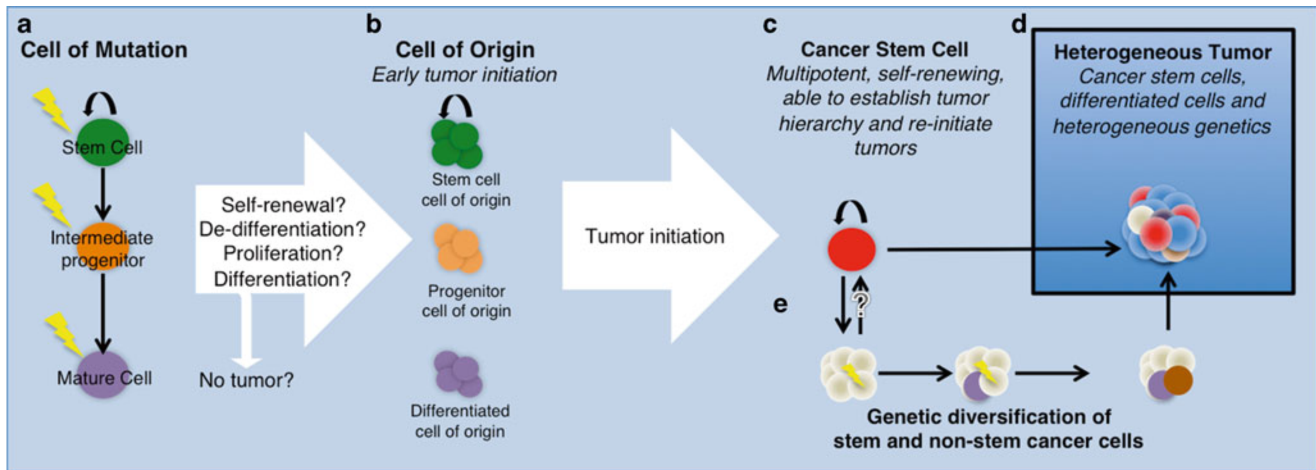

FIG. 2.2. Model of gliomagenesis. (**a**) Representation of a normal cellular hierarchy where the cell of mutation may give rise to the cell of origin (**b**). Cancer stem cells (**c**) are self-renewing and tumor re-initiating cells found in the tumor. Both cancer stem cells and non-stem cells experience genetic changes (**e**), contributing to the heterogeneous tumor (**d**). Different colors represent different cell types emphasizing intratumoral heterogeneity.

or dedifferentiate into another cell type before proliferating, so a distinction must be made for the cases where this happens (see reviews [17, 48]). An important concept to clarify is the difference between the cell of origin and BTSCs. Both can theoretically form the mature tumor, but the cell of origin is responsible for initiating the tumor and may or may not be a stem cell. The cell of origin is of interest in the discussion of BTSCs because evidence from mouse models has pointed to the possibility that NSCs within the brain may serve as the cells of origin. Given that BTSCs have stem cell properties, identifying the cell of origin may provide insight into how BTSCs are derived and how their ability to self-renew or differentiate differs from that of normal stem cells and the cell of origin. It is still unclear if multiple cell types within the brain can give rise to the same type of tumor and whether different types of tumors share the same cell of origin harboring different genetic mutations. To answer some of these questions, glioma models have been developed and used successfully to uncover key aspects of glioma biology.

Models of glioma aim to recapitulate human glioma pathology most commonly through two model systems: genetically engineered rodent models with mutations found in human gliomas, or xenografts of primary human glioma lines derived from patients. Other model systems also exist, including in vitro cultures of glioblastoma multiforme (GBM) cell lines, allografts of rodent glioma lines and virally mediated oncogenesis. These approaches have been used to provide reproducible platforms to study many aspects of tumor biology, including BTSCs, the cell of origin, and therapeutic implications [19, 21, 22, 49–54].

Primary lines derived from patient tumors carry a distinct advantage due to their genetic make-up, which most faithfully represents the disease [49]. The drawbacks of primary lines include: (1) the necessity to inject the mouse brain to create a xenograft, thus potentially altering the microenvironment; (2) the possibility that, in derivation of each culture, a subpopulation of the tumor is selected for; and (3) the fact that these tumors are grown in an immunodeficient background. Murine models have the disadvantage of a rodent genetic background and are limited in recapitulating the order and number of mutations that occur in a sporadic human glioma. Modeling efforts will likely continue to sample the phenotype produced by different combinations of mutations, location of the tumor, and the developmental point of induction of these mutations. In both cases, the tumor is grown in the mouse brain microenvironment, which raises an additional degree of separation from human glioma biology for both the mouse and xenograft model systems.

Experimental evidence from murine models of glioma suggests the cell of origin to be adult NSCs or proliferating progenitor/precursor cells, but not mature glia. However, this is highly controversial and remains an open question for the many different subtypes of glioma (see reviews [53, 55]). Following the discovery of adult neurogenesis, there was a paradigm shift in thinking about the origins of glioma, as the discovery of NSCs and their progenitor/precursor cell progeny became new candidates (Fig. 2.2). In a landmark effort to link the differentiation stage to tumor initiation, Holland et al. found that NSCs or neural progenitor cells expressing Nestin in the mouse brain were preferentially forming tumors with GBM characteristics after activation of the K-Ras/Akt pathway [25]. The same oncogenic insult did not produce tumors under the control of a GFAP promoter, suggesting that not all cell types within the same lineage could serve as the cell of origin [25]. Parada and colleagues have developed multiple tumor suppressor knockout models of gliomagenesis via inducible loss of p53, PTEN, and NF1, which are some of the most commonly mutated tumor suppressors in

GBM [56, 57]. Analysis of the high-grade gliomas generated from these models revealed that Nestin-expressing cell types found in the SVZ are likely to contain the cell of origin. More recent studies with PDGF-driven tumor formation and p53/NF1 knockout have shown that oligodendrocyte precursor cells (OPCs) are capable of giving rise to GBM tumors in mice [20, 58].

Mouse models using Ink4a-ARF loss, K-ras activation, or PDGF signaling give rise to gliomas in areas and cell types found outside of the neurogenic niches, suggesting that mature glial cells can produce malignancy when given the correct combination of oncogenic mutations [54, 59–62]. Interestingly, it has also been reported that mature neurons, in addition to GFAP-positive astrocytes, are capable of acting as the cell of mutation in a p53/NF1 model of glioma by undergoing dedifferentiation [24]. Some consideration should be given to the fact that some of these murine models represent functional genetic alterations that may or may not be the initiating events in glioma formation despite their oncogenic transforming abilities in this context. It remains an open question as to what the initial events in the different subtypes of glioma are, and how restricted the cell of origin truly is for any given tumor type, considering the unique combination of microenvironment, genetic changes, and organism.

It should be highlighted that in many of the aforementioned murine models, there is a propensity of early events in murine gliomagenesis to occur near the SVZ when the NSC population is targeted [56, 57, 63]. There is clinical evidence in humans that initiating events in glioma formation occur in or near the neurogenic zones of the brain. Human GBM has a propensity to occur most frequently in the periventricular area and less so in the surrounding cortex, albeit this evidence is controversial [63–65]. GBM also occurs infratentorially, but with much lower frequency [66]. The tendency for GBM to occur in the periventricular area within the cerebrum suggests that a cell-of-origin also resides within the same region; however, this correlation has not been directly linked to human neurogenesis. It is possible that tumors found far from neurogenic regions may be initiated by migrating cells that originated in the neurogenic niche. This is an interesting but unexplored hypothesis.

Grade II/III gliomas mutated for isocitrate dehydrogenase (IDH) tend to arise in different anatomic locations as compared to their grade IV GBM counterparts. Although IDH1 normally functions to convert isocitrate to α-ketoglutarate, the mutation leads to the production of oncometabolite 2-hydroxyglutarate (2HG) which stereo-chemically resembles α-ketoglutarate and is hypothesized to cause tumorigenic epigenetic changes [67–70]. In IDH-mutated gliomas, the cytosolic variant IDH1 is most frequently mutated, whereas mutations in IDH2 can be found less commonly [71]. Strikingly, the IDH1-mutated gliomas are most commonly found in the frontal lobe in an area that overlaps with the rostral and medial migratory streams used by neuroblasts to replenish interneurons in the olfactory bulb and frontal cortex, respectively [30, 63]. Nevertheless, IDH1-mutated tumors can also be found in other regions of the brain, albeit at a lower frequency. The fact that low- and high-grade tumors tend to arise in different anatomic locations raises the possibility of differing cells of origin. Alternatively, it may signify that IDH1 mutations promote gliomagenesis only in restricted cell types or lineages. The lack of mouse models that produce IDH1-mutated tumors has inhibited the dissection of the cell of origin in this class of tumors [72–74].

Despite the possibility that the cell of origin may originate from a stem cell or a more restricted progenitor/precursor cell, there is evidence that the tumor either gains (via dedifferentiation) or maintains a portion of its population as cells with stem cell properties. The continuum between the cell of origin and BTSCs is not well understood (Fig. 2.2). Murine models have allowed the detection and study of BTSCs in the context of gliomas and medulloblastomas primarily. Much of our understanding of BTSC biology has derived from the study of primary human GBM.

Identification of Brain Tumor Stem Cells

As mentioned earlier, the concept of cancer stem cells was initially developed in studies involving leukemias, where the cellular hierarchy is well established [14]. In such tumors, an abundance of lineage-specific cell surface markers made the isolation of distinct cell types within this hierarchy feasible. Some of the same surface markers were later used to isolate cancer stem cells in solid tumors. Before going into the details of surface markers and molecular characteristics of BTSCs, we will describe the main approaches used to identify them.

BTSCs, which are a subpopulation of cells within the tumor, are defined by their ability to *initiate tumors* in animal models that recapitulate patient tumor phenotype and heterogeneity [7, 8, 49]. As mentioned earlier, two critical properties of BTSCs are *self-renewal* and *multipotency* (Fig. 2.3). Self-renewal is tested with the following two standard assays. First, clonogenicity is assessed by in vitro tumor sphere formation ability over serial passaging [49, 75]. Briefly, cells that have been isolated according to their surface markers are seeded in suspension in low density or as single cells and the formation of spheres is analyzed. Serial sphere formation over time shows that cells have clonogenic potential, consistent with the ability of BTSCs to self-renew. A second critical assay is xenograft tumor formation, where these isolated cells form tumors when injected into immunodeficient or isogenic mice [15]. Such xenograft tumors are expected to recapitulate the original disease phenotype. Re-isolation of BTSCs from xenograft tumors and secondary tumor formation from those cells shows in vivo self-renewal.

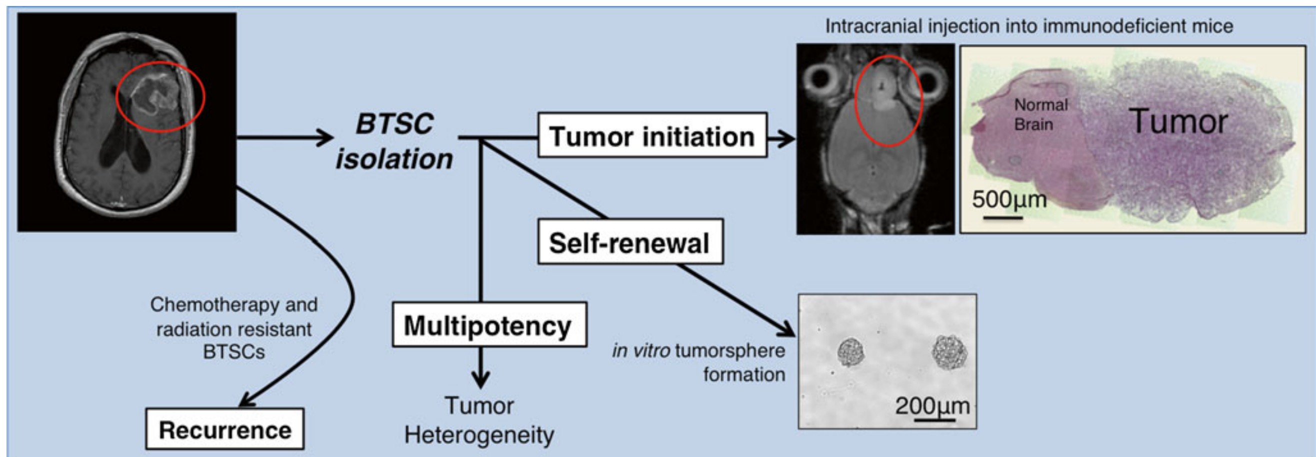

Fig. 2.3. Hallmarks of BTSC biology. Upon surgical resection and primary culture generation, BTSCs are isolated via expression of molecular markers. Isolated BTSCs are studied for their *tumor initiation*, *self-renewal*, and *multipotency*. Therapy-resistant BTSCs cause disease *recurrence.*

Differentiation potential, or multipotency, is another required functional property of BTSCs. In the case of gliomas, for example, BTSCs have been shown to give rise to glia, neurons, endothelium and pericytes [12, 13, 76–79]. These findings underscore the stem-like properties of BTSCs and provide a mechanism for BTSC-driven tumor heterogeneity.

By using these important assays, initial evidence for BTSCs came from pediatric brain tumors. Isolating cells by fluorescence-activating cell sorting (FACS) using cell surface markers originally found in human fetal brain, cells with tumorigenic properties were identified [80]. Shortly thereafter, Dirks and colleagues successfully isolated tumor-initiating cells in human GBM, the most malignant form of glioma, where they showed that CD133, a surface marker also expressed in embryonic NSCs and other adult stem cells, identifies cells with the ability to generate tumors when injected into immunodeficient animals [7, 8, 81]. After these seminal papers, the field of brain tumor stem cells expanded exponentially. However, we still need a better understanding of these cells in terms of their molecular signatures and niches, as well as their relevance to tumor growth and recurrence.

Molecular Characteristics of Brain Tumor Stem Cells

Molecular Markers

Functional similarities between BTSCs and NSCs directed researchers to analyze the expression of markers that were shown to be important for NSC biology and neural development. One of the best-defined molecular markers for brain tumors, including pediatric tumors, ependymoma, and especially GBM, is the cell surface marker CD133. CD133 is a pentaspan, glycosylated transmembrane protein. Apart from being expressed in fetal brain NSCs during embryonic development, its expression is highly associated with other tissue-specific stem cells and cancer stem cells of blood and solid malignancies [14, 81–85]. CD133-knockout murine models show photoreceptor degeneration, but the signaling functions of CD133 remain unknown [86]. In the context of GBM and medulloblastoma, it was shown that when injected into animals in limiting dilutions, CD133+ cells generate tumors more efficiently than their CD133− counterparts, suggesting that they have BTSC properties [87]. Furthermore, downregulation of CD133 via short hairpin RNA (shRNA) suppresses self-renewal of BTSCs in GBM [88].

Although CD133 is one of the best-studied markers in brain tumors, it is now well established that some CD133− cells do possess tumorigenic potential, suggesting that CD133 is not a universal marker and that CD133− BTSC populations exist as well [11, 89–91]. Furthermore, the fact that not all GBM tumors have CD133+ cells supports the hypothesis that CD133− BTSCs exist in these tumors [92–94]. Another important marker associated with BTSCs is the intermediate filament protein Nestin, a well-established NSC marker. Nestin+ cells were shown to have tumor-initiating ability in animal models and to generate tumor recurrence after chemotherapy [10, 61, 95]. BTSCs are also enriched for other NSC and stem cell markers, such as Nanog, Musashi-1, Bmi-1, Sox2, and Oct4 [96–98]. In addition, some BTSCs were shown to express other surface markers and transmembrane proteins, such as CXCR4, integrin α6, SSEA-1/CD15, L1CAM, and A2B5 [11, 99–101]. Finally, the side population (SP), defined as cells with the ability to extrude Hoechst dye via ABC-type transporters on the cell surface on flow cytometric analysis, has been shown to contain stem-like cells in a variety of brain tumors [102, 103].

Besides from traditional coding genes, the importance of noncoding RNAs in the regulation of gene expression has been increasingly recognized in recent years, making them important biomarkers in cancer biology. In particular, microRNAs are responsible for the posttranscriptional fine-tuning of gene expression by binding the 3′ UTR of messenger RNAs (mRNAs) and causing their translational arrest or degradation. MicroRNAs have been implicated in the regulation of stem cell self-renewal and differentiation, as well as the control of cell cycle and apoptosis [104].

MicroRNAs that are upregulated in gliomas are mostly associated with antiapoptotic, pro-proliferative, pro-invasion, and antidifferentiation pathways [105, 106]. On the other hand, some microRNAs, which are important for neural differentiation, were shown to be downregulated in gliomas, functioning as tumor suppressors [107]. An example of a microRNA critical to brain tumor biology is miR-124. Normally, miR-124 is known to promote neural differentiation in the brain. In gliomas, however, it was shown to be downregulated and its overexpression promotes differentiation [108, 109].

Many other microRNAs, whose targets include mRNAs encoding important survival and oncogenic molecules, such as PI3K, AKT, EGFR, and MAPK, were shown to play important roles in glioma and medulloblastoma [105, 108, 110].

Signaling Pathways

Dissecting major signaling pathways that are important for BTSC biology have been, and will continue to be, a challenge due to the complex interplay and cross talk between different signaling pathways. Besides molecular markers shared between NSCs and BTSCs, signaling pathways are also conserved between these two populations. This conservation has highlighted several signaling pathways in BTSC biology, some of which are described below.

Pathways Supporting Self-Renewal

Similar to NSC culturing, BTSCs are propagated as suspension culture, in serum-free media, under the influence of two mitogens, epidermal growth factor (EGF), and fibroblast growth factor (FGF), which are believed to induce self-renewal of BTSCs in vitro [49]. However, in the in vivo scenario, signals supporting self-renewal are dependent on the complex interplay of multiple pathways.

The Notch signaling pathway was originally identified by genetic screens in Drosophila as a master regulator of neural development [111–113]. Further investigation showed that Notch signaling is essential for maintaining NSCs in an undifferentiated state and represents a key component of fate decisions in neural and glial lineages [114, 115]. Apart from its critical role in neural development, Notch signaling has been highly associated with tumorigenesis, regulating both the self-renewal and differentiation of BTSCs in GBM [12, 77, 116–118]. Notch signaling is critical for the self-renewal of CD133+ GBM BTSCs, as evidenced by the fact that blockade of Notch signaling with γ-secretase inhibitors leads to depletion of CD133+ GBM BTSCs and decreases tumorigenicity [119, 120]. Notch signaling is activated in the vascular niche where GBM BTSCs reside [121]. More specifically, in this niche the endothelium provides Notch ligands to maintain the undifferentiated state of BTSCs [122].

The PI3K/Akt/mTOR pathway is critical for gliomagenesis and glioma BTSC self-renewal [2–5, 123, 124]. Commonly find mutations in gliomas are found in the components of PI3K/Akt pathway, such as loss of function of PTEN or gain of function of EGFR [3]. Furthermore, CD133 knockdown leads to inhibition of Akt activation and impaired self-renewal and tumorigenicity of glioma BTSCs, further confirming its crucial role in BTSC biology [123, 124].

Hedgehog-gli signaling has been implicated in medulloblastoma formation [125, 126]. It is important for BTSC clonogenicity, survival, tumorigenicity, and proliferation by operating through the key cell cycle regulators Cyclin D and Cyclin E [87, 127].

The Wnt/β-catenin signaling pathway functions by inducing progenitor cell proliferation and differentiation in gliomas [128]. Some reports also show that Wnt signaling is important for GBM BTSC self-renewal [91, 129].

Recent studies have shown that transforming growth factor-β (TGF-β) signaling regulates GBM BTSC biology [130–132]. TGF-β plays a role in regulating GBM BTSC self-renewal via acting through Sry-related HMG box factors (Sox2 and Sox4) [131]. Furthermore, inhibition of TGF-β in GBM tumors decreases perivascular $CD44^{high}/Id1^{high}$ BTSCs by repressing inhibitors of DNA-binding proteins Id1 and Id3 [130].

Another important signaling pathway relevant to BTSC self-renewal is mediated by hypoxia. GBM tumors are histologically heterogeneous and include regions that lack blood vessels and are highly necrotic and hypoxic [133]. Hypoxia has been previously shown to promote self-renewal of embryonic and adult stem cells [102, 134]. Along these lines, in gliomas hypoxia induces BTSC self-renewal via hypoxia-induced factors (HIF-1α and HIF-2α) [135–139]. These same factors also induce angiogenesis and neovascularization via upregulation of vascular endothelial growth factor (VEGF) [140]. Hypoxia is also known to reprogram CD133− BTSCs to become CD133+ [102]. Furthermore, hypoxia induces Notch signaling, whose importance in BTSC self-renewal was mentioned above [134]. Microscopic analysis has shown CD133 immunoreactivity around necrotic areas in GBM, a finding consistent with a hypoxic niche for BTSCs [141].

Pathways Promoting Differentiation

Bone morphogenetic protein (BMP) signaling functions as a strong differentiation signal. BMP4, important for astrocytic differentiation, induces GBM BTSC differentiation. BMP4

treatment was shown to block GBM BTSC self-renewal by suppressing asymmetric division, thereby depleting the stem cell compartment within tumors and leading to differentiation and proliferation block [76]. However, in a subset of glioma BTSCs, BMP-driven differentiation is impaired due to epigenetic silencing of the BMP receptor 1B (BMPR1B). This modification desensitizes glioma BTSCs to normal differentiation cues, thereby leading to their proliferation [142].

In GBM, some reports have suggested that the Notch pathway is critical for tumor-driven endothelial cell transdifferentiation of BTSCs [12, 13]. Similar to Notch signaling, TGF-β is known to regulate glioma progression by modulating the tumor microenvironment, including angiogenesis and immune response. TGF-β was also shown to induce differentiation of glioma BTSCs into vascular pericytes, which leads to further tumor growth by supporting vessel formation [78].

Stem Cell Niche and Tumor Microenvironment

Understanding the stem cell niche for BTSCs is highly important in unraveling the processes responsible for their self-renewal and signals inducing differentiation. Furthermore, the stem cell niche and microenvironment are highly critical in the context of drug design and delivery. Without understanding the different niches and signals provided within them, effective drug design is not possible.

Vascular Niche

NSCs were shown to reside within a vascular niche, adjacent to endothelial cells, which are believed to provide signals required for self-renewal [143]. BTSCs were also shown to reside closer to endothelial cells [144]. In medulloblastoma, CD133+ cells where shown to be in proximity to endothelial cells [121, 122]. Similarly, glioma BTSCs acquire a vascular niche, in which CD34+ endothelial cells present Notch ligands to BTSCs, keeping them in an undifferentiated state via activation of Notch signaling [122]. However, the complex architectural features of GBM suggest that BTSCs may also reside in relatively avascular microenvironments.

Hypoxic Niche

The importance of hypoxia in promoting self-renewal in embryonic stem cells and NSCs suggests that it may also regulate the self-renewal of BTSCs, especially in GBM, which is a highly hypoxic tumor. When considering this possibility, the question that arises is whether there are hypoxic areas within GBM. One such plausible histologic area is represented in pseudopalisading necrosis (PPN), in which densely packed cells surround necrotic regions [145]. The etiology and biological significance of PPNs is not well understood. However, others and we speculate that they may represent areas of active tumor growth and revascularization. A tantalizing hypothesis to explain such tumor growth and angiogenesis is, in turn, the presence of BTSCs within a hypoxic niche. Indeed, some studies have shown enriched CD133 immunoreactivity in PPNs, supporting this hypothesis [141]. Importantly, the putative presence of BTSCs in hypoxic areas devoid of blood vessels raises doubts about the effectiveness of systemic drug delivery methods.

Invasion

Invasion of glioma cells through the brain parenchyma represents perhaps their most malignant feature [146]. Single GBM cells have been shown to infiltrate normal brain tissue and travel more than several centimeters from the bulk of the tumor [147–149]. After surgical resection, the recurrent tumor occurs within the borders of the resection cavity, suggesting that these infiltrating cells have the capacity to regenerate tumors.

Although the mechanisms of invasion of BTSCs are not clear, C–X–C chemokine receptor type 4 (CXCR4) and its ligand, stromal-derived factor-1α (SDF-1α), which are highly important for vascularization, were shown to be crucial for the invasive behavior of GBM cells [140, 150]. Enrichment of SDF-1α/CXCR4 expression in glioma BTSCs highlights their invasive properties. This signaling was also shown to mediate recruitment of BTSCs toward endothelium, leading to further invasion and differentiation. Furthermore, this chemoattractant signaling induces endothelial proliferation via attracting tumor cells and inducing VEGF expression in gliomas and other systems, such as the gastrointestinal tract [151].

Therapeutic Importance of Brain Tumor Stem Cells

Besides understanding how tumors are formed and how the cellular hierarchy within the tumors is maintained, BTSCs are of particular interest because of their intrinsic resistance to current chemoradiotherapeutic approaches (Fig. 2.3). In GBM, postsurgical therapy consists of concomitant temozolomide administration and radiotherapy.

Therapy Resistance

Chemotherapy Resistance

The side population of GBM cells is believed to have the ability to actively transport chemotherapeutic agents to the extracellular space, due to the expression of ABC-type transporters on their plasma membrane [152]. Furthermore, analysis of cells that are resistant to lethal doses of chemotherapeutic drugs has revealed that they express stem cell markers, such

as CD133, CD117, CD90, CD71, and CD45, and are able to regenerate tumors when injected into immunodeficient mice [153]. Gene expression analysis of CD133+ BTSCs in glioma showed increased expression of antiapoptotic genes. In relation to this finding, CD133 expression was shown to be significantly higher in recurrent tumors, further suggesting that BTSCs have intrinsic mechanisms of chemoresistance [99].

Recently, Parada and colleagues used a mouse model for GBM to demonstrate that upon treatment with temozolomide, an alkylating agent, which represents the standard of care in GBM, a restricted Nestin+BTSC population repropagated the tumor. Selective ablation of this population arrested tumor growth, suggesting that BTSCs are the reason for GBM recurrence [10].

Radioresistance

Besides their resistance to chemotherapy, BTSCs were shown to be highly resistant to radiation. Similar to chemoresistant populations, radioresistant GBM cells express BTSC markers. Molecular mechanisms involved in glioma BTSC self-renewal, such as Notch and TGFβ signaling, are thought to underlie such radioresistance [116, 154]. CD133+ BTSCs were also shown to have increased DNA repair capacity via selective activation of Chk1 and Chk2 kinases [155].

Most chemotherapeutic drugs target cycling cells. By the same token, response to radiation depends on cell cycle checkpoints. However, BTSCs are mostly dormant and quiescent, which spares them from cell cycle-dependent therapeutic approaches, and further highlights the importance of developing new therapies that directly target BTSCs [156].

Updates on Clinical Trials Targeting BTSCs

Because of their central role in promoting growth and recurrence of primary brain tumors, BTSCs are major candidates for targeted therapeutic approaches. In particular, signaling pathways promoting BTSC self-renewal and inducing therapy resistance represent critical drug targets.

As mentioned earlier in the chapter, the Notch pathway plays a crucial role in regulating self-renewal and therapy resistance in BTSCs [12, 77, 116–120, 122]. Therefore, inhibition of Notch signaling in a clinical setting has always been of major interest. Due its contribution to several other diseases processes as well, there are several Notch pathway inhibitors being tested in clinical trials. However, a major limitation of Notch inhibitors is its well-known role in normal tissue-specific stem cells and the risk of systemic toxicity [157]. In the context of brain tumors, there are active clinical trials testing the γ-secretase inhibitor RO4929097 (ClinicalTrials.gov NCT01269411, NCT01189240, NCT01122901).

Another important therapeutic target is the TGF-β signaling pathway, which plays a role in glioma BTSC self-renewal [130–132] and contributes to radioresistance of these cells as a part of the tumor microenvironment [151, 154]. Preclinical data with TGF-β receptor kinase inhibitors and neutralizing antibodies have shown that inhibition of TGF-β signaling sensitizes BTSCs to radiation [154]. TGF-β inhibition is being explored in clinical trials with high-grade glioma patients (ClinicalTrials.gov NCT00431561, NCT00761280).

Discussion

In this chapter, we summarized the current understanding of stem cell biology in brain tumors, as well as emerging concepts. We have approached the issue from two perspectives: the cell of origin of brain tumors and cancer stem cells in brain tumors (BTSCs). Throughout the chapter, we discussed the possibility of neurogenic niches in normal brain as the putative origin of brain tumors and we highlighted molecular signatures and signaling pathways implicated in BTSC biology.

Due to their intrinsic resistance to chemoradiotherapy and their highly tumorigenic nature, BTSCs represent attractive therapeutic targets. However, lack of universal molecular markers identifying BTSCs and complex interplay between signaling pathways regulating BTSC biology have thus far impaired the successful clinical implementation of directed therapeutics toward these cells. Furthermore, the overlap between molecular signatures in BTSCs and normal adult stem cells complicates the issue further due to putative toxicity. We believe that a better understanding of cellular heterogeneity and hierarchy in these tumors will be crucial to overcoming these issues and designing effective therapies against brain tumors and other malignancies.

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3 Molecular Pathology Techniques

Matija Snuderl

The field of neuro-oncology has undergone a number of significant changes over the past decades. One of the most striking, however, has been the rapid pace of discovery in the field of molecular genetics, especially over the past few years. As a result, the genomic landscape of the most common entities has been defined, including a discovery of recurrent genetic alterations, leading to the establishment of diagnostic, prognostic, and predictive biomarkers. Some of these genetic markers, such as 1p/19q loss, O^6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation, and isocitrate dehydrogenase 1 (*IDH1*) mutations, have already entered routine clinical diagnostics and are considered a standard of care. While the clinical utility of other molecular genetic biomarkers, such as epidermal growth factor receptor (*EGFR*) amplification, proto-oncogene B-Raf (*BRAF*) mutation/duplication, or molecular subclassification based on gene expression profile is not firmly established yet, some can be utilized for diagnostic purposes. Furthermore, given the development of targeted therapy, the molecular signature can be also utilized to identify the appropriate target population, substratify patients for clinical trials, and validate candidate predictive biomarkers. As in other tumors and diseases, advanced molecular diagnostics will not replace traditional histopathology, but provide valuable additional information to increase diagnostic accuracy and precision.

Given the limitations of standard cytotoxic therapies, such as chemotherapy and radiation therapy, in the treatment of brain tumors, it has become clear that major progress will require novel approaches. As a result, significant efforts are being made to develop more targeted or selective approaches, based on the specific molecular signature of the tumor. A variety of technical assays have been designed to analyze gene expression, as well as large chromosomal losses and gains, gene rearrangements, focal copy-number changes, point mutations, and epigenetic changes. Genome, transcriptome, and epigenome analyses will likely become a focus for diagnostics to identify new therapeutic targets.

Gliomas are the most common tumors of the central nervous system (CNS) and often require additional molecular work-up, either for diagnosis or biomarkers. In clinical practice, focused assays are usually performed. The most commonly used assays include analyses of 1p/19q loss, *MGMT* promoter methylation, and *IDH1* mutation status. Most commonly performed technical assays are fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR) and its variants, variety of methylation specific assays and sequencing or immunohistochemistry (IHC). These assays are particularly useful in clinical management and diagnostics of adult diffuse gliomas. Although genetic changes and expression profiles have been well studied in other brain tumors as well, the routine clinical use of molecular testing in meningiomas, ependymomas, or medulloblastomas is not yet established. Whole genome expression profiling and DNA analysis of medulloblastomas have pioneered molecular and biological subclassification of a morphologically relatively uniform disease. Similar results were shown in gliomas and specific molecular classes have also been defined in meningioma and ependymoma using next-generation sequencing (NGS) and/or expression profiling. With the costs of whole genome approaches decreasing, we can expect a decline in number of single target assays in molecular laboratories in favor of broad genome-wide analyses in the future.

Molecular Techniques in Clinical Practice

Copy-Number Analysis

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) is one of the oldest and most commonly used techniques in molecular pathology [1]. FISH uses fluorescently labeled DNA probes which attach/hybridize to specific targets in the DNA, providing the

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information on copy-number changes on the level of single cells while preserving the morphology of the underlying tissue. Using different fluorescent dyes enables the investigation of multiple DNA targets simultaneously. FISH can be used on formalin-fixed paraffin-embedded tissue (FFPE) and allows identification of genomic changes in situ. A disadvantage is that FISH probes/signals have to be relatively large to be detected by fluorescent-light microscopy and therefore are not useful for identifying small genomic changes, such as small insertions/deletions. Also, due to the relatively broad optical spectrum of fluorescent dyes, the number of dyes (and therefore, probes) is limited to four at most on a single slide.

FISH offers numerous applications for the routine detection of cytogenetic biomarkers. It can assess ploidy, large chromosomal gains and losses, focal amplifications/deletions, and large structural gene rearrangements. Because the assay is performed directly on the tissue, it allows for the detection of genetic changes even in a small biopsy, or when only a limited number of tumor cells are present among normal tissue. In contrast with whole genome assays, FISH also provides information of whether different genetic changes are present in the same tumor cell or in a different tumor subclone, i.e., genetic mosaicism.

Because of the diagnostic utility of FISH in clinical practice, its application for a variety of tumors is now considered standard of care, and standard protocols are well established. Therefore, any molecular pathology or neuropathology laboratory should be able to implement it. Probes are available commercially and automated systems are used in large laboratories. However, several issues and limitations have to be noted. The assay is labor intensive, with the maximum number of slides managed by a single technician ranging between 10 and 20 per single run, depending on the technician's experience. Larger sample volumes can be managed more efficiently with deparaffinization, protease digestion, and pre- and post-hybridization washes performed by an automated system. Automated systems also allow for standardized and uniform treatment of all specimens. Protease digestion is particularly important, since the brain tissue has a strong autofluorescence and insufficient digestion will result in strong background and weak hybridization signals. On the other hand, excessive digestion will damage the tissue and may lead to a technical failure. Also, the tissue fixation and processing can have a deleterious effect on the ability to perform FISH. Particularly heavy acid decalcification, which is fortunately rare in CNS specimens, almost always leads to FISH failure. The time required for scoring can vary greatly. While the 1p/19q assay, for example, is quite time-consuming and requires scoring ~100 nuclei and two slides, one for chromosome 1 and one for chromosome 19, EGFR and other amplification assays can be detected relatively quickly. However, in the light of recent observations about minor amplified subpopulations and the potential impact of different levels of EGFR amplification on survival, careful scoring of the entire tumor specimen is warranted [2].

For interpretation, appropriate cutoffs must be determined according to specificity and sensitivity for each test. The most common findings in neuropathology FISH are deletions, low-level copy-number gains/losses, and high-level copy-number gains, i.e., amplifications (Fig. 3.1). Gene rearrangements are less common. For 1p/19q deletions, one of the possible methods of interpretation is the median percentage of nuclei with two reference/control signals (e.g., 1q or 19p) and one test signal (e.g., 1p or 19q) plus three standard deviations as the cutoff values for deletion. Another possibility is to use the ratio of target versus reference signals, with most control specimens being near 1.0 and cutoff around 0.75–0.85, depending on the laboratory standards. In addition, there is increasing evidence in the 1p/19q literature that there are different clinical implications of absolute deletions with one target and two reference signals per nucleus compared to so-called relative deletions in tumors with polyploidy/aneuploidy. These tumors can show variable numbers of target and reference signals such as duplication with two target signals and four reference signals per nucleus, or 3:6, 4:8 ratios. This finding would be misinterpreted as absolute deletions by PCR loss of heterozygosity (LOH) methods. However, recent studies have shown that relative deletion, also known as a superloss, is an important prognostic marker and patients with relative deletion have shorter progression-free survival [3, 4].

Gene amplification testing is most commonly applied for *EGFR*, although the other two most commonly amplified receptor tyrosine kinase (RTK) genes platelet-derived growth factor receptor alpha (*PDGFRA*) and mesenchymal–epithelial transition factor (*MET*) RTK gene are gaining increasing attention, partly due to the increasing clinical availability of kinase inhibitors against these targets [5]. Also, while *EGFR* amplification is generally limited to adult GBMs, *PDGFRA* amplification is common in lower grade and pediatric gliomas [6]. Typically, RTK gene amplifications involve the majority of cells within a given tumor and with high levels of amplification. However, tumors with scattered amplified cells, which represent a minority of the tumor, are also encountered in clinical practice. This phenomenon is most commonly observed in *MET*-amplified GBMs, where cells with amplification can be rare and scattered throughout the tumor. Another recently described phenomenon is mosaic heterogeneity, where tumors are composed of subclones with mutually exclusive amplification of RTK genes [7–9]. Up to three coexisting subclones with amplifications of *EGFR*, *MET*, and *PDGFRA* within a single tumor have been described. More importantly, these studies have shown that during glioma progression, subclones have different propensities to infiltrate normal brain and genomic changes can vary widely among different parts of the same tumor [7]. In addition, studies have also shown that some GBM cells contain simultaneous amplification of different RTK genes. Although the significance of these findings is not clear, they emphasize the complexity of the disease and raise several

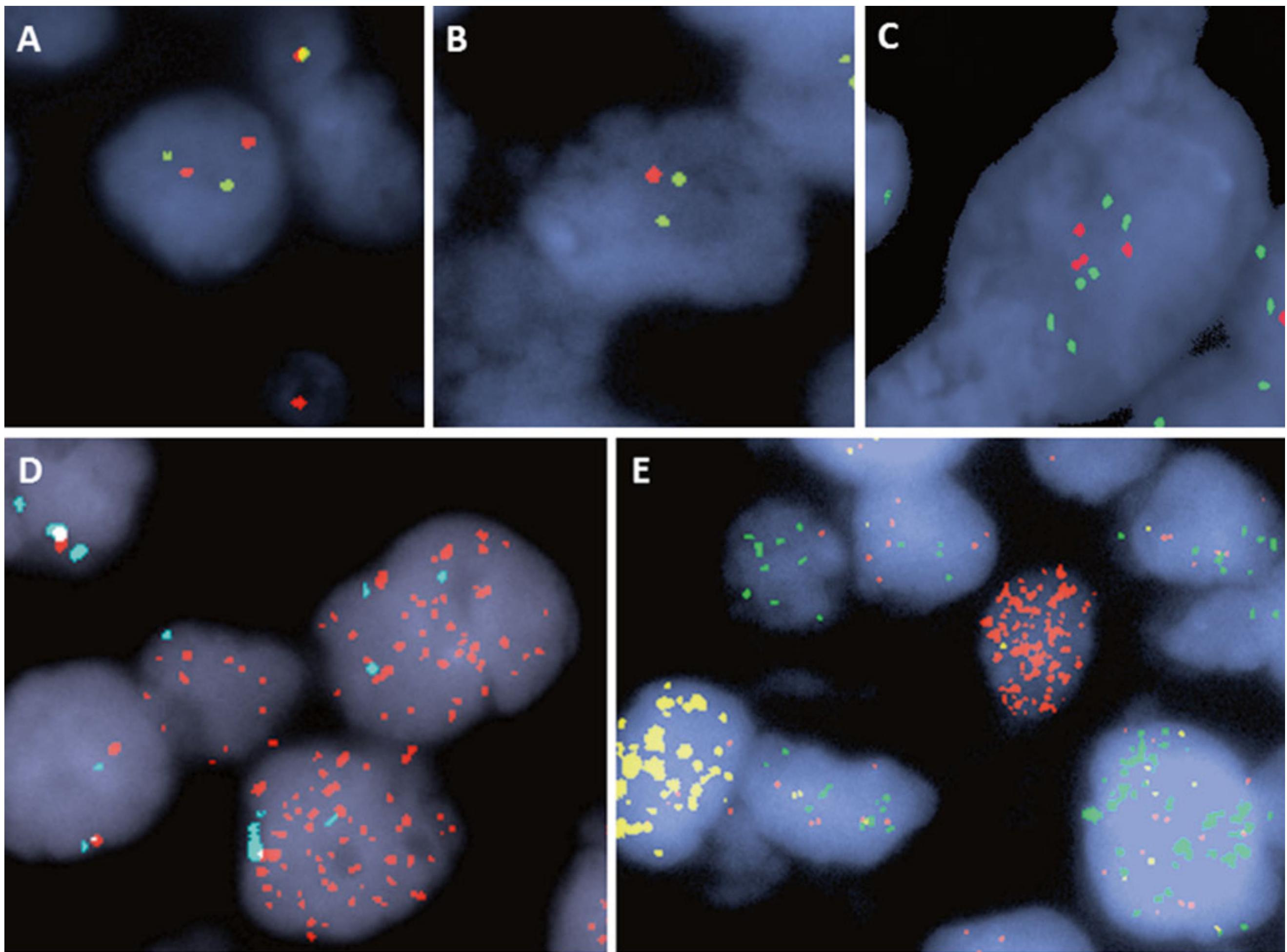

Fig. 3.1. Examples of FISH applications in molecular neuropathology: (**a**–**c**) 1p evaluation, 1p red, 1q green: (**a**) Maintenance of 1p (2:2 signals), (**b**) 1p loss (1:2 signals), (**c**) relative deletions/superloss (4:6). (**d**, **e**) RTK genes evaluation: (**d**) high level amplification of *EGFR* (*red*: *EGFR*, *blue*: CEP7), (**e**) mosaic heterogeneity with mutually exclusive amplifications of *EGFR* (*red*), *PDGFRA* (*green*), and *MET* (*yellow*) in subclones within a GBM. All panels: nuclei are counterstained with DAPI.

challenging implications for molecular pathology and clinical practice. This also raises an important practical question: whether the molecular analysis should be focused on a single target or multiplexed, i.e., analyzing several targets, or even the whole genome. This issue is also discussed in the section on array comparative genomic hybridization (aCGH) below. While specific criteria may differ among laboratories, it is reasonable to suggest that the presence of any subpopulation with gene amplification should be reported. Lastly, FISH can be used to evaluate for translocations. The most typical indication would be for *EWS* gene rearrangement in small, round blue cell tumors when Ewing's sarcoma/peripheral primitive neuroectodermal tumor (PNET) is in the differential.

The 1p/19q analysis can be performed by several techniques, most commonly by FISH, single nucleotide polymorphism (SNP) array, or PCR-based microsatellite LOH. However, FISH is the most commonly used assay and offers additional prognostic information compared to PCR LOH. Fluorescent test probes are commercially available and hybridize to so-called minimally deleted regions [10, 11]. The test probe localizes to 1p36 and a control/reference probe localizes on the opposite arm to 1q25. Target and control/reference probes for chromosome 19 localize to 19q13 and 19p13, respectively. One FFPE section cut at 4–5 μm is used for each chromosome. A few caveats apply for FISH 1p/19q. A normal copy-number LOH resulting from mitotic recombination would not be detected by FISH and could in theory result in false negatives [12]; however, this would be rare in 1p/19q co-deleted oligodendroglioma. More importantly, FISH cannot assess multiple markers to cover the entire arm of the chromosome. Therefore the observed loss might only represent a relatively small "probe-size" deletion on 1p or 19q. However, only the whole arm deletions are truly associated with a favorable prognosis. While the result would be read as positive technically, biologically this would represent a false-positive finding. Many tumors with these

minimal deletions are in fact astrocytomas, rather than oligodendrogliomas, and are actually associated with a worse prognosis. GBMs in particular contain these minimal deletions, and a misdiagnosis of GBM with oligodendroglial component could be made based on a biologically false-positive finding. To avoid this pitfall, some laboratories avoid commercially available probes and choose home-brewed probes on 1p32 and 19q13.4, which are outside the minimal regions of deletion. Although the sensitivity is decreased, this strategy increases specificity of the assay.

The size of FISH probes, ~1 Mb, and staining with either a green or orange/red spectrum fluorescent dye, allows localization against the DAPI counterstained nucleus. As discussed above, four main patterns can be recognized: maintenance of 1p and 19q with two control probes and two target probes, absolute deletion with two control probes and one target probe, polysomy with several copies of target and control regions, and polysomy with deletion of target regions. This pattern known also as relative loss or superloss consists of four control signals and two target signals, for example. However, the ratio of signals can vary and show rations such as 6:3 or 8:4.

Multiple studies have confirmed high reproducibility between SNP/LOH analysis and FISH [13]. While SNP/LOH analysis has an advantage of analyzing multiple markers on chromosomal arms, FISH offers the ability of evaluating the tumor in situ, with small biopsies and without patient's matched normal blood. With growing evidence of implications of polysomy, FISH seems to offer additional prognostic value compared to PCR LOH. There is a strong association between histology and 1p/19q loss. Tumors with classic oligodendroglial features have a higher likelihood of 1p/19q codeletion [14, 15]. It is important to keep in mind that there is no need to select the most oligo-like area when choosing the best section for 1p/19q analysis. It seems that 1p19q codeletion is a very early event in the tumor development, and therefore is present in both oligodendroglial and astrocytic components of an oligoastrocytoma. Another interesting association exists between tumor site and genetics, with frontal oligodendrogliomas having a significantly higher likelihood of 1p/19q loss than temporal lobe tumors [3].

Array Comparative Genomic Hybridization

DNA arrays provide a whole genome analysis of copy number changes. Many arrays offer both copy-number variant and SNP content for LOH analysis in a single array. Genomic DNA can be isolated from FFPE tissue after deparaffinization and protease digestion. A normal male/female DNA standard is usually used for comparison. However, the patient's germline DNA from the peripheral blood can also be utilized. This is particularly useful for SNP analysis. The cancer arrays usually contain a high-resolution backbone with an average spacing approximately one oligo probe every 25–50 kb, which ideally avoid regions containing common copy-number variants (CNV) to minimize detection of benign CNVs. The probe density is usually higher: one every 5 kb in regions defined by International Standards of Cytogenomics Arrays (ISCA). Furthermore, some arrays contain an increased density of probes in known cancer-related genes with up to a single exon resolution, where the density of the probes can be up to one probe every 50 bp. This is particularly useful for genes with known specific deletions, duplications or mutations in cancer. One must keep in mind that although aCGH is a genome-wide technique, the distribution of probes highly depends on the purpose of the array. The design is specific for each clinical indication, and therefore laboratories performing aCGH testing for different clinical questions cannot use the same array for all of them. Although the backbones might be the same or very similar, DNA coverage distribution with highest probe density are significantly different based on whether the array was designed for autism, epilepsy, or cancer, for example.

While a simple PCR LOH does not provide a significant advantage, the aCGH + SNP arrays offer several advantages compared to FISH. The aCGH + SNP provides a whole genome view of the DNA (Fig. 3.2). The same reaction can be performed for all gliomas in the laboratory, regardless whether the diagnosis is GBM or oligodendroglioma, which decreases costs necessary for storing, optimizing, and running several different FISH probes. For example, in a small cell GBM variant where three separate FISH reactions, 1p, 19q, and EGFR, are needed, a single array can provide a definitive answer. In medulloblastoma, aCGH can be utilized for the subgroup classification since different subgroups carry characteristic chromosomal changes. In addition, the array provides information about other genomic changes in brain tumors such as *PTEN*, *CDKN2A/p16*, *PDGFRA*, *NF1*, and *MET*, which are not routinely tested. This information, while not utilized in current clinical care, will increasingly play a role for design of molecularly driven studies, including clinical trials. For example, clinical outcome predictions can be made by evaluating several loci of DNA rearrangements in medulloblastomas, where a number of FISH reactions could be replaced by a single aCGH [16]. If all potential targets are to be tested by FISH, the costs and labor intensity would be significantly higher than a single aCGH + SNP array. An additional advantage is the interpretation software which allows quick review of genomic changes and automated variant call. The software allows manually adjusting levels at which variants can be called and minimizes the possibility of false negatives. While the genome still has to be reviewed manually, the amount of time spent analyzing the array data seems to be equal or shorter in comparison with 1p/19q analysis, which is clearly the most labor-intensive assay in regard to data evaluation. A disadvantage of aCGH technique in comparison with FISH is that it might not be able to detect changes if only scattered infiltrating cells are present in the tissue [7] and might be challenging with small biopsies since approximately 1.5 μg of DNA is needed.

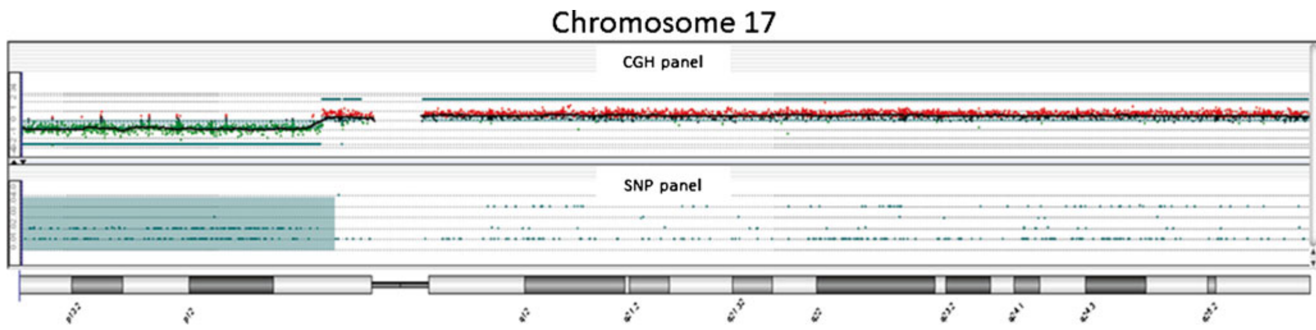

Fig. 3.2. Example of aCGH result in molecular neuropathology: view at chromosome 17 in a medulloblastoma shows a deletion of the short arm of the chromosome 17. This loss occurs in ~25–50 % of medulloblastoma. 17p loss has been associated with a poor survival in some studies suggesting that loss of a tumor suppressor gene located on 17p plays a role in the genesis or progression of medulloblastoma. A novel candidate gene, CTD nuclear envelope phosphatase 1 (*CTDNEP1*), was identified as a recurrent target of mutation in Group 3 and Group 4 medulloblastomas. *CTDNEP1* is located on chromosome 17p13.1 in a hotspot of deletion and LOH.

Mutation Analysis

Mutation-Specific Antibodies

Until recently, the only way to analyze point mutations was by Sanger sequencing. A truly revolutionary event was the introduction of mutation-specific IDH1 R132H antibody into clinical practice. That was quickly followed by a novel BRAF V600E mutation-specific antibody [17–20]. The advantage of using a mutation-specific antibody is undisputable. The staining can be performed in a clinical immunohistochemistry laboratory on FFPE on standard 5 μm sections (Fig. 3.3). Provided the antibody is robust and validated as being highly sensitive and specific, detection is fast, inexpensive, reliable, and allows identification of single infiltrating tumor cells. In comparison with rather nonspecific antibodies such as p53, the mutant protein is not expected to be present in any reactive or inflammatory conditions that may lead to overexpression of nonspecific markers. As a consequence, tumor mutation-specific antibodies are of great value in distinguishing not only reactive astrocytes from tumor cells but also oligodendroglioma/oligoastrocytoma from their morphological mimickers [21]. Although there is strong correlation between *IDH1* mutation and 1p/19q loss, the 1p/19q testing cannot be replaced by IDH1 antibody and several caveats must be noted. For IDH1, the antibody detects only one of several known mutations. While R132H is the most common mutation and represents ~90 % of *IDH1* mutations, other mutations at that site will not be detected by the antibody. Furthermore, mutations of *IDH2* at the residue R172 can also be found in gliomas, although rarely [22]. The R172 residue in *IDH2* is the exact analogue of the R132 residue in *IDH1*. The residue is located in the active site of the enzyme and forms hydrogen bonds with the isocitrate substrate [23]. Therefore, *IDH1* and *IDH2* sequencing provides a definitive answer in IDH1 R132H antibody-negative tumors.

BRAF V600E antibody can be used for the same purpose. However, it is most useful in supratentorial tumors. Although BRAF alterations in pilocytic astrocytoma of the cerebellum are common, they are usually due to a tandem repeat producing a fusion BRAF:KIAA1549 gene [24, 25], which would not be detected by the antibody. BRAF V600E is present in supratentorial pilocytic astrocytoma and pilomyxoid astrocytoma, pleomorphic xanthoastrocytoma (PXA), ganglioglioma and dysembryoplastic neuroepithelial tumor [26]. The antibody could be particularly useful in distinguishing between a PXA and a GBM on a small biopsy, since BRAF V600E mutation would be highly unusual in a GBM, but they are common in PXA [27].

Another example of a clinically important antibody detecting a molecular aberration is *INI1*. The loss of protein expression in an embryonal brain tumor is virtually diagnostic of atypical teratoid-rhabdoid tumor (AT/RT), a highly aggressive neoplasm of early childhood. Immunohistochemistry for INI1 should be performed on every medulloblastoma or primitive embryonal tumor in childhood to avoid misdiagnosis of the AT/RT [28].

Sequencing

Until recently, Sanger sequencing represented the most common way to investigate mutations in brain tumors. Considering that many genes commonly mutated in gliomas such as *TP53* and *NF1* are large and can be altered by several different mutations and the predictive value is unknown, sequencing played a minimal role in clinical laboratories for brain tumors. One of the relevant applications is *IDH1* and *IDH2* sequencing for tumors negative for IDH1 R132H by immunohistochemistry, when the suspicion for less common mutations is high based on clinical presentation. NGS methods are still mostly used in research. However, they are being adopted by clinical laboratories, usually as focused cancer gene panels (Fig. 3.4). As the cost of sequencing continues to decline, and the methods themselves including data analysis become easier to manage in the clinical setting, they will

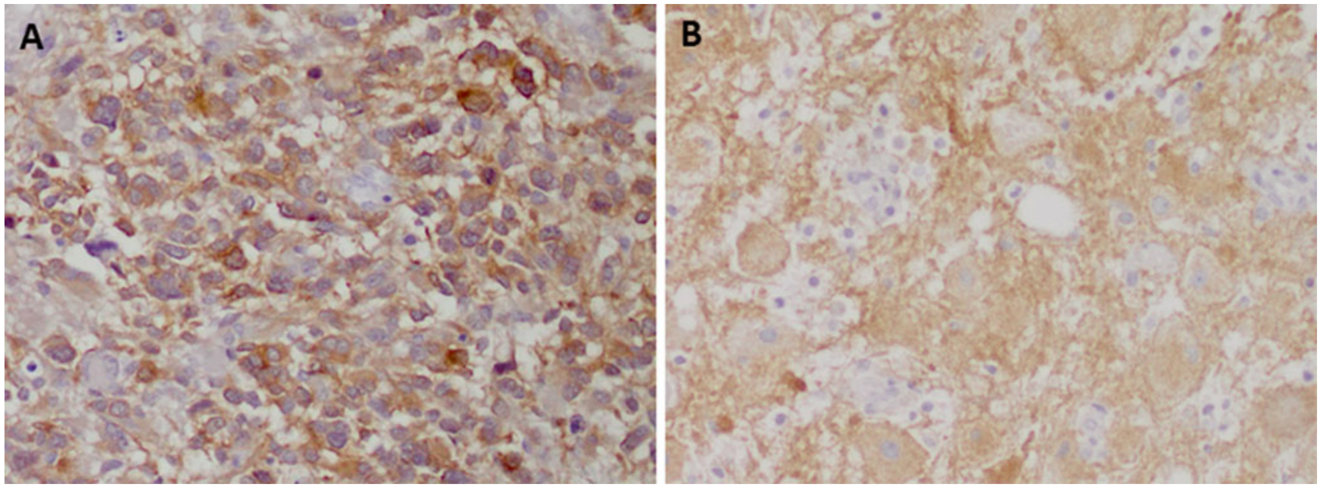

Fig. 3.3 Examples of utility of mutation specific antibodies in neuropathology: the immunohistochemistry with a specific antibody against (**a**) IDH1 R132H in a case of a diffuse astrocytoma and (**b**) BRAF V600E in a cerebellar ganglioglioma shows strong immunoreactivity specific for tumor cells. Reactive cells in the background are negative (**b**)

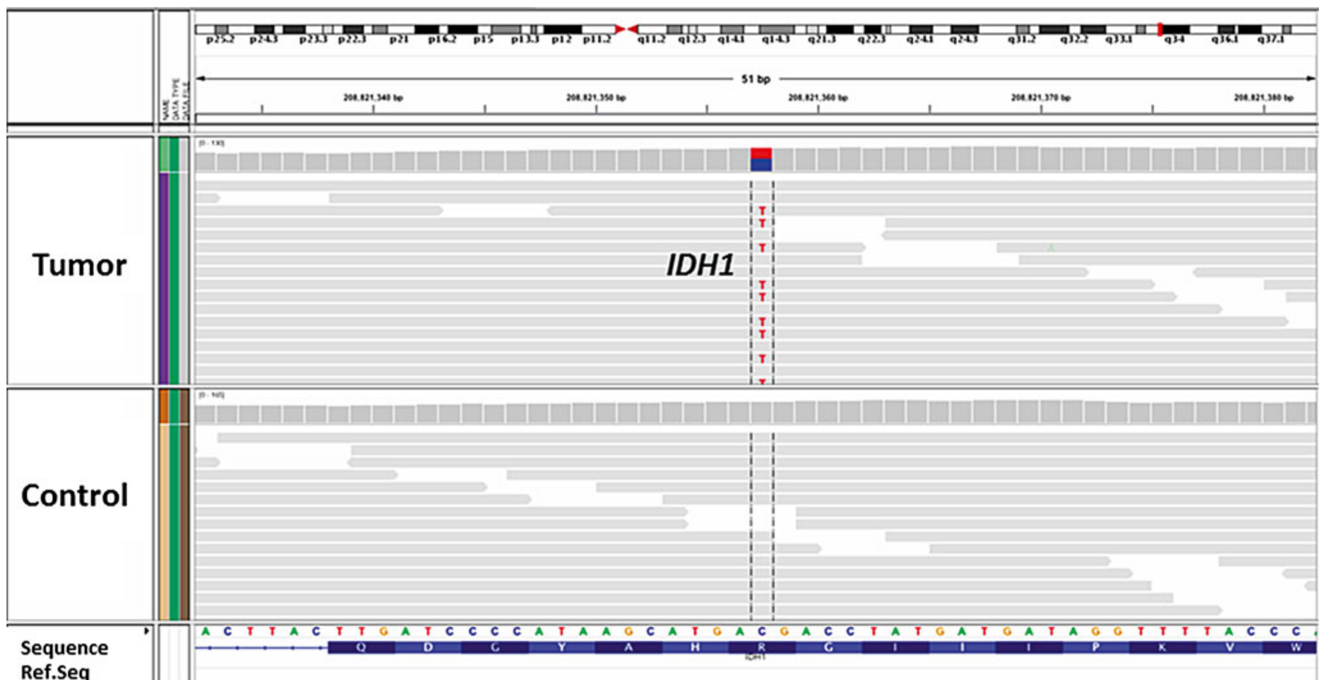

Fig. 3.4 Example of the next-generation sequencing in molecular neuropathology: in oligodendroglioma, whole genome sequencing (Illumina platform) identifies mutation in the IDH1 (c. 395 C>T, p.R132H) gene. The majority of *IDH1* mutations in gliomas are p.R132H. The example shown is in the form of the IGV browser view (Courtesy of Dr. Stephen Yip, BC Cancer Agency)

likely become increasingly available for routine use. In the future, NGS will most likely cover tens to hundreds of cancer-specific genes. However it is only the matter of time before the whole exome or whole genome sequencing cost will not be that much different from a focused panel. Additionally, whole exome/genome sequencing will allow identification of gene rearrangements, which were previously unappreciated phenomena in gliomas [29].

MGMT Testing

MGMT promoter methylation has been confirmed by several clinical studies as a biomarker in patients with gliomas. The MGMT gene is located on chromosome l0q26.34 and contains five exons, the first of which is noncoding. Transcription of the MGMT gene is initiated at a single site within a GC-rich, non-TATA box-containing promoter. Expression of the MGMT gene is epigenetically regulated by methylation-dependent silencing.

Temozolomide (TMZ) methylates DNA at position 6 of guanine nucleotides. The resultant O^6-methylguanine adducts pair with thymidine, and when DNA mismatch repair (MMR) enzymes attempt to excise O^6-methylguanine, they generate single- and double-strand breaks which lead to apoptosis. MGMT can rescue the cell by restoring the normal guanine, which leads to resistance to alkylating chemotherapy. Gliomas with MGMT promoter methylation are less capable to repair DNA and are more sensitive to TMZ.

Several methods have been established for detection of MGMT in glioma (reviewed in [30]). In general, they can be divided into methods requiring or not requiring bisulfite treatment. The three most common methods are methylation-specific PCR (MSP), real-time PCR or MethyLight PCR, and methylation-specific sequencing or pyrosequencing, and they all require bisulfite treatment of the tissue. Detection can be performed on FFPE tissue, as well as on the frozen tissue. For practical purposes, FFPE-based methods are preferable. Other methods that do not require bisulfite conversion and can be used in the clinical setting are methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) and IHC for MGMT protein expression.

There is a significant heterogeneity of MGMT expression and promoter methylation within a glioma. In contrast to 1p/19q testing, MGMT testing requires careful sample selection with a neuropathologist evaluating the case, providing an estimate of the percentage of neoplastic cells and selecting the section with the least amount of necrosis and contaminating non-tumor cells. If normal brain is present on the same section, microdissection of the tumor from the unstained slide is warranted. Many laboratories also require a minimum 50 % of a viable tumor in a given sample to perform testing.

DNA can be extracted from the FFPE tissue using available protocols and kits. The most common methods for MGMT promoter methylation require sodium bisulfite treatment of DNA, which converts unmethylated cytosine into uracil. Methylated cytosine in a CpG island remains unchanged. This bisulfite-modified DNA is used as a template for PCR and sequence differences between methylated and unmethylated DNA after bisulfite treatment allowing for the design of PCR primers that are specific for each template. Bisulfite treatment of DNA is the most difficult part of the assay since it causes further DNA fragmentation. Furthermore, partial conversion could lead to false-positive results. Therefore, appropriate methylated and unmethylated controls are necessary and must be treated in parallel to patient samples to ensure that complete conversion occurred.

MSP is the most commonly used method and allows for the evaluation of methylation status at 6–9 CpG sites. Two primer sets are usually used. One pair is used for amplification of sequences with converted cytosine after bisulfite treatment detects an unmethylated MGMT. A second pair of primers is used for sequences with unconverted cytosine (mC) and detection of a methylated MGMT. PCR product can be visualized by capillary gel electrophoresis, after fluorescent labeling, or by agarose gel electrophoresis (Fig. 3.5). With numerous established protocols available, this method can be easily established in a molecular laboratory and does not require specialized laboratory equipment. An advantage is an easy-to-read result; however a disadvantage is lack of the quantitative assessment of methylation. Another disadvantage is that this method does not include a control for bisulfite conversion, and incomplete conversion of unmethylated cytosines may be interpreted as methylation, leading to false-positive results.

The qMethylation-Specific RT-PCR-MethyLight assay is a simple, quantitative real-time PCR method to determine the methylation status of MGMT CpG islands. It utilizes the TaqMan PCR with forward and reverse primers. It also contains a fluorescent oligo probe, which emits only after it is degraded by the 5′–3′ exonuclease activity of Taq polymerase. It requires a second set of primers and a probe, for amplification of a housekeeping gene, which are used as amplification controls for quality and quantity of the DNA. Control gene primers and probes are designed for the regions with no CpG islands and complementary to the

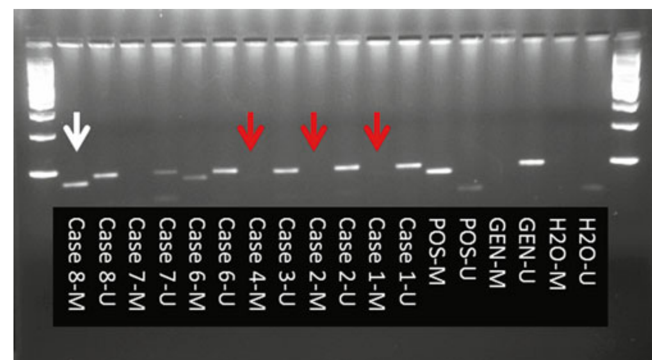

Fig. 3.5. Example of methylation specific PCR evaluation of MGMT in GBM: Agarose gel electrophoresis show examples of MGMT evaluation. *H_2O* water, *GEN* genomic control (negative for promoter methylation), *POS* positive control (positive for MGMT promoter methylation), *U* unmethylated primers, *M* methylated primers. Cases 1, 2, and 3 show no PCR product in the methylated lane and are examples of tumors without MGMT promoter methylation (*red arrows*). Case 6 shows a product in the methylated lane and is an example of a tumor with MGMT promoter methylation (*white arrow*).

bisulfite-converted sequence. This allows control for assessing the efficiency of bisulfite conversion and quantifying MGMT methylation.

The qRT-PCR assay is more specific, and rarely produces false-positive results. The assay is relatively easy to set up, but requires a real-time PCR instrument, which is available in most laboratories. The results are easy to interpret, and inclusion of the standard curve gives numerical values for copy numbers of methylated MGMT sequences, as well as housekeeping gene sequences. However, the percentage of contaminating stromal cells cannot be accurately assessed, and therefore quantitation of MGMT promoter methylation by qRT-PCR is not recommended.

Primers can be designed to cover both the upstream and downstream regions of CpGs, as well as methylated and unmethylated sequences. Sanger bidirectional DNA sequencing can be used to provide a semiquantitative measure of MGMT promoter. However, standard Sanger sequencing has not been established in MGMT analyses compared to methylation specific PCR. On the other hand, pyrosequencing, or sequencing by synthesis, has been used in some laboratories. Pyrosequencing also requires bisulfite treatment of genomic DNA and PCR amplification with primers surrounding CpGI, followed by pyrosequencing. The main advantage of pyrosequencing is the ability to quantify methylation at each CpG site and identify cases with low levels of methylation reliably. However, costs of equipment are high and it is therefore more appropriate for high volume laboratories. NGS methods are still mostly research applications; however, they will likely become available in clinical settings, and can be used for methylation analysis.

Methylation-specific MLPA utilizes unique approach with the ligation of oligonucleotide probes, followed by a digestion of the genomic DNA-probe hybrid complex with methylation-sensitive endonucleases. When the CpG locus is not methylated, methylation-sensitive restriction endonuclease cleaves its restriction site, resulting in lack of PCR amplification. When the CpG locus is methylated, the restriction site is protected from endonuclease digestion and PCR product is generated. The methylation-specific probes are designed so that the sequences detected contain a methylation-sensitive restriction site GCGC. The advantage of this semi-quantitative method, in which the level of methylation at each site can be determined, is that it does not require bisulfite treatment. MLPA can detect changes in both CpG methylation and copy-number of up to 40 chromosomal sequences in a single reaction. Capillary electrophoresis is necessary to identify and quantify PCR products of the individual probes. The sample DNA is split and one part is subjected to a single ligation step, whereas for the other part ligation is combined with the methylation-sensitive digestion. Subsequent PCR reaction amplifies either total DNA or the methylated fraction. Comparison of the peaks of the ligated fraction and the fraction that is digested with endonuclease provides the methylation ratios. The disadvantage of this method is the need for special equipment and expensive reagents. However, for laboratories performing MLPA assays for other indications, the methylation-specific MLPA is easy to establish.

The use of IHC for the detection of MGMT has been investigated in a number of studies, with lack of concordance between MGMT expression by immunohistochemistry and MGMT promoter methylation. Furthermore, lack of MGMT expression by IHC was not as robust of a biomarker as MGMT promoter methylation. The conclusion from these studies is that MGMT promoter methylation and MGMT protein expression detected by immunohistochemistry cannot be used interchangeably to predict survival for patients with malignant gliomas [31]. Therefore, immunohistochemistry is not the method of choice for the detection of MGMT activity and its use should be discouraged.

Expression Profiling

Gene signatures have been shown to be capable of distinguishing molecular subtypes of tumors that appear indistinguishable histologically, but reflect different disease biologies as evidenced by differences in clinical presentation and/or outcomes. A number of groups have attempted to identify individual genes as well as signaling pathways from microarray data that are prognostic in malignant glioma and medulloblastoma [32–34]. Expression profiling was able to identify specific subgroups within each disease that were associated with improved or decreased survival. Despite some prognostic value [35], the application in clinical practice has been limited due to a variety of reasons such as costs, equipment requirement, and the need of frozen material for good-quality whole genome expression profile studies. Overall, as with all molecular tests in clinical practice, the use of FFPE-based assays is critical to the wide-scale acceptance of a biomarker due to the limited availability of fresh/frozen tissues. In GBM, a multigene profile compatible for FFPE samples is currently used as a stratification factor in a large Phase III clinical trial (RTOG-0825) [36]. The 9-gene set was validated with an independent sample set and was shown to be an independent predictor of clinical outcome after adjusting for clinical factors and MGMT status. Another approach is to use a selected set of genes that have been firmly associated with the subtype using FFPE samples on a platform such as NanoString® to molecularly classify brain tumors such as medulloblastoma or GBM (Fig. 3.6). RNA-based methods such as NanoString® that can utilize FFPE tissues are easier to implement in clinical laboratories than assays that require high-quality RNA from the frozen tissue, which will likely not be implemented in standard clinical practice.

Summary

Several well-established techniques are currently used in molecular neuropathology. Analyses include copy-number changes, mutations, and epigenetic modification assessed

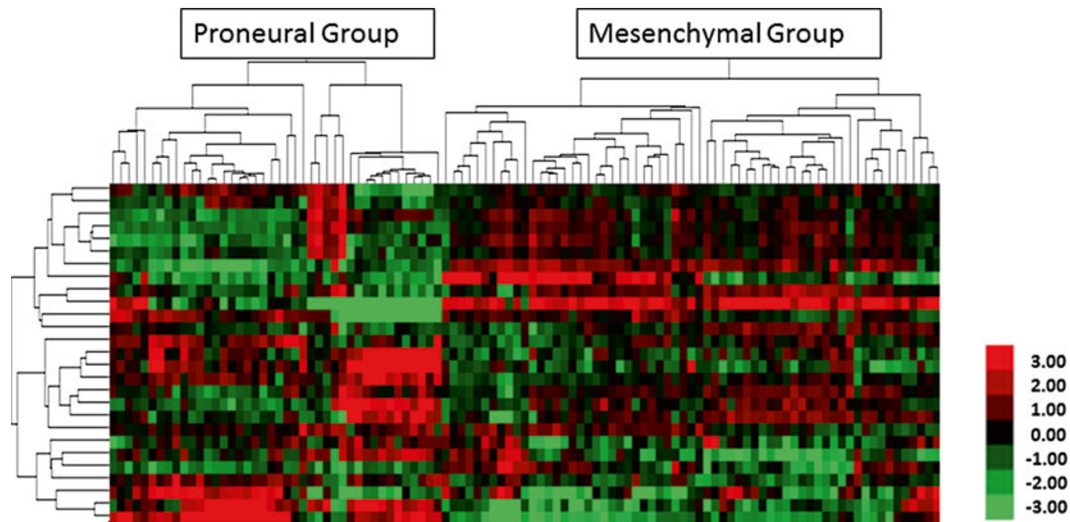

Fig. 3.6 Example of expression profile evaluation of GBM: RNA isolated from the FFPE tissues and can be used for the next-generation of expression profile techniques. Using an expression of a core of validated genes for GBM and unsupervised hierarchical clustering, tumors are segregating into different molecular subgroups such as Proneural and Mesenchymal. *Red–green* scale shows the change in the expression level (Figure created in collaboration with Dr. Stephen Yip, BC Cancer Agency)

most commonly by FISH, PCR, sequencing of mutation-specific antibodies. From the technical perspective, once validated, these techniques are robust and require minimal troubleshooting. The most commonly performed tests with the largest clinical impact include MGMT promoter methylation, 1p/19q status and *IDH1* mutation. These assays should be incorporated both within routine clinical care and within clinical trial designs. When used in the right clinical context after neuropathology review and with appropriate interpretation guidelines, they can provide diagnostic, prognostic, and predictive information that can help guide clinical management.

At the present time, the number of assays that can be performed on brain tumors and the information that can be obtained is significantly greater than what can be used for practical diagnostic and clinical purposes. Careful retrospective and prospective validation of molecular genetic alterations and profiles for prognostic and predictive value will be required in clinical studies before implementation into routine diagnostics and clinical care.

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4 Low-Grade Gliomas

Fausto J. Rodriguez and Daniel C. Bowers

Low-grade gliomas encompass multiple histological subtypes, including pilocytic astrocytomas (PA), pilomyxoid astrocytomas, diffuse astrocytomas (DA), oligodendrogliomas, subependymal giant cell astrocytomas (SEGA), pleomorphic xanthroastrocytomas (PXA), and others. Low-grade gliomas are grouped together because they share low mitotic rates, slow growth rates, and affected patients often achieve long-term survival with surgery alone. Also, upon instances of tumor progression, they are often treated with identical chemotherapy or radiation therapy regimens. Recent retrospective studies have suggested several genetic and molecular markers that are associated with specific tumor subtypes and may help with diagnostic and prognostic evaluation. Importantly, several of the identified molecular pathways involved are potentially targets for new chemotherapy agents, and their associated markers may also have predictive value in terms of therapeutic response.

However, except for PA arising in patients with neurofibromatosis type-1 (NF1) and SEGAs in children and young adults with tuberous sclerosis, little was known until recently about the molecular underpinnings of low-grade gliomas. Low-grade gliomas among patients without NF1 do not inactivate the *NF1* gene, and generally lack changes to the oncogenes and tumor suppressors altered in adult diffuse astrocytomas [1, 2]. Until recently, cytogenetic studies of pilocytic astrocytomas were notable for a lack of detectable chromosomal alterations, with largely normal karyotypes in the more than 100 cases initially studied [1].

Mutations in *IDH1* or *IDH2* have been identified in the majority of low-grade gliomas among adults, but interestingly are almost never detected in pediatric low- or high-grade glioma, and when present have mostly been reported in children at least 14 years old [3–5]. This suggests that adolescents with *IDH*-mutant tumors may represent the youngest patients with “adult” low-grade gliomas.

The purpose of this chapter is to describe the histopathology, cytogenetics, gene expression profiles, and molecular genetics of low-grade gliomas. This information will be useful in identifying molecular signaling pathways, defining prognostic groups and ultimately leading to the development of novel, molecularly targeted therapies for low-grade gliomas.

Pathology

Low-grade gliomas represent a spectrum of neoplasms that may be broadly separated into circumscribed and diffuse groups. Circumscribed gliomas encompass pilocytic astrocytoma (PA), SEGA, and PXA (Figs. 4.1 and 4.2). These tumors usually have discrete borders at the imaging and gross pathology levels, which allow for gross total resection and cure by surgery depending on anatomic location (Table 4.1). The diffuse glioma group includes diffuse astrocytoma and oligodendroglioma (Figs. 4.3 and 4.4). They demonstrate a more infiltrative pattern of growth on both imaging and histologic sections. Compared with the circumscribed group, the diffuse gliomas have a higher propensity for histologic progression to higher grade tumors and therefore more aggressive behavior.

Circumscribed Gliomas

Pilocytic Astrocytoma

PAs represent the most frequent glioma subtype in children. They are characterized by elongated, bipolar astrocytes usually with bland nuclear features. The classic architecture is a biphasic pattern, with alternating Rosenthal fiber-rich compact areas and loose, microcyst rich regions. Eosinophilic granular bodies may be present in these regions and even abundant. Additional variable features include hyalinized or glomeruloid vessels, hemosiderin deposition, and degenerative nuclear pleomorphism or multinucleated cells. Monotonous oligodendroglial-like cells may predominate in some examples. Occasional mitoses and non-pseudopallisading necrosis may be present, but they have an inconsistent relation with clinical outcome [6, 7]. As other astrocytomas, these

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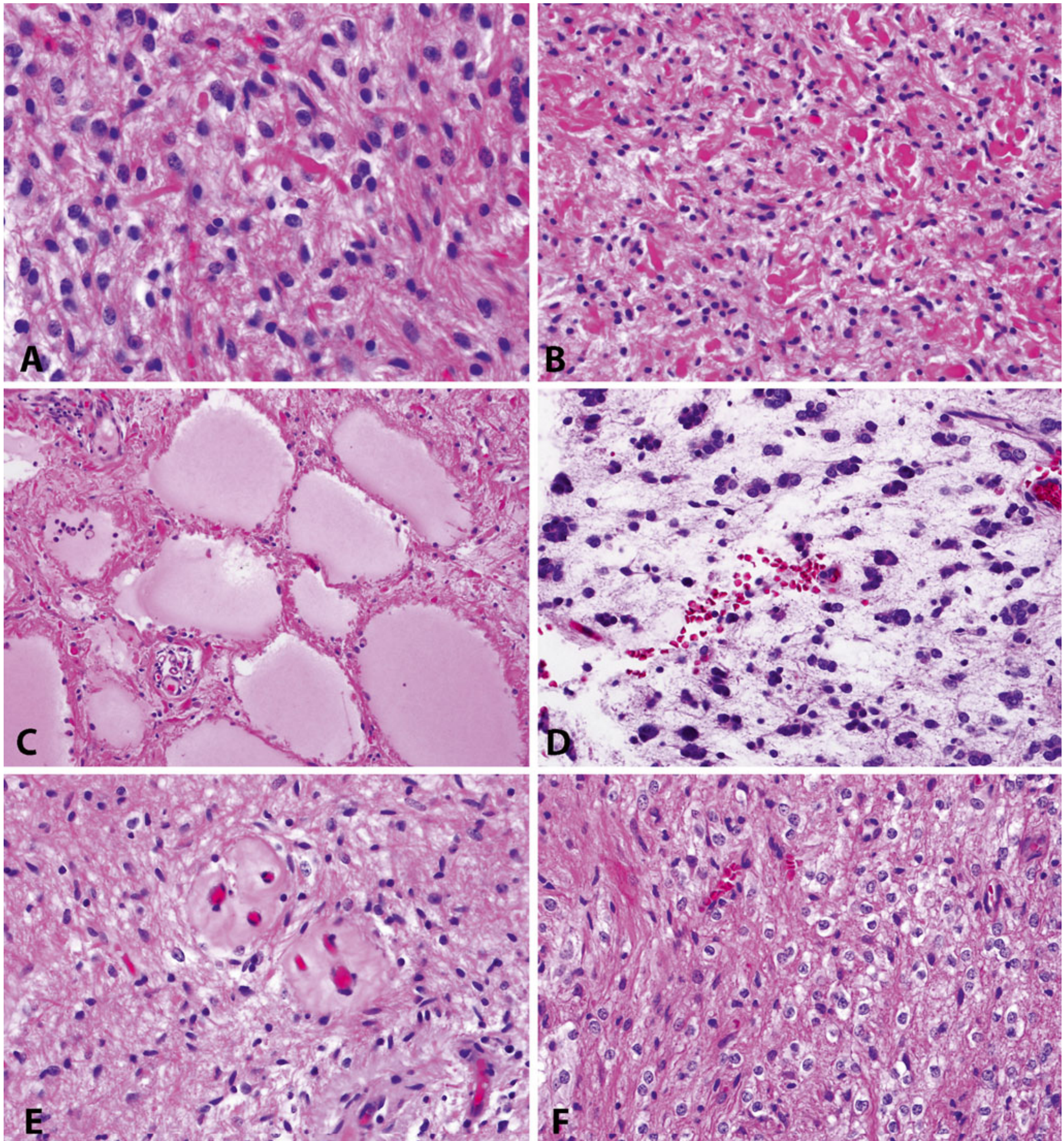

Fig. 4.1. Pathologic features of pilocytic astrocytoma (PA). PA is histologically characterized by the presence of neoplastic bipolar astrocytes in compact areas (**a**). Rosenthal fibers are a frequent feature of pilocytic astrocytomas and may be numerous (**b**). The second architectural pattern of PA is characterized by loose stroma, containing microcysts (**c**) and occasionally multinucleated cells and clusters of small nuclei (**d**). Microvascular hyalinization is frequent in PA (**e**), as it is typical of long standing, slow growing tumors. A variable component of round cells with perinuclear halos may be present in a subset of PA (**f**), and raises the important differential diagnosis with oligodendroglial neoplasms.

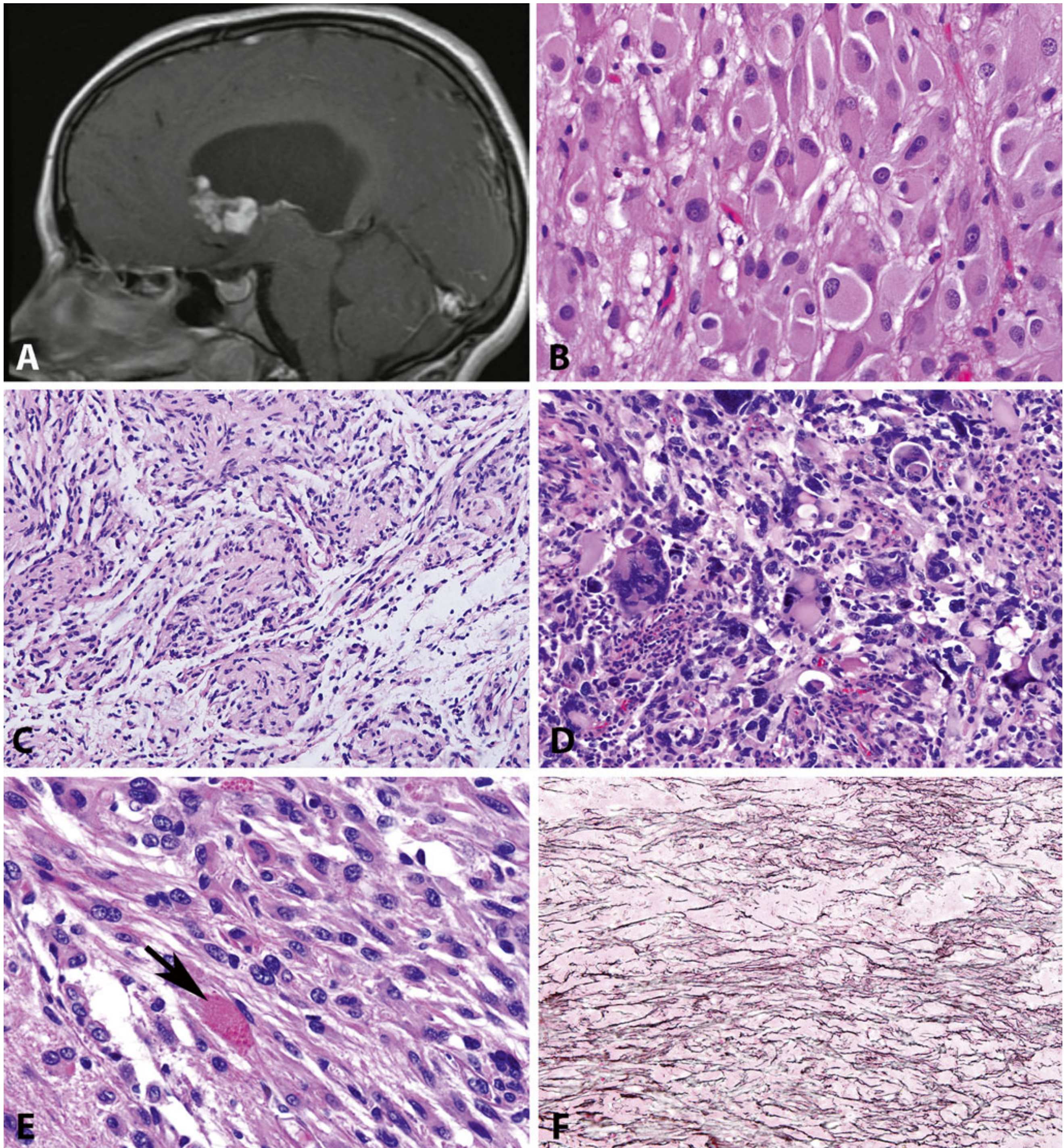

Fig. 4.2. Pathologic features of circumscribed gliomas. Subependymal giant cell astrocytoma (SEGA) develops within the lateral ventricle, almost always near the Foramen of Monro (T1-weighted MR image post-contrast) (**a**). The histology of SEGA is distinctive, containing large cells with voluminous eosinophilic cytoplasm and prominent nucleoli (**b**). Pilomyxoid astrocytoma is a distinctive variant of pilocytic astrocytoma, usually developing in the hypothalamus of young children, characterized by perivascular arrangements in a loose myxoid stroma (**c**). Pleomorphic xanthoastrocytoma is another distinctive astrocytoma characterized by conspicuous pleomorphic cells (**d**), usually with low proliferative activity, as well as a fascicular arrangement and eosinophilic granular bodies (*arrow*) (**e**). Unlike other gliomas, PXAs tend to be reticulin-rich, particularly in superficial regions juxtaposed to the leptomeninges (**f**).

TABLE 4.1. Histopathology and molecular features of low-grade gliomas.

Tumor type	Location	Histology	Grade	IHC	Cytogenetics	Molecular genetics	Signaling pathways
Pilocytic astrocytoma	Cerebellum > optic pathways, brainstem, supratentorial, spinal cord	Bipolar cells, compact and microcystic areas, Rosenthal fibers, eosinophilic granular bodies	I	GFAP, OLIG2+, p53−	7q34 duplication, whole Ch7 or 8 gain	*BRAF-KIAA1549* fusion, *BRAF* (V600E) mut, *RAF1* fusions, *NF1* inactivation (syndrome associated cases)	MAPK, mTOR
Pilomyxoid astrocytoma	Hypothalamic region > brainstem, hemispheres, spinal cord	Monomorphic spindle cells, myxoid stroma, perivascular pseudorosettes	II	GFAP+	Similar to PA	Similar to PA	Similar to PA
SEGA	Lateral ventricle (near foramen of Monro)	Large cells, round nuclei, macronucleoli, amphophilic cytoplasm	I	S100, GFAP+++, synaptophysin, neurofilament+	–	*TSC1* and *TSC2* mutations	mTOR
Angiocentric glioma	Cortex (temporal lobe)	Monotonous spindle cell with perivascular arrangement	I	GFAP+, EMA + (dot-like)	6q23.3 gain/ deletion	*MYB* alterations	Cell cycle regulation
Pleomorphic xanthoastrocytoma	Hemispheric (temporal lobe)	Cellular pleomorphism, xanthic change, fascicular arrangement, eosinophilic granular bodies	II	S100 > GFAP+, CD34, synaptophysin +/−, p53−	–	*BRAF* (V600E) mut; mTOR pathway (*NF1*, *TSC2*, *PI3R1*), *TP53* (rare)	MAPK, mTOR
Diffuse astrocytoma	Throughout CNS	Infiltrative pleomorphic cells, rare to absent mitotic activity	II	GFAP+++, OLIG2+++, IDH1 (R132H) (frequent), p53 (frequent)	7q, 8q gain	*IDH1/2*, *TP53*, *ATRX*, *PTEN*, *CDKN2A*, mutations	MAPK, PI3K/mTOR, cell cycle regulation
Low-grade oligodendroglioma/ oligoastrocytoma	Hemispheric (frontal > temporal, parietal, occipital)	Round cells with perinuclear halos, chicken-wire microvasculature	II	S100+++, OLIG2+++, IDH1 (R132H) (frequent), p53−	1p19q co-deletion	*IDH1/2*, *CIC*, *FUBP1* mutations; *TERT* promoter mutations	
Pediatric diffuse astrocytoma		Same as adult	II	GFAP, OLIG2+++, IDH (R132H) (usually −), p53+	8q13.1 gain/ deletion; 6q23.3 gain/deletion	*FGFR1*, *MYB*, *MYBL1*, *BRAF* (V600E) alterations	MAPK, PI3K/mTOR, cell cycle regulation
Pediatric low-grade oligodendroglioma	Hemispheric (frontal–temporal lobes)	Same as adult	II	S100, OLIG2+++, IDH1 (R132H) (usually −), p53−	1p19q co-deletion (rare)	*FGFR1*, *MYB* alterations; IDH1 (R132H) (rare)	MAPK, PI3K/mTOR, cell cycle regulation

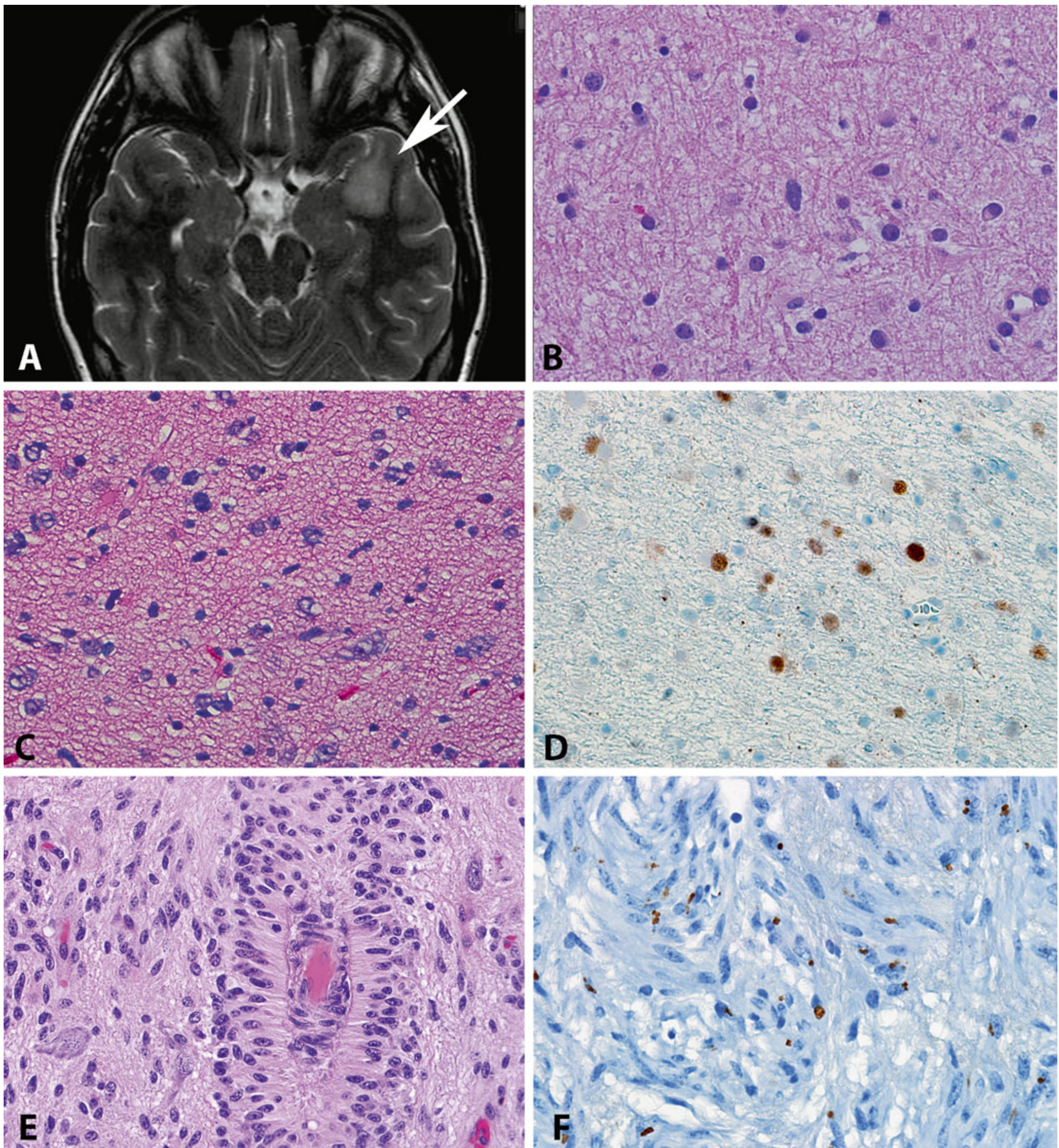

FIG. 4.3. Pathologic features of diffuse astrocytomas and angiocentric gliomas. Diffuse astrocytomas (WHO grade II) present as areas of hyperintensity in T2 weighted MR images (*arrow*, **a**). As the histologic level, diffuse astrocytomas usually have low cellularity but have definite atypia manifesting by irregular nuclear contours and hyperchromasia (**b**). Some diffuse astrocytomas may show obvious hypercellularity, but by definition mitotic activity is rare to absent (**c**). Immunohistochemistry frequently demonstrates strong p53 immunolabeling, a surrogate for *TP53* mutations (**d**). Angiocentric glioma is a distinctive low-grade neoplasm characterized by monotonous cells with elongated nuclei infiltrating cortex but also arranged around vessels (**e**). Despite their infiltrative nature, angiocentric glioma shares biologic properties with ependymomas, including the presence of microlumens highlighted by EMA immunohistochemistry (**f**).

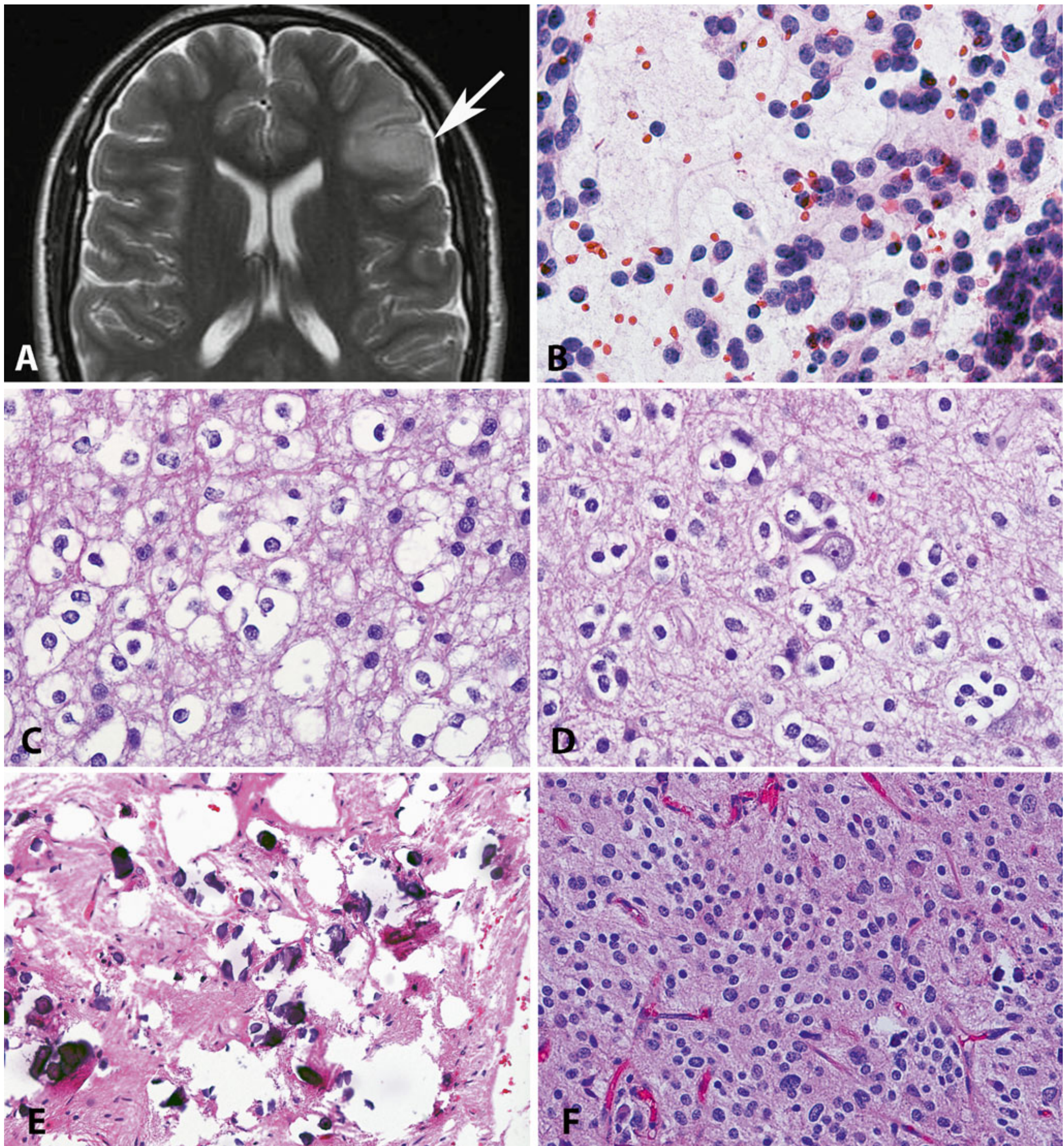

Fig. 4.4. Pathologic features of low-grade oligodendrogliomas. Oligodendrogliomas frequently involve the frontal cortex, with expansion of infiltrated gyri (Axial T2 weighted MR) (**a**). On cytologic preparations, oligodendrogliomas demonstrate round cells with a small nucleolus (**b**). The nuclear uniformity and characteristic halos are best appreciated in formalin-fixed paraffin-embedded sections (**c**). Perineuronal satellitosis is a characteristic feature of infiltrating gliomas, particularly oligodendroglioma (**d**). Microcalcifications are not uncommon in oligodendroglial neoplasms (**e**). The designation of oligoastrocytoma is reserved for tumors that have areas with astrocytic and oligodendroglial morphology. Most of these tumors are morphologically ambiguous as this H&E shows (**f**), and lead to prominent interobserver variability.

tumors express GFAP and OLIG2. P53 and Ki-67 show low labeling indices. Elevated Ki-67 labeling indices have been associated with worse outcome in some studies [8] but not in others [9, 10]. Unlike the category of diffuse gliomas, the development of anaplasia/histologic malignancy in PA is a very rare event (<2 %), and has been defined as the presence of brisk mitotic activity with or without necrosis [11].

Pilomyxoid Astrocytoma

Pilomyxoid astrocytoma (PMA) is considered a variant of PA, characterized by monophasic morphology within a myxoid background and conspicuous aggregates around blood vessels [12]. Unlike conventional PA, PMA lacks a biphasic pattern and Rosenthal fibers. Eosinophilic granular bodies are rare to absent. The classic presentation of PMA is the hypothalamic region of young children. Because of its higher likelihood for aggressive behavior and leptomeningeal dissemination [13], the WHO assigns a grade II to this variant. Morphologic and molecular overlap occurs with conventional PA, which is supported by the recognition of tumors with intermediate features between PMA and PA or PMAs that mature into PA over time [14].

Subependymal Giant Cell Astrocytoma

SEGA is a unique astrocytoma subtype that arises almost always within the lateral ventricles near the foramen of Monro in tuberous sclerosis patients. It is composed of large cells with voluminous eosinophilic cytoplasm, round nuclei and prominent nucleoli. The mitotic activity is very low. At an immunohistochemical and ultrastructural level, these tumors demonstrate evidence of neuronal differentiation in addition to a glial phenotype, which is more accurately consistent with a glioneuronal neoplasm [15]. This includes immunoreactivity for synaptophysin and neurofilament protein in addition to GFAP.

Pleomorphic Xanthoastrocytoma

PXA is a rare neoplasm with distinctive histology. It is composed of spindle neoplastic cells with often conspicuous pleomorphism, sometimes including giant cell forms. Mitotic activity is very low in most cases, and this disconnect between the cellularity and pleomorphism of the tumor and its relative lack of mitoses is one of the first clues to the diagnosis. Eosinophilic granular bodies, particularly large pale forms, are also frequent.

PXA may also demonstrate immunoreactivity for neuronal markers in addition to S100 and GFAP. P53 immunolabeling is typically weak or negative, while CD34 expression is relatively frequent [16]. An additional characteristic finding is the presence of pericellular staining with reticulin special stains, particularly in areas juxtaposed to leptomeninges.

Although PXA is low grade (WHO grade II) by definition, among the circumscribed gliomas it has the highest propensity for recurrence and biologic aggressiveness. A subset develops anaplastic features in the form of brisk mitotic activity and/or necrosis and microvascular proliferation [17]. However, these changes do not consistently predict an adverse outcome, and pending more definite data, a grade III is not yet allowed under the WHO classification.

Diffuse Gliomas

Angiocentric Glioma

Angiocentric glioma is a distinctive neoplasm that was added to the WHO 2007 classification [18, 19]. It is slow growing, frequently associated with epilepsy and therefore shares many clinical properties with dysembryoplastic neuroepithelial tumor. Despite gross circumscription, it is histologically infiltrative. The most characteristic feature of angiocentric glioma is the presence of thin elongated nuclei with little pleomorphism and a perivascular aggregation in a parallel and perpendicular pattern (Fig. 4.3). GFAP expression is a constant feature by immunohistochemistry, but in addition EMA frequently stains in a dot-like fashion, and microlumina may be present on electron microscopy, which suggests a dual astrocytic/ependymal phenotype.

Diffuse Astrocytoma

Diffuse astrocytoma (DA) is a specific subtype characterized by neoplastic astrocytes with nuclear hyperchromasia, atypia, usually low cellularity and rare to absent mitotic figures (Fig. 4.3). Unlike the circumscribed glioma group, DA demonstrates an exquisite infiltration of underlying brain parenchyma, making them surgical challenges. They also have a strong tendency for progression to higher grade neoplasms (anaplastic astrocytoma, glioblastoma), particularly in adults.

Oligodendroglial Tumors

Oligodendroglial tumors include oligodendrogliomas and mixed oligoastrocytomas. They may be low (grade II) or high grade (grade III) (Fig. 4.4). Oligodendroglial morphology is defined by round nuclei with perinuclear halos and little internuclear variability. A delicate “chicken-wire” type microvasculature may be present. Limited mitotic activity may be present, but brisk mitotic activity, endothelial hypertrophy and necrosis define higher grade tumors. Adult oligodendroglioma in particular has classic molecular alterations involving chromosomal arms 1p and 19q, and has almost become a combined pathologic/molecular diagnosis.

Pediatric Diffuse Gliomas

Prior observations have hinted that morphologically similar neoplasms in the adult and pediatric populations may have different clinical behavior. DA, for example, may not always

have the same predictable progression to anaplasia in children as in adults. This has been confirmed by molecular studies [20]. Diffuse intrinsic pontine gliomas represent a distinct subset of diffuse gliomas in children, and are essentially defined by their anatomic development within the brainstem. Although in theory, a subset of these may be identified as grade II early in their course, high-grade histologic features are almost always present postmortem [21]. Oligodendrogliomas in children for example lack 1p19q codeletion and *IDH1/2* mutations in contrast to adult tumors [22–24]. Furthermore, the mutational landscape uncovered by recent, large scale sequencing efforts is different, and simpler, in pediatric diffuse gliomas.

New and Unclassifiable Entities

After accounting for well-defined histopathologic categories, a subset of low-grade gliomas remain diagnostic challenges, particularly in the pediatric population. In fact, approximately 20 % of pediatric brain tumors are unclassifiable according to traditional schemes, most of which involve low-grade gliomas (Burger PC, personal communication). One particular entity that has been recognized for a while, but has been the recent focus of several larger series is a low-grade oligodendroglioma-like tumor that remains indolent despite widespread superficial CNS dissemination [25–28]. This tumor has frequent 1p chromosome arm loss but lacks IDH1 (R132H) mutations. There is also a rare subset of pediatric low-grade tumors that have been termed descriptively "massively calcified low-grade glioma" that lack alterations associated with other low-grade gliomas [29].

Gene Expression Profiling

Global gene expression profiling studies have provided important biologic insights into the biology of gliomas in general and low-grade gliomas in particular. In PA, gene expression profiling studies have highlighted differential gene expression signatures related to anatomic regions and NF1 vs. sporadic occurring tumors [30]. These studies have also uncovered possible biomarkers associated with worse clinical outcome in PA including overexpression of *Matrilin-2* [31], and underexpression of *ALDH1L1* [32] and myelin basic protein (*MBP*) [33].

Analogous studies focusing on DA have also provided important insights particularly highlighting phenotypic and genetic differences between adult and pediatric DA. These included expression changes in genes involved in neural stem cell maintenance, CNS development, DNA replication, and cell cycle [20], which may explain in part the more aggressive behavior of these tumors when occurring in adults. Integration of mutation, copy number, and transcriptome analysis has also allowed the separation of distinct molecular subgroups of grade II and III diffuse gliomas with biological and prognostic relevance. Using this approach, Gorovets et al. classified diffuse astrocytic tumors into three molecular classes denoted "preglioblastoma" (PG), "neuroblastic" (NB), and "early-progenitor-like" (EPL) [34]. The NB and EPL subclasses were associated with a higher frequency of *IDH* and *TP53* mutations and 8q gains, as well as better clinical outcome compared with the PG class. Interestingly, 8q gain is one of the most frequent cytogenetic abnormalities of diffuse astrocytoma [35].

Molecular Genetics and Signaling Pathways

Genetic Predisposition to Low-Grade Glioma

The earliest insights into the molecular alterations contributing to glioma formation have evolved from the study of inherited tumor syndromes. Neurofibromatosis type 1 (NF1) is associated with germline mutations in the *NF1* gene that encodes for the protein neurofibromin, a negative regulator of RAS signaling. These patients are predisposed to gliomas of various grades, particularly PA of the optic pathways. Furthermore, biallelic *NF1* inactivation is a feature of these patients tumors [36]. Tuberous sclerosis complex is associated with germline mutations in *TSC1* or *TSC2*, which leads to increased mTOR activity and a predisposition to SEGA. Conversely, Li–Fraumeni syndrome is associated with *TP53* mutations, which predisposes to a variety of tumors, including infiltrating astrocytomas [37]. These early observations suggested that the pathways deregulated by these germline alterations were important for gliomagenesis.

More recent studies have also highlighted a role for germline polymorphisms that predispose to diffuse glioma development. For instance, genotyping efforts have uncovered single nucleotide polymorphisms (SNP) variants at 8q24.21 (near the *MYC* gene) to be associated with an increased risk for gliomas with *IDH1/2* mutations, both astrocytic and oligodendroglial [38].

Circumscribed Gliomas

BRAF and MAPK Alterations

One of the most remarkable discoveries in pediatric neurooncology in the recent years has been the identification of *BRAF* duplications in the majority (53–77 %) of PA tumors [39–44]. Subsequent studies demonstrated that this tandem duplication always involves the kinase domain of BRAF and leads to a novel fusion (usually *BRAF-KIAA1549*) [42, 45]. This novel fusion has oncogenic properties resulting in ERK/MAPK pathway activation (Fig. 4.5). Furthermore, it induces glioma-like lesions in mice when introduced into neural stem cells [46]. This alteration is almost always restricted to PA, particularly those arising in the cerebellum or optic

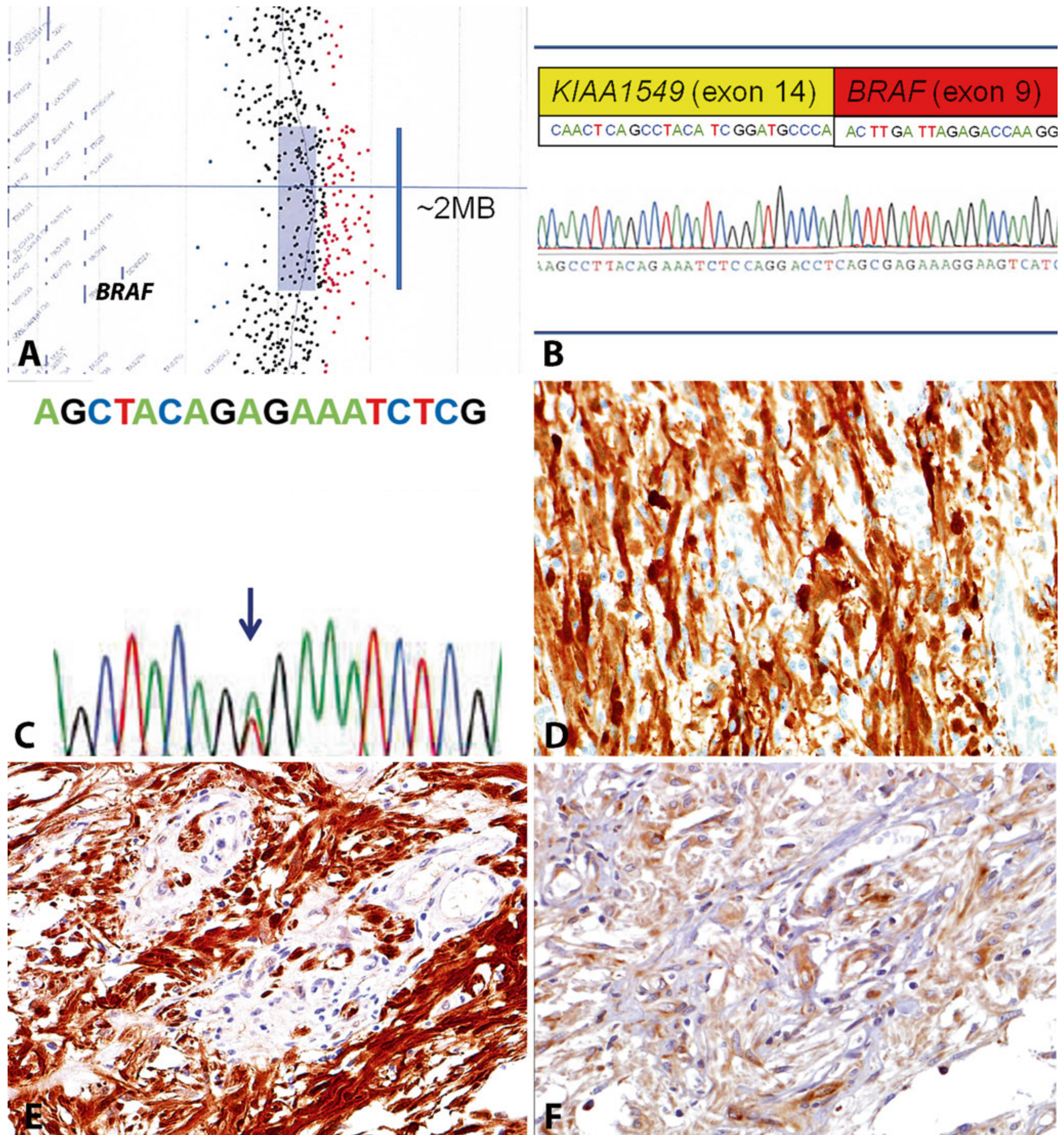

Fig. 4.5. Molecular features of circumscribed gliomas. The most frequent molecular alteration in PA is a *BRAF* duplication that may be identified by array CGH (**a**). This duplication usually leads to a gene fusion, usually involving the neighboring gene *KIAA1549* (**b**). In contrast, most pleomorphic xanthoastrocytomas contain a *BRAF* (V600E) mutation resulting from a single nucleotide change (**c**). Activating *BRAF* alterations may result in induction of the phenomenon of oncogene-induced senescence, and associated with increased p16 expression (**d**), which may explain the low proliferation rates in many of these tumors. The genetic alterations present in PA and other circumscribed gliomas result in near universal activation of the MAPK and mTOR signaling pathways, which may be identified by detection of phospho-ERK (**e**) and phosphor-S6 (**f**) protein, respectively.

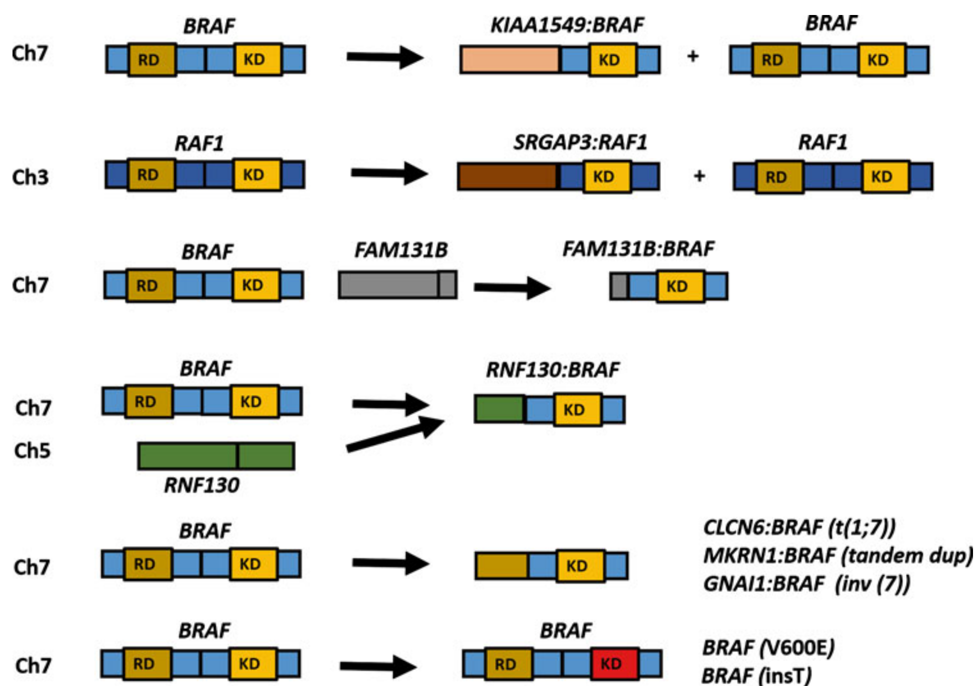

Fig. 4.6. Spectrum of BRAF alterations in pilocytic astrocytoma/circumscribed gliomas. Although tandem duplications involving the *BRAF* kinase domain (KD), excluding the regulatory domain (RD) and leading to a *KIAA1549*:*BRAF* fusion are the most frequent alterations associated with pilocytic astrocytoma in specific, alternative alterations occur in a small proportion of cases. These include an interstitial deletion leading to *FAM131B*:*BRAF* fusion, fusion events involving alternative partners, as well as point mutations (e.g., *BRAF V600E*) and small activating insertions (insT). A similar rearrangement involving the related gene *RAF1*, also occurs on rare occasions.

pathways [40, 41]. Conversely, *BRAF* fusions are less frequent in PA arising in the cerebral hemispheres. However, a small subset of unclassifiable low-grade astrocytomas and neuroepithelial/glioneuronal tumors may also have it [47]. In addition, it appears that the frequency of *BRAF-KIAA1549* fusion varies by age, being less frequent in PA of adults [48].

The development of low-grade astrocytomas in patients with germline *NF1* loss and the high frequency of somatic *BRAF* alterations in sporadic circumscribed low-grade gliomas provide strong evidence that the MAPK/ERK signaling pathway is critical for the biology of these tumors. Other studies have shown rarer genetic alterations leading to activation of this pathway, including small *BRAF* insertions, *RAF1-SRGAP3* fusions (at 3p25), activating *RAS* mutations, and a *FAM131B-BRAF* fusion mediated by an interstitial deletion [45, 49, 50] (Fig. 4.6). Some of these rearrangements seem to be facilitated by sequence microhomology [51]. A recent whole genome/transcriptome sequencing study of PA identified single MAPK pathway activating alterations, predominantly through *BRAF-KIAA11549* fusions, but also novel *BRAF* fusion partners in very rare cases (i.e., *RNF130-BRAF*, *CLCN6-BRAF*, *MKRN1-BRAF*, and *GNAI1-BRAF*) [52]. Alterations in other genes not involving *BRAF* were also identified in a small subset of non-cerebellar PA (*NTRK2* rearrangements, as well as *FGFR1* and *PTPN11* mutations). Of interest, in this study every PA had a genetic alteration in the MAPK pathway, which was almost always exclusive (except for *PTPN11* mutations which occurred only in combination with *FGFR1* alterations) [52].

BRAF (V600E) mutation, reported in numerous cancer types, is less restricted to histopathology and has been reported to occur with variable frequency in many brain tumor subtypes [53–58], including PXA, gangliogliomas, desmoplastic infantile gangliogliomas, PA, diffuse gliomas and even in dysembryoplastic neuroepithelial tumors. However, the frequency of *BRAF* (V600E) mutation appears to be higher in PXA, occurring in over half of cases [57, 59, 60].

At the current time, the prognostic significance of *BRAF* alterations in low-grade gliomas remains unclear. Several studies have not found a significant association with outcome in patients with low-grade gliomas containing *BRAF* fusions [13, 47, 49]. In a study by Hawkins et al. focusing on a clinically relevant group of 70 pediatric low-grade astrocytoma patients (i.e., sporadic, subtotally resected tumors in non-cerebellar locations), the investigators found *BRAF-KIAA1549* fusions to be significantly associated with better clinical outcome [61]. Conversely, Horbinski et al. in a study of 198 cases, found on multivariate analysis midline location and *p16* deletion (but not *BRAF* rearrangement) as independent prognostic factors [62]. In a meta-analysis of *BRAF* alteration data encompassing approximately 700 pediatric

low-grade astrocytomas, specific *BRAF-KIAA1549* fusion variants have independent prognostic implications in extra-cerebellar PA, but *BRAF* fusions in general were not independently associated with outcome (Jones D et al., unpublished data). RT-PCR and FISH based methods that are able to detect this fusion in formalin-fixed paraffin-embedded tissue have been developed [63], and have been increasing applied for clinical use.

Given the high frequency of somatic *BRAF* genetic alterations in circumscribed low-grade gliomas, the possibility of pharmacologic inhibition as a therapeutic strategy is very appealing. However, targeted therapeutics for BRAF must be taken with caution, since recent pharmacologic evidence suggests that inhibitors that are effective against BRAF (V600E) may have paradoxic pro-growth effects in tumors that are *BRAF* wild type or contain activating *BRAF* fusions [64].

Oncogene-Induced Senescence

Senescence, i.e., irreversible growth arrest, is a cellular phenomenon that may occur as a result of oncogene activation. Clinical observations have documented stabilization, or even regression, of a subset of PA. Furthermore, PA shares frequent alterations in the *BRAF* oncogene with another limited neoplastic proliferation, cutaneous melanocytic nevi, which are known to senesce. Recent studies [65, 66] have shown markers of senescence, including p16 and acidic senescence-associated β-galactosidase, in primary PA and low passage cultures. Furthermore, senescence was also induced after introduction of *BRAF* (V600E) in neural stem cells, and p16 loss in clinical samples was associated with worse clinical outcome [66].

PI3K/mTOR

PI3K/mTOR signaling has been implicated as a frequent molecular property of a variety of tumor types. This pathway is of great interest for targeted therapeutics, since pharmacologic inhibitors (i.e., rapamycin and its analogs) are widely available. mTOR exists as part of two multiprotein complexes: mTORC1 and mTORC2 (mTORC1 is composed of RAPTOR, mLST8 and GBL), and signaling through this complex leads to increased protein translation, cell growth, and survival. In mTORC2, mTOR interacts with RICTOR, mSin1, and Protor, activation leads to AKT activation (identified by phosphorylation at S473)/PKC signaling, and subsequently increased cell survival and regulation of cytoskeletal dynamics [67].

Of relevance to low-grade glioma, particularly pediatric, is studies demonstrating increased mTOR signaling in the context of *NF1* loss. These include mouse models of NF1-optic glioma [68] and unusual low-grade gliomas in NF1 patients characterized by increased cell size [69]. mTOR activation is also more frequent in rare PA that develop anaplasia [70], and regulates proliferation of murine stem cells containing activating *BRAF* fusions [46]. A recent study of 177 pediatric low-grade gliomas and PA showed significant mTOR activation (~60 % of cases) as measured by pS6 protein [71]. In addition, mTOR inhibition led to decreased cell growth of two pediatric cell lines in vitro. Of great clinical interest, mTOR inhibitors have pharmacologic efficacy in SEGA and other manifestations of tuberous sclerosis [72], a syndrome essentially defined at the molecular level by constitutive mTOR activation. PI3K/mTOR pathway activation is also a frequent feature of both adult and pediatric diffuse gliomas, through alterations in *PTEN*, *NF1* and genes encoding for receptor tyrosine kinases (e.g., *FGFR1*) [73].

Diffuse Gliomas

One of the earliest molecular alterations described in diffuse gliomas, with morphologic and prognostic relevance was the identification of 1p19q co-deletion, particularly in tumors with oligodendroglial morphology (Fig. 4.7). Subsequent studies highlighted a strong association between 1p19q co-deletion and therapeutic response, particularly in anaplastic oligodendroglioma [74]. This alteration may be identified by a variety of methods that work in formalin-fixed paraffin-embedded tissue, most commonly FISH [75], but also array based platforms, such as SNP arrays [76]. Although partial deletions involving chromosome arm 1p and/or 19q are not uncommon in gliomas with various histologies, it is whole arm 1p and 19q co-deletion that is most closely associated with oligodendroglial histology, which is mediated by an unbalanced (t1;19) translocation [77, 78]. Subsequent whole exome sequencing studies have identified recurrent mutations in *FUBP1* (Ch 1p) and *CIC* (Ch 19q) as likely tumor suppressor genes inactivated at these locations [79, 80].

Another remarkable subsequent discovery in the biology of diffuse gliomas was the identification of recurrent point mutations in genes encoding for the cytosolic metabolic enzyme *IDH1* (and less frequently *IDH2*) through exome sequencing efforts. Although initially identified in a subset of glioblastomas, it was subsequently noted that these mutations were highly prevalent (>80 %) in diffuse gliomas grade II and III, both astrocytomas and oligodendrogliomas as well as in secondary glioblastomas [81–83]. These IDH mutations occur almost always at the same site (Arg132 of IDH1 and analogous Arg172 site in IDH2) and result in a neoenzymatic function leading to increased 2-hydroxyglutarate (2HG) [84], which has numerous cellular effects including inhibition of histone and DNA demethylation and global epigenetic alterations (reviewed in [85, 86]). *IDH1/2* mutations appear to be early events in tumorigenesis, since they show similar prevalence in grade II astrocytomas and oligodendrogliomas, and may occur earlier than 1p19q loss and *TP53* mutations. Diagnostically, an antibody directed against the most frequent IDH1 mutant protein in gliomas (IDH1 R132H), is in clinical use and valuable in differentiating infiltrating gliomas from other tumors and non-neoplastic conditions (i.e., gliosis) [87, 88].

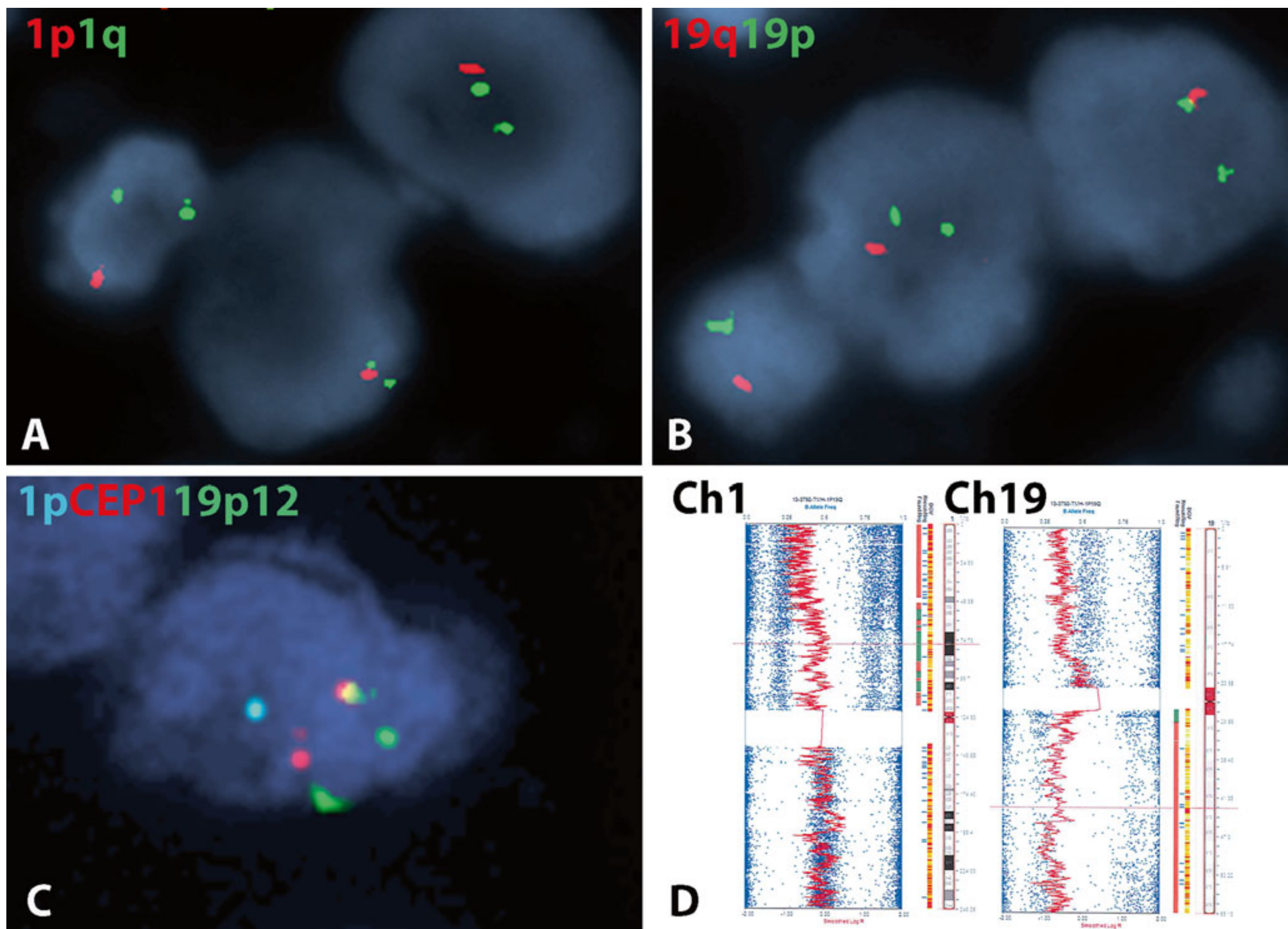

FIG. 4.7. Molecular genetic alterations in oligodendroglial neoplasms. The most characteristic molecular alteration in oligodendroglial tumors is combined deletions of 1p (**a**) and 19q (**b**) which is mediated by a t(1;19) translocation (**c**). The 1p19q co-deletion may also be recognized by array based methods (e.g., SNP platforms) in formalin-fixed tissue. As this case demonstrates, the classic co-deletion of oligodendroglioma involves the whole 1p and 19q chromosomal arms (**d**) (SNP figure courtesy of Christopher Gocke, MD).

Another molecular property of a subset of infiltrating astrocytomas is the presence of a telomerase independent mechanism of telomere maintenance known as the alternative lengthening of telomeres (ALT) (Fig. 4.8). Although present in a small subset of cancers of various types, this phenotype is enriched in DA (WHO grade II), anaplastic astrocytoma (WHO grade III), as well as secondary and pediatric glioblastoma (WHO grade IV). Subsequent studies found this phenotype to be strongly associated with mutations in the gene encoding the chromatin remodeling protein ATRX [89]. These mutations lead to ATRX protein loss and are strongly associated with *IDH* mutations [90–92], but are mutually exclusive with 1p19q co-deletion/*CIC*/*FUBP1* mutations [93].

All these recent studies highlighted an important role for telomere maintenance in the biology of diffuse gliomas, findings subsequently reinforced by the finding of mutations in the *TERT* promoter which leads to increased transcriptional activity [94]. Interestingly, among diffuse gliomas, *TERT* promoter mutations are more frequent in oligodendrogliomas and primary glioblastomas and mutually exclusive with *ATRX* mutations and the ALT phenotype. An expanding picture is now emerging with distinctive molecular signatures separating various low-grade glioma subtypes (Fig. 4.9).

Whole Genome Sequencing Studies of Pediatric Low-Grade Glioma

Whole genome/exome sequencing studies have also provided recent, important insights into the molecular genetics important for pediatric low-grade glioma development (Fig. 4.10). Zhang et al. in a whole genome sequencing study of 39 pediatric low-grade gliomas and glioneuronal tumors found very few genetic alterations, with 24 tumors (62 %) containing single relevant (non-silent) somatic alterations [73]. They found rearrangements of *MYB* and duplications of the gene segments of *FGFR1* encoding for the tyrosine kinase domain in approximately half of pediatric DA.

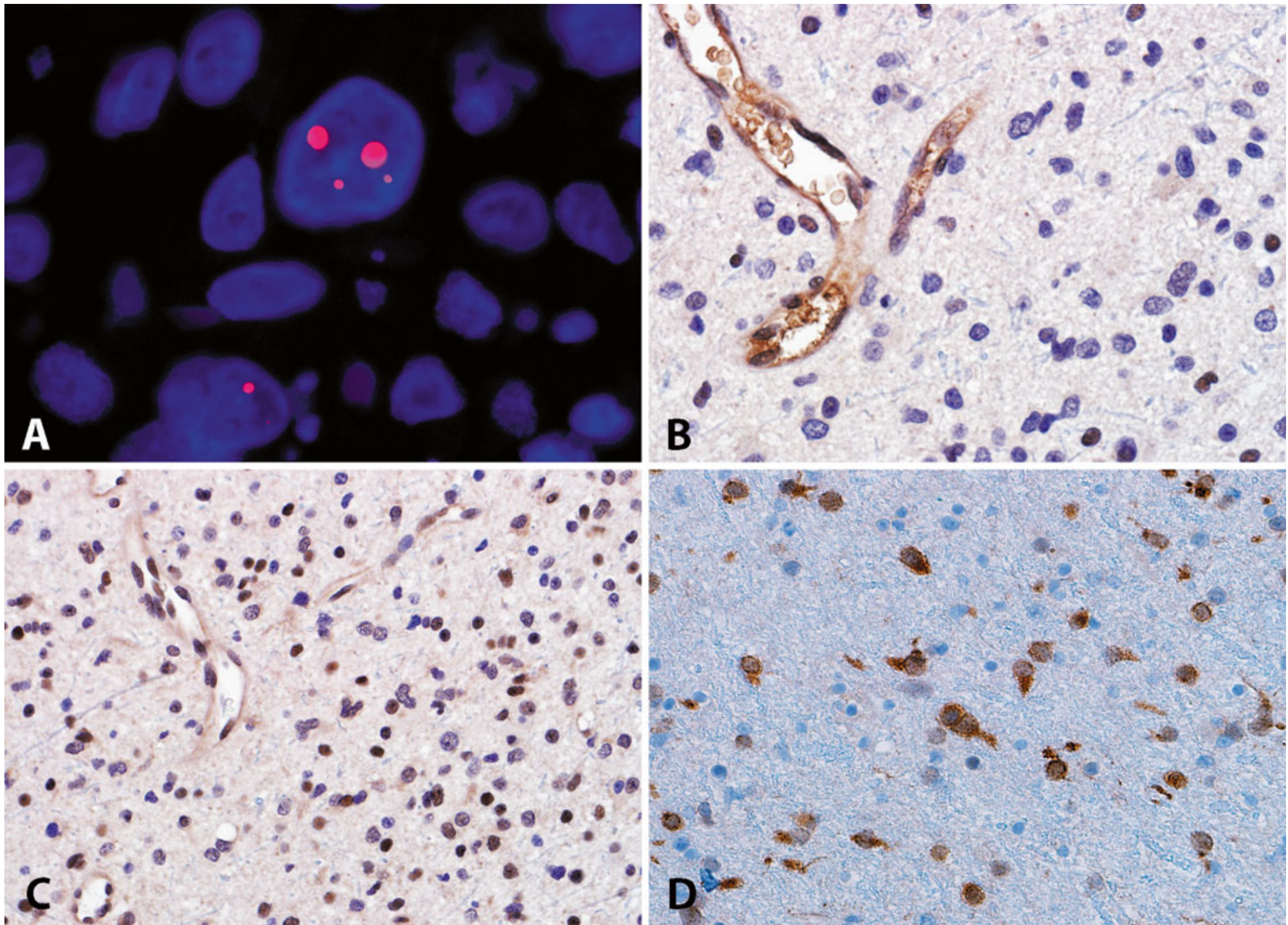

FIG. 4.8. Molecular features of adult diffuse astrocytomas. The alternative lengthening of telomeres (ALT) is a frequent phenotype identifiable in diffuse astrocytomas by telomere specific FISH, which demonstrates ultrabright signals (Courtesy of Christopher Heaphy, Ph.D.) (**a**). The ALT phenotype is frequently associated with *ATRX* mutations and protein loss in neoplastic cells (**b**). Conversely DAXX is usually preserved in most CNS tumors (**c**). IDH1 mutations are frequent in diffuse gliomas, and may be recognized by an antibody directed against the most frequent mutated protein product (R132H) (**d**).

Ramkissoon et al. studied 44 pediatric diffuse low-grade gliomas using high resolution copy number analysis and identified 8q13.1 alterations in 28 % of cases leading to *MYBL1* gain [95] (Fig. 4.11) The authors also found a similar alteration involving the related gene *MYB* in two angiocentric gliomas. These alterations frequently result in a duplication as well as truncation of a C-terminal regulatory domain.

In their study, Zhang et al. also identified various alterations in the small subset of the rarer pediatric oligodendroglial tumors [73]. For example, duplications of *FGFR* TK were present in 3 (of 5) pediatric oligodendrogoliomas and 4 (of 8) oligoastrocytomas. *FGFR1-TACC1* fusion, *NAV1-NTRK2* fusion, *FGFR1*:*p.N544K*, and *BRAF*:p.G503 > EYSG were present in each of the two remaining oligoastrocytomas, and a *MYB-MAML2* fusion and adult oligodendroglioma alterations (*IDH1*, *CIC* mutations, 1p19q co-deletion) in one additional oligodendroglioma each. Prior studies have also found low frequencies of 1p19q co-deletion and IDH1 (R132H) in pediatric oligodendroglioma [22–24], and when present they tend to occur in older children (>15 years of age). Pending the study of additional cases, it therefore appears that there is genetic overlap between pediatric DA and oligodendrogliomas, unlike the morphologically similar tumors in adults.

Compound Genetic Alterations

Although initial studies suggested separation of different tumor types by molecular features, specifically by *BRAF-KIAA1549* fusion and *IDH1/2* mutations [96], subsequent studies have documented tumors with overlapping molecular alterations. For example, Badiali et al. found coexisting *IDH* mutations and *BRAF-KIAA1549* fusions in <10 % of 185 adult diffuse gliomas, particularly tumors with oligodendroglial morphology [97]. *BRAF* (7q34) gain was also even

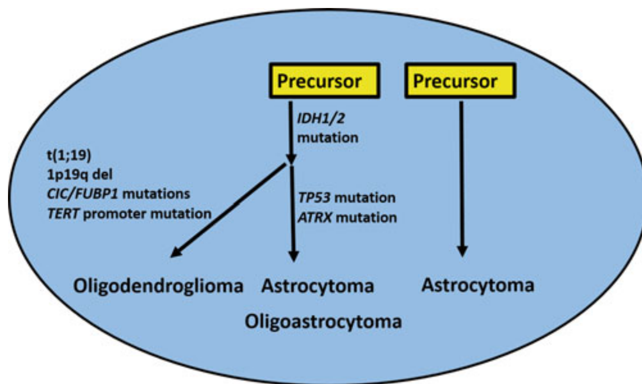

Fig. 4.9. Molecular pathogenesis of adult low-grade diffuse gliomas. Recent studies have also refined our classification of diffuse gliomas in adults. IDH mutations appear to be an early event, shared by oligodendroglial tumors and a subset of diffuse astrocytomas with a relatively better prognosis. Additional alterations (e.g., t(1;19)) are associated with the oligodendroglial subgroup, while ATRX alterations are associated with the astrocytic pathway. Other astrocytomas lack these alterations, and are associated with a worse prognosis. Often, they have molecular alterations more typical of primary glioblastoma. Oligoastrocytoma is an heterogeneous group, and may share molecular properties with oligodendrogliomas or astrocytomas.

more frequent in tumors with 1p19q loss (~40 %) in another study [98].

When focusing on activating alterations in the MAPK pathway, in most instances only a single alteration is encountered, particularly in PA. However, overlapping alterations (*BRAF-KIAA1549*, *BRAF* (V600E), NF1 syndrome) may also occur in a small proportion of cases [47, 49].

Epigenetics

One of the most important insights into the molecular biology of gliomas in the past several years is the presence of genetic mutations that lead to profound global alterations in the epigenetic landscape. For example, global methylation analysis of glioblastomas as part of the Cancer Genome Atlas (TCGA) led to the discovery of a CpG island methylator phenotype (CIMP) group of tumors that is strongly associated with *IDH1/2* mutations [99]. Interestingly, *IDH1* mutations are sufficient to induce this CIMP phenotype [100]. Subsequently, similar epigenetic alterations have been identified in lower grade tumors including oligodendrogliomas [101]. Mutations in genes encoding for chromatin remodelers (e.g., *ATRX/DAXX*) or components (*H3F3A*)

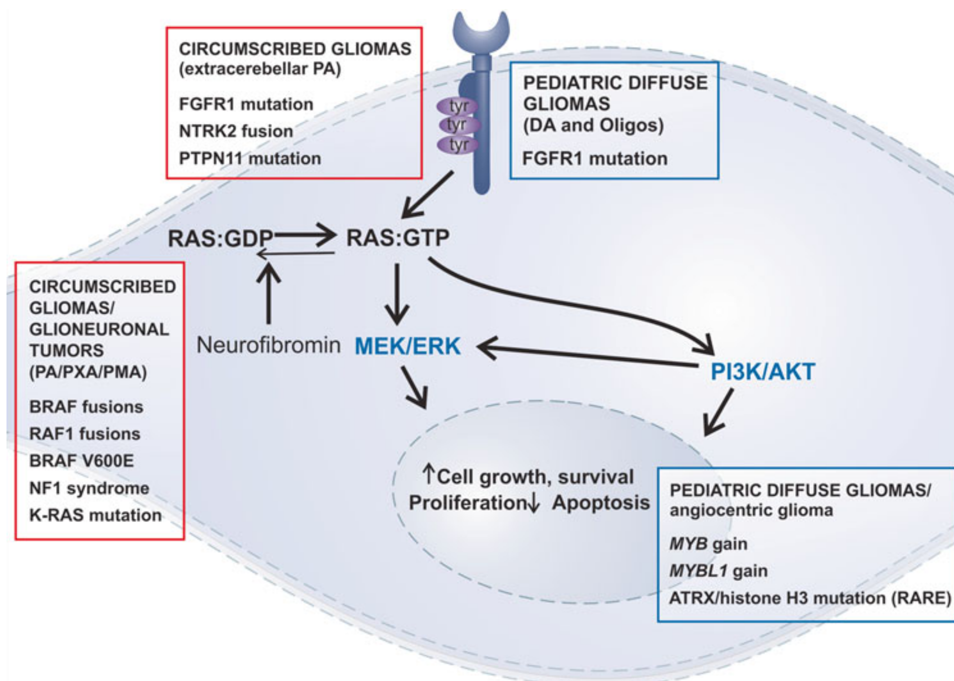

Fig. 4.10. Signaling pathways in pediatric low-grade gliomas. Recent genomic studies have clarified the molecular genetic alterations associated with pediatric low-grade gliomas and circumscribed gliomas, identifying alterations in BRAF, FGFR1 and transcription factors MYB or MYBL1. Many of these alterations particularly lead to MAPK and PI3K/mTOR pathway activation, an almost universal feature of these tumors.

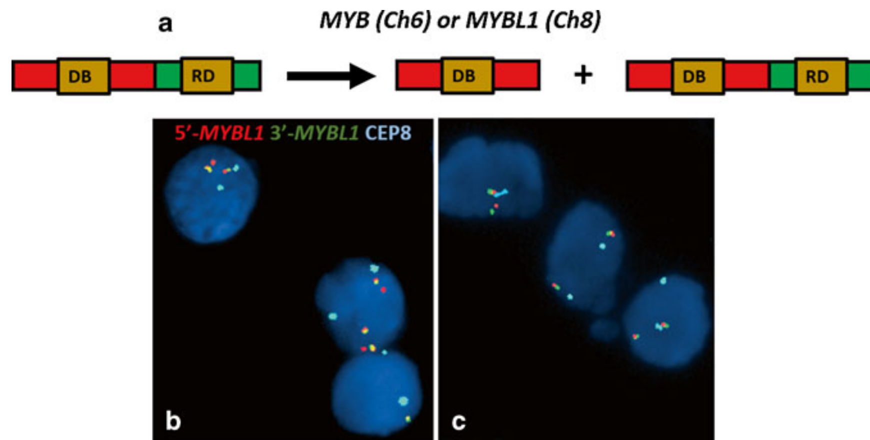

Fig. 4.11. Molecular alterations in *MYB* or *MYBL1* in pediatric diffuse gliomas. Diffuse gliomas in children, particularly astrocytic, and angiocentric gliomas have a relatively high frequency of MYB or MYBL1 rearrangements, often leading to a gain containing the DNA binding (DB) and activating domains, but lacking a C-terminal regulatory domain (RD) (**a**). FISH strategy identifies a pediatric glioma with *MYBL1* gain (three *red* copies) (**b**). Cells lacking *MYBL1* alteration for comparison (**c**) (FISH images courtesy of Azra Ligon, Ph.D.).

have also been identified in specific subsets of diffuse gliomas, particularly pediatric high-grade ones.

Epigenetic alterations, including dysregulation in microRNA levels [102] and DNA methylation [103] are also a recently recognized feature of PA, and may identify biologically and/or clinically relevant subsets that deserve further study. For example, the *AKAP12* tumor suppressor gene is underexpressed and methylated in DA and other infiltrating gliomas in contrast to PA [104].

Molecular Targeted Therapies

MAP Kinase Pathway: BRAF Duplication/BRAF V600E/MAPK/ERK Targeting Agents

Uncontrolled growth is a necessary step for the development of all cancers. In many cancers, a defect in the MAP Kinase Pathway has been demonstrated to regulate cell proliferation, mitosis, survival, and apoptosis. As a result of the relative high frequency of *BRAF* duplication/fusion mutations and activation of the MAP kinase pathway described among pediatric low-grade gliomas, including PA and PMA [39–41, 44, 47, 49, 61, 70, 105], there is considerable interest in targeted MAP kinase pathway inhibition as a potential therapy for these tumors (Fig. 4.12).

Sorafenib (Nexavar, Bayer and Onyx Pharmaceuticals) is an inhibitor of mutated BRAF including B-RAF and C-RAF (it has less potency against BRAF V600E). Sorafenib is approved the United States Food and Drug Administration (FDA) for the treatment of renal cell carcinoma and liver cancer. A recent phase II study of Sorafenib enrolled 11 patients with recurrent or progressive pediatric low-grade gliomas. Nine of 11 (82 %) patients enrolled had tumor progression at 3 months and thus the clinical trial was stopped early [106]. Furthermore, *KIAA1549-BRAF* fusions were identified among three of the nine patients with tumor progression, demonstrating a lack of activity among these patients. One patient with a ganglioglioma of the spinal cord completed six cycles with stable disease. Another patient with a PMA of the brainstem achieved a partial radiological response. The authors proposed that sorafenib may lead to ERK activation in both BRAF wild type and KIAA1549-BRAF mutant cell lines, and proposed that this paradoxical effect may be the mechanism by which Sorafenib promoted tumor growth in the patients with low-grade gliomas.

In general, BRAF V600E mutations are rare among pediatric low-grade astrocytomas, with the exception of being relatively common among PXA, gangliogliomas and a small subset of extra-cerebellar PA [57]. *Vemurafenib* (Plexxikon, Daiichi Sankyo) is a competitive inhibitor that is specific for the ATP binding domain of mutant BRAF V600E. As a result, it has activity against tumors with BRAF V600E, but not other mutant forms of BRAF [107, 108]. Following impressive, albeit transient, responses of recurrent melanoma to Vemurafenib [108], the United States Food and Drug Administration (FDA) approved Vemurafenib for the treatment of BRAF V600E mutation positive, inoperable or metastatic melanoma. A phase I clinical trial of Vemurafenib against BRAF V600E mutant pediatric low-grade gliomas is expected to be open in the near-term future. *Dabrafenib* (Tafinlar, GlaxoSmithKline) is another selective inhibitor of V600E-mutant BRAF and has been approved by the FDA for unresectable or metastatic melanoma. Recently, a phase I/II clinical trial of Dabrafenib has opened for pediatric brain tumors. *LGX818* (Novartis) is another selective BRAF V600E inhibitor that is undergoing clinical trials in adults,

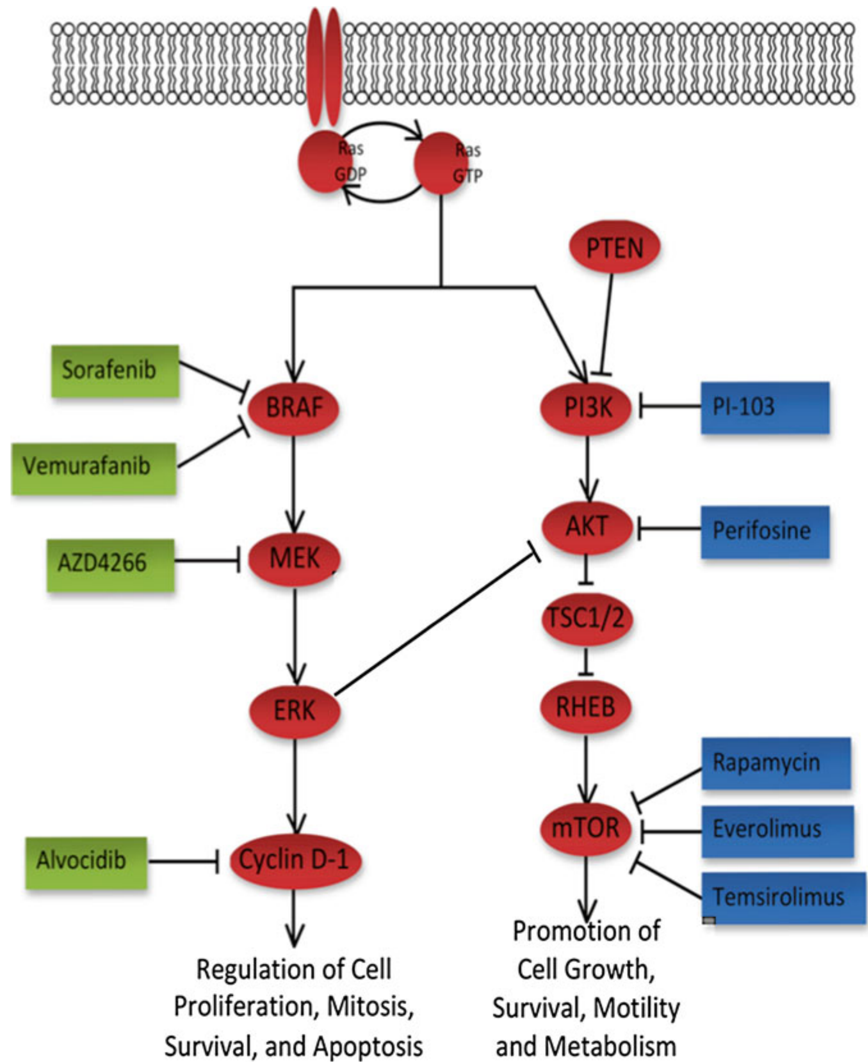

FIG. 4.12. Activated signal pathways and signal transduction inhibitors of potential clinical activity for pediatric low-grade glioma. Note that the agents listed below are just a few of the many new agents currently under development.

but there are no clinical trials of LGX818 for pediatric brain tumors at this time.

Selumetinib (AZD6244, AstraZeneca and Array BioPharma), a potent inhibitor of MEK, immediately downstream of BRAF, is an investigational therapy currently in clinical trials for non-small cell lung cancer and several other cancer types. AZD6244 is currently in phase I/II trials for pediatric low-grade astrocytomas through the Pediatric Brain Tumor Consortium.

Alvocidib (Flavopiridol, Sandofi) is a cyclin dependent kinase (CDK) inhibitor that has some activity against adult relapsed chronic lymphocytic leukemia, although it has not yet been approved by the FDA as treatment for cancer. Alvocidib has completed phase I trials in children which established the maximum tolerated dose, dose limiting toxicities were neutropenia and diarrhea. However, there are currently no ongoing studies of Alvocidib for pediatric brain tumors.

mTOR Pathway: PI3-K/AKT/mTOR Targeting Agents

The mTOR Pathway has been implicated as an important mechanism for tumor growth in many pediatric low-grade astrocytomas. Methylation of the PTEN promoter is associated with PI3-K activation and Akt phosphorylation in PLGAs [109]. Because of the clinical significance of PTEN promoter methylation and its effects on the PI3-K pathway, therapies that target tumors with PI3-K activation may be of clinical benefit in PLGAs. The mammalian target of rapamycin (mTOR) is downstream to the PI3-K and AKT and is therefore an ideal target for PLGAs with PTEN promoter methylation [110–115].

PI-103 is a dual inhibitor of PI3-K/mTOR and has activity in preclinical trials against malignant glioma cell lines. Unfortunately, because of its rapid in vivo metabolism, PI-103

is not being pursued further as an anticancer agent. Nonetheless, demonstration of dual PI3-K and mTOR inhibition suggests promise for this class of targeted agents. Currently, *SF-1126* (Semafore Pharmaceuticals) and *XL765* (Exelixis) are dual PI3-K/mTOR inhibitors that are currently in early phase trials in adults. New agents that are specific PI3-K inhibitors that are in early phase trials in adults include PX-866 (Oncothyreon) and XL147 (Exelixis). None of the PI3-K inhibitors have yet undergone phase clinical trials in children.

Akt, also, known as Protein Kinase-B, is a protein that is believed to play an important role in regulating development and growth of cancer cells. *Perifosine* (Keryx Biopharmaceuticals. Aeterna Zentaris) was the first drug that belongs to a class of agents known as "Akt-inhibitors." Perifosine has completed phase I testing in children with recurrent solid tumors [116]. However, results from the phase III trials of perifosine for recurrent colon cancer and myeloma were disappointing and it does not appear that there will be future studies of this agent. *GSK1120212* (GlaxoSmithKline) and *AZD5363* (Astex) are other Akt-inhibitors currently in early phase clinical trials in adults with recurrent cancer, but there are no clinical trials of these agents for children with brain tumors at this time. *MLN0128* (Millennium Pharmaceuticals) is a potent and selective small molecule active-site TORC1/2 kinase inhibitor that has demonstrated in vitro anticancer activity. MLN0128 is currently in early phase I, dose-finding clinical trials for adult malignancies.

SEGAs, found nearly exclusively among children with tuberous sclerosis, essentially always have activation of the AKT/mTOR pathway due to germline mutations in the *TSC1* or *TSC2* genes. Clinical trials have demonstrated that the mTOR inhibitors, *Sirolimus* (Pfizer) and *Everolimus* (Novartis Pharmaceuticals) have demonstrated activity against SEGAs [72, 117]. As of result of these studies, Everolimus has been approved by the FDA for the treatment of SEGAs not amenable to surgical resection. In addition, mTOR signaling may have a role in the biology of pediatric low-grade gliomas, especially among children with NF1 [118]. A report of a phase I/II study of Sirolimus and Erlotinib (Genentech) examined 16 patients with recurrent pediatric low-grade astrocytomas [119]. Of the seven children with NF1 in this clinical trial, all patients had either stable disease or tumor responses. Kieran and colleagues recently reported 23 patients with low-grade gliomas (median age: 9 years; range, 3–17 years) who were treated with single agent Everolimus after tumor progression following prior treatment with a carboplatin-containing chemotherapy regimen. Four of 23 patients had a partial response (>50 % decrease in tumor size) and 13 additional patients had stable disease. Therapy was generally well tolerated; two patients discontinued therapy due to mouth sores ($n=1$) and withdrawal of consent ($n=1$) [120].

As described above, recent advances in the understanding of PLGA biology suggest that pharmacologic targeting of these pathways will yield new therapies for PLGAs in the near- to intermediate-term future. However, multiple interactions and feedback loops exist between the MAP Kinase Pathway and the mTOR pathway which may explain the lack of clinical effect of single agent BRAF targeting. However, combination therapies with multiple signal transduction inhibitors or signal transduction inhibitors targeting of both the MAP kinase and mTOR pathways and conventional cytotoxic agents may yield antitumor activity [121, 122].

IDH1/2 Mutation Inhibition

Specific mutations in the isocitrate dehydrogenase genes IDH1 and IDH2 (IDH1/2) are often found in several adult brain tumors including low-grade (WHO grade II) diffuse gliomas, oligodendrogliomas, and nearly all cases of secondary glioblastomas which develop from lower grade gliomas [83, 123–125]. IDH1/2 mutations are also found in approximately 15 % of adults with acute myelogenous leukemia [126]. IDH1/2 mutations are rarely found in pediatric gliomas, with the exception being that IDH1 mutations are often found among children older than 14 years old with high-grade gliomas [4]. Although there is some conflicting data, reports of adults with low-grade gliomas whose tumors had an IDH1 and IDH2 mutations had longer survival [83, 127–129].

Investigators are examining strategies to inhibit mutant IDH1 activity and slow growth of IDH1-mutant gliomas [130, 131]. As one example, glutaminase is necessary for generation of α-KG from glutamine. Inhibition of glutaminase by either siRNA or the small molecule inhibitor, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) slowed growth of glioblastoma cells expressing mutant IDH1 compared with those expressing wild-type IDH1 [132]. AG-221 (Agios Pharmaceuticals) is an IDH2 inhibitor that is currently in early clinical trials for adults with IDH2 mutant-acute myelogenous leukemia. However, despite recognized potential for therapeutic strategies of IDH1 and IDH2 inhibitors, there are as yet no clinical trials of IDH1 or IDH2 inhibitors against low-grade gliomas.

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5 Ependymoma

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Ependymomas constitute about 10 % of pediatric brain tumors, with an annual incidence of 0.3/100,000 children. Median age at diagnosis is 6 years and the male to female ratio is 1.2/1. Ependymomas are thought to arise from radial glia cells in the subventricular zone of the brain [1], and 2/3 of all ependymomas are located in the fourth ventricle [2]. Thus, clinical symptoms are often caused by obstruction of the ventricular system, and patients frequently present with headache, nausea, and vomiting. In addition, cerebellar ataxia or weakness of the abducens nerve can be present. Other common tumor locations include the supratentorial ventricular system, brain stem, and spinal canal.

Ependymomas pose a major challenge in pediatric oncology due to their large clinical and biological heterogeneity, as well as their limited sensitivity towards classical chemotherapy. The treatment of ependymomas therefore has rested largely on surgery and radiation therapy, with limited treatment options for patients with recurrent disease. In recent years, however, significant progress has been made in our understanding of the tumor genetics and biology of ependymoma. Based on molecular genetic information, it has become clear that histologically indistinguishable tumors can differ fundamentally in terms of disease biology. Although most of this new knowledge has yet to be translated successfully into clinical practice, the coming years promise to become a period of progress by incorporating disease biology into diagnostic and therapeutic algorithms. It is expected that further insights into tumor biology continue to be gained by rapidly evolving experimental technologies, such as next-generation sequencing and epigenetic profiling (DNA methylation, histone modifications, miRNA profiling), as well as improved preclinical models. We anticipate that these advances will improve risk stratification of newly diagnosed patients and allow for the development of novel, risk-adapted treatment protocols. Furthermore, the incorporation of molecular targeted therapies holds promise for future therapies that are less toxic and/or more effective than current standard approaches.

The following chapter will therefore attempt to summarize our current body of knowledge about the molecular genetics and biology of ependymomas, its application to clinically relevant risk stratification of patients, and the opportunities for developing novel molecular targeted therapies.

Histopathology

Macroscopically, ependymomas appear as well-circumscribed tumors. Histological features typically seen in ependymomas include perivascular pseudorosettes composed of glial tumor cells that are radially arranged around blood vessels, and true ependymal rosettes of tumor cells that form a central lumen. Perivascular pseudorosettes occur in the great majority of these neoplasms, whereas true ependymal rosettes are only present in a minority of tumors. Notably, these features can be found in ependymomas across all molecular subtypes. Regressive changes include areas of myxoid degeneration and calcifications. The current World Health Organization (WHO) classification [3] recognizes two histological grades of ependymomas: grade II ("classic" ependymoma) and III (anaplastic ependymoma).

The following histopathological variants of *WHO grade II ependymomas* can be distinguished [3]: (1) *Cellular ependymoma* shows conspicuous cellularity without a significant increase in mitotic activity. (2) *Papillary ependymoma* shows well-formed papillae in which tumor vessels are covered by a layer of tumor cells. (3) *Clear cell ependymoma* displays an oligodendroglia-like appearance with a perinuclear halo. These tumors appear to be preferentially located in the cerebral hemispheres and frequently progress to high-grade ependymomas. (4) Tanycytic ependymoma consists of cells which are arranged in the fascicles with variable width and cell density. These tumors are more frequent in the spinal cord. In addition, rare ependymoma variants including lipomatous ependymoma, giant cell ependymoma, melanotic ependymoma, signet ring cell ependymomas, and

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ovarian ependymoma have been described. Occasional non-palisading tumor necrosis may be observed, and is compatible with ependymoma WHO grade II. Immunohistochemistry of GFAP is usually applied for routine diagnostics of ependymal tumors, together with the EMA antibody that typically reveals dot-like immunostaining with a predominant localization along the luminal surface of ependymal rosettes.

Anaplastic ependymomas (WHO grade III) tend to remain as well-demarcated lesions, but are sometimes frankly invasive. Occasionally, extraventricular location with extensive infiltration of white matter can be noted. Microscopically, these tumors include highly cellular and poorly differentiated areas with rare pseudorosettes, brisk mitotic activity and frequent microvascular proliferation and necroses with palisading cells. However, histological classification of ependymomas into WHO grades II and III can be challenging, and even experienced neuropathologists commonly differ in their grading [4].

Cytogenetics and Molecular Genetics

Although our knowledge of tumorigenesis, biology, and progression of ependymoma has advanced significantly during the past 10 years, we are still in the early stages of translating this knowledge into clinical research and practice. Several candidate oncogenes and tumor suppressor genes have emerged as potential therapeutic targets and a novel molecular staging system was recently proposed with the potential to improve future stratification of ependymoma patients in clinical studies [5].

Germline mutations of the tumor suppressor gene *NF2* on chromosome 22q are associated with a variety of central nervous system (CNS) tumors, including ependymomas, schwannomas, and meningiomas. In sporadic ependymoma, however, *NF2* mutations appear to be restricted to a subset of spinal ependymomas in adult patients [6].

Early cytogenetic and comparative genomic hybridization studies provided the first evidence that ependymoma represents a biologically heterogeneous group of diseases [7, 8]. These initial studies, however, were limited by low genomic resolution, limited numbers of tumor samples from different CNS locations, and lack of detailed clinical and patient outcome data. In contrast, more recent studies using larger sample sizes and at higher resolutions have identified genetic signatures that could readily distinguish ependymomas arising in different anatomic localizations [1].

The most frequent genetic abnormalities in primary pediatric ependymoma involve gains of chromosome 1q, 5, 7, 9, 11, 18, and 20 and of losses chromosome 1p, 3, 6q, 6, 9p, 13q, 17, and 22. Several groups reported chromosome gain at 1q with an incidence of approximately 25 % to be the most frequently detected aberration in childhood ependymoma. Notably, gain of chromosome 1q has also been identified as the most consistent biomarker being associated with poor outcome and fossa posterior location in independent studies [6, 7]. These findings suggest that chromosome 1q may host candidate genes involved in ependymoma tumorigenesis and/or progression. Potential driver oncogenes located on chromosome 1q, especially within the hotspot region 1q21–32, include *DUSP12* (1q23.3), *S100A10* (1q21), *CHI3LI* (1q32.1), *TPR*, *SHC1*, *JTB*, and *HSPA6* (1q32) [9].

Loss of chromosome 22, especially complete or partial monosomy of chromosome 22, was reported as one of the most common aberrations in sporadic ependymomas. Aside from *NF2*, reduced expression of other candidate genes contained in the minimally deleted region at chromosome 22q has been observed, including *SULTA4*, a gene widely expressed in several compartments of the human brain, as well as *CBX7*, *G22P1*, and *MCM5*, which may be involved in cellular DNA repair and/or replication [8, 10].

Another recurrent finding in pediatric intracranial ependymoma is loss of chromosome 6q, which has been linked to an increased risk of recurrence. In particular, deletion of chromosome 6q23 has been associated with poor progression-free survival. Several genes located within this region were found to be downregulated in tumors with a heterozygous deletion, including *SASH1*, *TCP1*, *ADM1*, and *CDK11* [9].

Recently, chromosome 9q33–34 was identified as one of the most frequently gained regions (up to 36 % of patients), mainly occurring in posterior fossa tumors arising in children. The prognostic value of 9q gain remains controversial, since one study showed an association with increased risk of relapse, whereas a more recent one found this aberration to define a lower-risk group [5, 11]. Since different markers were used to identify 9q gains in these studies, one likely explanation for these differing results is that the precise genomic location is of major importance. Nevertheless, the biological relevance of this genomic region is supported by the fact that it harbors two oncogenes, namely *NOTCH1* and *TNC* which had previously been linked to brain tumorigenesis [11]. The *TNC* gene was shown to be upregulated in infant ependymomas, and overexpression was associated with a short time to relapse and poor prognosis [11, 12]. In addition, Notch pathway members, including receptors (Notch1 and Notch2), ligands (JAG1, DLL1, and DLL2), and downstream targets (HES1, HEY2, and MYC), were observed to be consistently overexpressed in ependymoma [11, 13]. The first hint towards involvement of Notch signaling came from a report by Taylor et al., in which activation of Notch signaling was observed in both supratentorial and spinal ependymomas [1].

Homozygous deletion of *CDKN2A*/*p16*$^{\mathrm{INK4a}}$ has repeatedly been detected in supratentorial ependymomas [1, 7, 14]. *CDKN2A*/*p16*$^{\mathrm{INK4a}}$, a tumor suppressor gene located at 9p21.3, regulates neural stem cell (NSC) proliferation, and its deletion has been shown to rapidly expand progenitor cell numbers in developing neural tissue.

Although we were able to demonstrate stepwise accumulation of genetic aberrations during disease progression for

the first time in a case with anaplastic ependymoma [15], much work remains to be done to define the molecular changes that underlie disease recurrence and progression in ependymoma.

Gene Expression Profiling

A decade ago, the first studies were published to reveal distinct gene expression patterns separating subgroups of ependymoma [8]. Supporting this initial finding, a comprehensive picture of tumor heterogeneity associated with disease localization has emerged on a transcriptional and cytogenetic levels [1, 13, 16–18]. Recently, two distinct variants of posterior fossa ependymomas were identified by gene expression profiling, defined as Group A (Group 1 in [16]) and Group B (Group 2 in [16]) [18]. Group A tumors were associated with very poor outcomes, recurred at significantly higher rates, and developed metastases in more than 80 % of cases. Patients diagnosed with this disease variant were younger on average and their tumors tended to be located laterally within the posterior fossa. Approximately half of Group A patients developed a relapse of their disease, which notably was independent of the extent of surgical resection. From the genomic point of view, it was somewhat surprising to find that the genomes of these aggressive Group A tumors were without large cytogenetic alterations. This variant of ependymomas, however, comprised activation of classic cancer-related signaling pathways, such as EGFR, PDGF, RAS, ECM, VEGF, MAPK, and integrins. Strikingly, Group B ependymomas showed a highly disparate molecular profile, featuring large chromosomal aberrations, partially affecting whole p- or q-arms of a chromosome or the entire chromosome. Transcriptome profiling of Group B ependymomas showed highly specific overexpression of genes involved in ciliogenesis and microtubule assembly, as well as mitochondrial metabolism.

The existence of two biologically distinct variants of posterior fossa ependymomas was confirmed in a subsequent study [16]. Wani and colleagues observed overexpression of genes associated with mesenchyme in Group 1 tumors, as well as an association with younger age and reduced recurrence-free survival, similar to the findings by Witt and colleagues in Group A ependymomas [18]. Comparable to Group B tumors [18], Group 2 tumors were associated with an excellent prognosis, tended to occur in adolescent children and young adults, and did not express genes associated with altered gene ontology terms in their transcriptomes [16]. In addition, Wani et al. were able to define and validate a 10-gene signature to reliably classify posterior fossa ependymomas into the two groups. This gene signature, which can be obtained from small amounts of routine formalin-fixed, paraffin-embedded tissue, is of major interest as a clinically feasible approach for patient stratification in future clinical trials.

Another study by Johnson and colleagues performed a gene expression analysis of 83 ependymomas, including both supra- and infratentorial locations [17]. They were able to identify nine molecular subgroups in total, although their clinical relevance was uncertain, as detailed patient information and outcome data was unavailable [17]. The following molecular subgroups related to localization were described: four supratentorial subgroups (A–D), two subgroups of posterior fossa ependymomas including some spinal tumors (E, F), and three subgroups consisting of tumors of posterior fossa localization only (G, H, I) [17]. Integrating these findings of two variants of posterior fossa tumors, the distribution would be as follows: one variant corresponds to Group A by Witt et al., Group 1 by Wani et al., and Cluster G, H, and I by Johnson et al.; the other variant corresponds to Group B by Witt et al., Group 2 by Wani et al., and Cluster E and F by Johnson et al. (Fig. 5.1).

In conclusion, several independent studies have confirmed the presence of at least two, genetically and biologically, different variants of posterior fossa ependymoma. At present, the standard treatment for patients with posterior fossa ependymomas remains maximal safe surgical resection followed by adjuvant radiation therapy. The role of additional adjuvant chemotherapy is being investigated in an ongoing phase III clinical trial by the Children's Oncology Group (COG), ACNS0831, which includes planned post hoc molecular subgroup analysis. Future studies will be needed to investigate experimental, intensified treatment regimens in prospectively selected high-risk patients.

Subgroup-specific preclinical models are being developed [17, 19] and are expected to help inform the rational selection of novel therapies for testing in future clinical trials.

Prognostic Stratification

The two most widely accepted factors used for patient stratification are extent of resection, metastatic status, and WHO grading. WHO grading, as an important, independent prognostic marker has been described early on [20], and has recently been confirmed by a large meta-analysis investigating 2408 ependymoma patients [21], whereas other studies have suggested that tumor grading is highly dependent upon the experience of individual neuropathologists [22, 23]. Regarding molecular markers, deletion of *CDKN2A* along with 1q gain was identified as the strongest indicator of poor prognosis in a cohort of 292 intracranial ependymomas [5]. The same study was able to identify reliable cytogenetic markers for standard, intermediate, and high-risk ependymoma, comprising the first molecular staging system for ependymoma that could be validated in a completely non-overlapping patient cohort [5]. This cytogenetic risk stratification model for intracranial ependymoma comprises three cytogenetic subgroups. Group 1 is associated with standard risk, with tumors displaying large aberrations of chromosomes

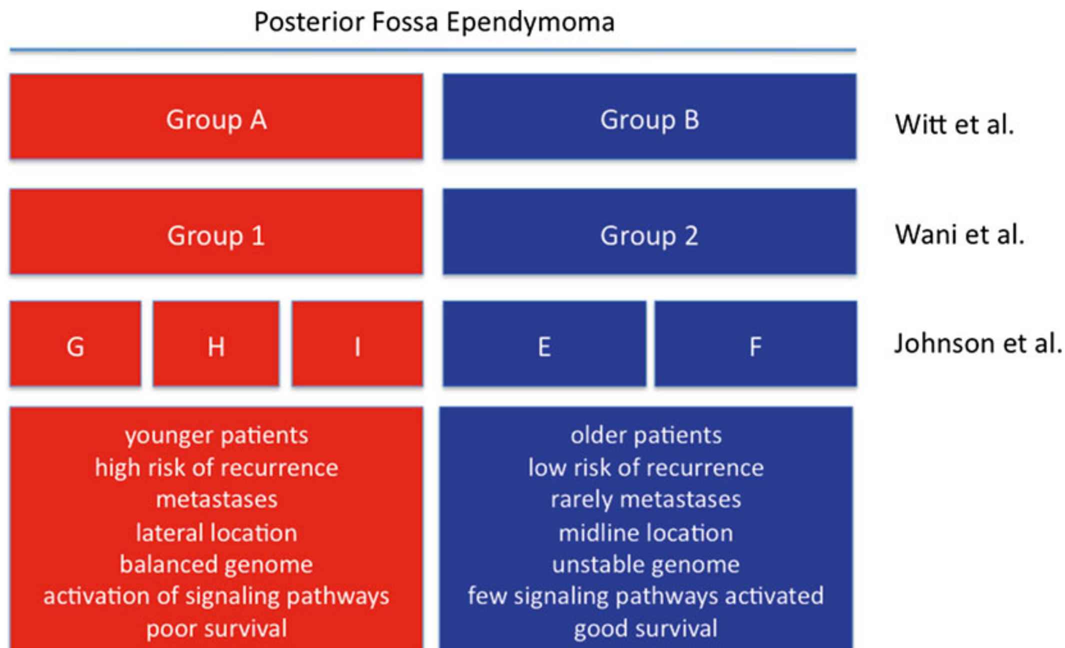

Fig. 5.1. Molecular subgroups of posterior fossa ependymoma, as described by Witt et al., Wani et al., and Johnson et al. Group A, Group 1, and clusters G, H, and I describe to same biological subgroup (*red*), as do Group B, Group 2, and clusters E and F (*blue*). Both subgroups differ significantly in their molecular and clinical variables.

6, 9, 15, and 18. Group 2 is associated with intermediate risk, and tumors show a balanced genome. Group 3 is associated with high risk, and defined by 1q gain and/or homozygous deletion of CDKN2A/B [5]. Gain of 1q25 as a negative prognostic marker has since been confirmed in three independent clinical cohorts (CCLG/SIOP, BBSFOP, and SIOP) [24]. A separate study confirmed gain of 1q as a negative prognostic marker in posterior fossa ependymoma [25].

In conclusion, copy number gain of chromosome 1q is the most widely published negative prognostic molecular marker and applies to both supra- and infratentorial ependymoma [5, 7, 18, 24–28].

Other prognostic markers that are based on immunohistochemistry, rather than cytogenetics, have also been identified. Tenascin C is an extracellular matrix protein and has been shown to be a negative prognostic marker in ependymoma [11, 12, 18]. The NSC marker Nestin is a negative prognostic marker identifying ependymoma with poor prognosis especially in WHO II tumors [29], possibly indicating a less favorable, undifferentiated phenotype. Conversely, expression of neurofilament light polypeptide 70 (encoded by *NEFL*) is a positive predictive marker in supratentorial ependymoma [30] and may indicate a more favorable, differentiated phenotype. The immunohistochemical markers LAMA2 and NELL2 delineate the two molecular subgroups in posterior fossa ependymoma described above: Group A tumors (with poor prognosis) are characterized by the pattern LAMA2 positive and NELL2 negative, and Group B tumors (with more favorable prognosis) by the pattern LAMA2 negative and NELL2 positive [18]. The delineation of two molecular subgroups by the expression of LAMA2 and NELL2, and their prognostic values, have since been confirmed in a separate study [16].

Finally, miRNAs associated with prognosis have been described in ependymoma: let-7d, miR-596, and miR-367 are associated with poor survival, and miR-203 is an independent predictor for time to relapse [31].

Molecular Signaling Pathways

Identification of molecular signaling pathways that can be targeted for therapeutic purposes will be crucial for the rational development of novel drug-based treatments. It is important to note that thorough characterization of molecular subgroups and establishment of faithful subgroup-specific models will be needed for successful preclinical testing. It has become evident that different molecular subgroups of posterior fossa ependymoma show distinct activation of molecular signaling pathways (Fig. 5.2): Group A shows activation of epidermal growth factor receptor (EGFR), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and mitogen-activated protein kinase (MAPK) among others [18]. Group B shows less activation of classic oncogenic signaling pathways; gene expression profiles, however, indicate activation of ciliogenesis, microtubule assembly and mitochondrial metabolism [18]. These promising findings display novel treatment opportunities in a

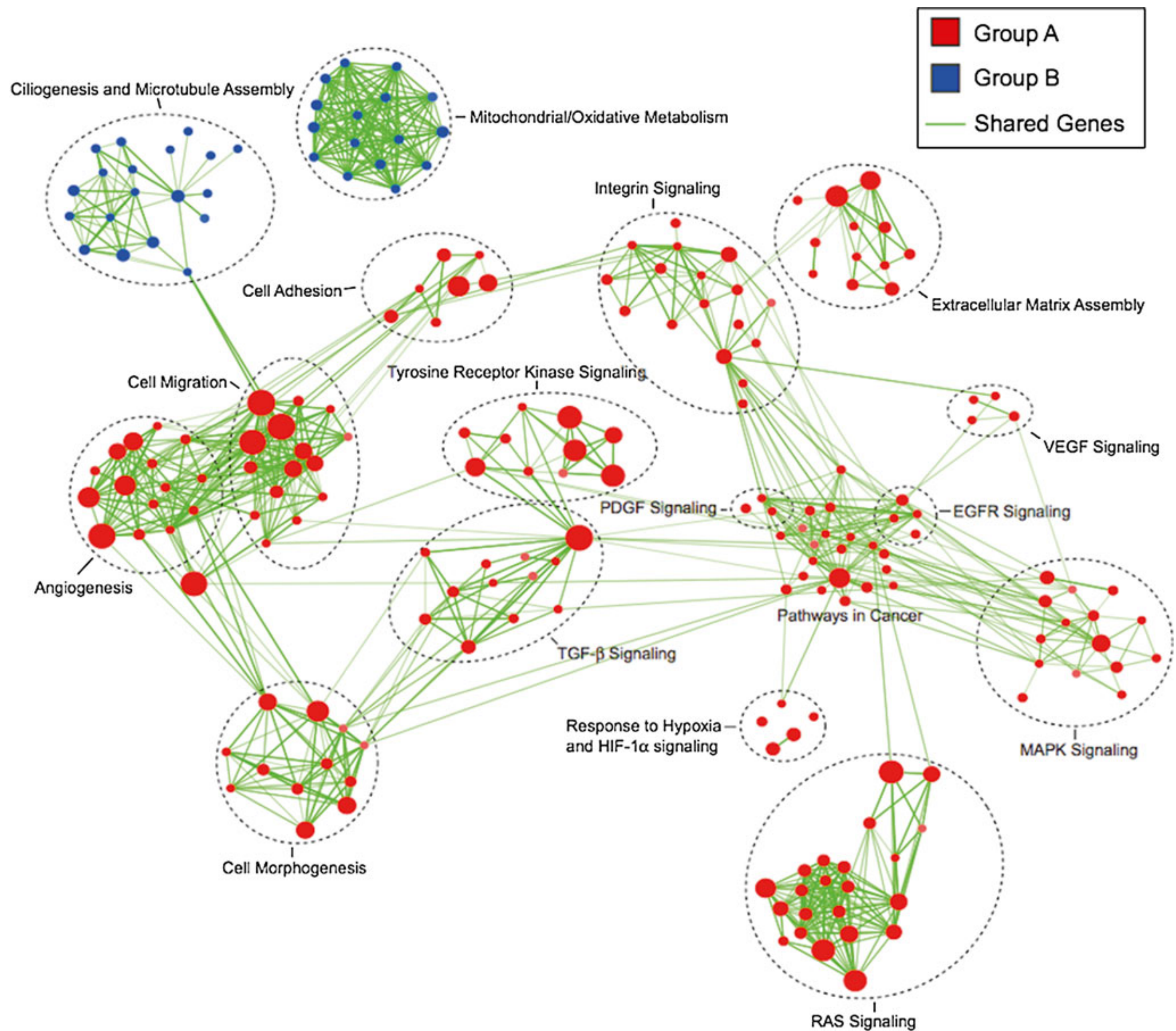

Fig. 5.2. Group A (*red*) and Group B (*blue*) posterior fossa ependymomas show distinct activation of signaling pathways and biological functions. The map was created by geneset enrichment analysis of transcriptomes of the two molecular subgroups of posterior fossa ependymomas, using Cytoscape and Enrichment Map (Adapted from Witt H, Mack SC, Ryzhova M, Bender S, Sill M, Isserlin R, et al. Delineation of two clinically and molecularly distinct subgroups of posterior fossa ependymoma. Cancer Cell. 2011;20:143-57, with permission).

fashion of subgroup-specific targeted therapies. A large number of therapeutic drugs inhibiting these molecular pathways (e.g. MAPK-, EGFR-, PDGF-, VEGF- and integrin-inhibitors) are already approved for other cancers and/or in various stages of clinical development, including those for pediatric patients. Carefully designed clinical studies will be needed to assess the potential of these agents to complement current standard therapies (surgery, radiotherapy) and/or other investigational therapies, such as chemotherapy. Of note, an integrated in vivo high-throughput drug screen using the preclinical supratentorial subgroup D-specific model [19] recently showed that the most active compounds against ependymoma which showed the least toxicity on NSCs were 5-FU and bortezomib, a proteasome inhibitor [19].

Other signaling pathways implicated in ependymoma biology are the Notch pathway, p53 and ERBB (EGFR and ERBB2/3/4), among others. Notch activation is associated with ependymoma progression [11], and inhibition of the Notch pathway using gamma-secretase inhibitors reduces neurosphere formation in vitro [11]. Aberrant expression of p53 was identified as an unfavorable prognostic marker in ependymoma, whereas its regulator MDM2 did not show an association with prognosis [12, 32]. The molecular mechanism of p53 pathway dysregulation in ependymoma is not

yet understood. Despite frequent p53 overexpression in ependymoma, known mechanisms such as *TP53* mutation or promoter hypermethylation, MDM2 overexpression, P14^{ARF} promoter hypermethylation or increased PAX5 expression are not observed in ependymoma. Furthermore, some studies implied the RTK1 family of proteins including EGFR, ERBB2, ERBB3, and ERBB4, in ependymoma biology, promoting growth, motility, and survival of ependymoma cells, whereas ERBB2 overexpression may potentiate radial glia proliferation [14]. In two studies, EGFR expression was found to be associated with an increased risk of disease recurrence [7, 12].

Molecular Targeted Therapies

Radical surgical resection, whenever feasible, remains the mainstay of ependymoma treatment and may be sufficient in a subset of patients with supratentorial ependymomas [33]. Because of high rates of local recurrence without additional therapy, adjuvant involved-field radiation therapy is generally employed in current standard treatment protocols. Nevertheless, local or distant disease recurrence is common, including for approximately half of all patients with posterior fossa ependymoma. In case of metastatic dissemination at diagnosis, craniospinal radiation therapy is typically used. The role of chemotherapy in the treatment of ependymoma is not well established, and response rates to single agent or combination chemotherapy in recurrent ependymoma are disappointing [34]. It has been shown, however, that chemotherapy can be effectively used to delay the beginning of radiotherapy in very young children, without compromising their prognosis [35]. Recent and ongoing clinical trials are examining the role of neoadjuvant chemotherapy in unresectable or disseminated disease, as well as in the adjuvant setting post radiation therapy for high-risk patients [36]. Due to the generally limited efficacy of classical chemotherapy in disseminated and recurrent ependymoma, however, novel therapies are urgently needed.

Based on the discovery of molecular signaling pathways relevant to ependymoma biology, several targeted therapy approaches are currently in development, including chromatin-modifying drugs. Early phase clinical trials are currently investigating compounds targeting Notch, EGFR, HDACs, and ERBB among others, as single agents or in combination with chemotherapy, in children with ependymoma.

Targeting the Notch pathway in a phase I clinical trial by using the gamma-secretase inhibitor MK-0752 showed that the MK-0752 is well tolerated in children, and response was seen in one ependymoma and one glioblastoma patient [37]. Promising preclinical data on EGFR inhibitors in ependymoma show success alone or in combination with phosphoinositide 3-kinase inhibitors [38, 39]. In vitro, ependymoma cells are sensitive to HDAC inhibitor (HDACi) treatment, which induced differentiation in subgroup C ependymoma cells [40] and increased apoptosis in others [41]. As a result, HDACis, such as vorinostat (suberoylanilide hydroxamic acid, SAHA), are being investigated in recent and ongoing clinical studies including patients with ependymoma [42, 43].

For both traditional chemotherapy and targeted agents, however, sufficient drug penetration into CNS tumor tissue and efficient target inhibition in vivo represent formidable challenges for successful translation of preclinical discoveries into effective clinical therapy. For example, a recent clinical and molecular biology study using the EGFR/ERBB2 inhibitor lapatinib in children with refractory brain tumors revealed that the drug failed to achieve meaningful concentration in the tumor tissues and, as a result, failed to inhibit the molecular targets [44].

Recently, two studies highlighted genetic and epigenetic alterations as therapeutic targets of different subtypes of ependymoma.

Posterior fossa ependymoma harbor nonrecurrent somatic mutations in a cohort of 47 tumors using whole-exome and whole-genome sequencing technologies [45]. Notably, a very low mutation rate was found in these tumors regardless of subgroups, with an average of only five somatic mutations per tumor (4.6 and 5.6 somatic mutations in Group A and Group B ependymomas, respectively). In contrast, DNA methylation patterns were highly dissimilar between both subtypes. When comparing only PF ependymoma subtypes, Group A ependymomas display a much higher proportion of methylated CpG-islands within the promoter regions as compared to Group B ependymomas. Based on this distinct pattern of epigenetic alteration, Group A tumors show a CpG-island methylator phenotype (CIMP). Additionally, Group A/CIMP-positive tumors show a greater extent of epigenetic silencing of targets of the polycomb repressive complex 2, including downregulation of differentiation genes through histone H3-lysine 27 (H3K27) trimethylation. To investigate if epigenetic agents can be used as potential novel treatment option for Group A tumors, in vitro and in vivo tests were performed. The preclinical treatment approaches using either 5-aza-2′-deoxycytidine, 3-deazaneplanocin A, or GSK343 (a selective inhibitor of the H3K27 methyltransferase EZH2) have shown very good response of cells and mice bearing Group A tumors. These results are promising treatment strategies targeting DNA CpG methylation, PRC2/EZH2, and/or histone deacetylases of this chemotherapy-resistant disease.

Another study, using whole-genome sequencing and/or RNA sequencing of 77 ependymomas, identified a novel gene fusion affecting *RELA* and *C11orf95* [46]. In line with findings of the study by Mack and colleagues, no recurrent somatic mutations were detected in posterior fossa ependymomas, including Group A and Group B. Notably, among supratentorial ependymomas Parker and colleagues discovered a frequent translocation within a region of chromosome 11q, which is possibly caused by chromotripsis (a recently discovered phenomenon of genomic rearrangement arising

during a single genome-shattering event) and resulted in a *C11orf95-RELA* gene fusion in about 70 % of cases. RELA is a downstream target of the NF-κB signaling pathway, acting as a transcription factor and regulating several biological actions of cell maintenance. Importantly, a genetically engineered mouse model was successfully developed based of the *C11orf95-RELA* gene fusion. NSC from a Ink4a/Arf-null background were transduced with the retroviruses carrying the *C11orf95-RELA* fusion. These transgenic NSCs were then implanted into the cerebrum and developed supratentorial ependymomas within a few days. Hence, this model delivers excellent opportunities for preclinical drug testing in vivo of a supratentorial subtype of ependymomas.

Summary

As has been shown, genomic and gene expression profiling in ependymomas not only identifies biologically distinct subgroups but also allows for the stratification of patients into clinically meaningful prognostic subgroups. As demonstrated by the delineation of Group A and B posterior fossa ependymomas, the tight association between molecular profile and clinical behavior is of high practical relevance for the individual patient. One simple consequence of the identification of a Group A vs. Group B tumor for the patient is the new possibility of truly risk-adapted adjuvant treatment of previously equally treated tumors. Thus, the identification of the molecular profile adds considerable additional information to the classical histopathological analysis, enabling better informed clinical decisions.

The first steps toward better tumor diagnostics and disease stratification have been completed on the molecular level, now the key to successful translation into the clinic lies in (1) faithful preclinical models, (2) appropriate patient selection, and (3) careful consideration of pharmacological issues in brain tumors.

The strong heterogeneity of the tumor biology between different ependymoma subgroups, such as Group A and B, implies that the therapeutic treatment of each subgroup needs to be addressed individually. As has been shown in subgroup specific mouse models, new drugs can be validated and "old" drugs rediscovered for a very specific subset of ependymomas. Thorough characterization of preclinical models and their molecular subgroup therefore has to be a prerequisite for preclinical studies in order to yield results that can be translated into the clinic. Accordingly, the appropriate patient selection is of paramount importance for the success of future clinical trials. Not only do the trial design and therapy need to be tailored to the molecular ependymoma subgroups, but individual patients' molecular subgroup and targets will need to be confirmed reliably and in real-time. Future clinical studies should therefore include thorough molecular characterization of the tumor to be treated. Finally, pharmacological issues such as clinically achievable concentrations of the drug of interest, as well as the blood brain barrier (BBB) need to be taken into account for clinical trial design, and confirmation of successful target inhibition in the tumor tissue itself would be highly desirable as part of trials exploring novel, molecular targeted drugs.

The advent of high-throughput molecular analyses such as whole genome sequencing or genome-wide methylome analysis at affordable prices will undoubtedly allow for rapid and comprehensive molecular characterization of individual patients' tumors not only for research, but also in routine clinical practice. The patients will benefit from these insights if we can succeed in the translation of the molecular knowledge into novel and more effective individual treatment strategies.

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6 Adult High-Grade (Diffuse) Glioma

Katharine McNeill, Kenneth Aldape, and Howard A. Fine

The central brain tumor registry of the United States (CBTRUS) estimates that there will be about 70,000 primary CNS tumor diagnosed in 2013. Of these, about 20 % will be malignant gliomas [1]. Glioblastomas (WHO grade IV, formerly termed glioblastoma multiforme or GBM) are the most common malignant primary brain tumors, with an incidence of about 3–4 per 100,000 [2]. Peak incidence for malignant glioma is in the fifth or sixth decade of life, and there is a slight male preponderance (1.1–1.3:1) [2]. Incidence and median age of diagnosis by histology is summarized in Table 6.1.

While traditionally high-grade "malignant" gliomas (grades III and IV) are distinguished from low-grade gliomas (grades I and II), this distinction does not correspond with the known biology of these tumors and is therefore probably outdated. Grade I gliomas are usually circumscribed, with a very low propensity for malignant transformation, and, while not the subject of this chapter, occur predominantly in children and have a molecular pathogenesis that is unrelated to diffuse glioma. In contrast, the diffusely infiltrating gliomas (grades II–IV) are prone to tumor recurrence and progression to higher grades, and their clinical behavior is malignant to varying degrees. As an added complexity, while the histologic diagnosis of glioblastoma (grade IV) is relatively straightforward, the histopathologic distinction of grade II from grade III glioma is ill-defined and subject to considerable inter-observer variability. In addition, the molecular genetics of grade II and III gliomas largely overlap, arguing that these are best considered within a spectrum of a single disease entity. Median survival varies with histologic diagnosis, and ranges from 5 to 7 years for diffuse (grade II) astrocytoma, 3–5 years for anaplastic astrocytoma [3] to 15–16 months for glioblastoma [4]. One-, 2-, 5-, and 10-year survival by histologic diagnosis are summarized in Table 6.2. There is some evidence that average survival times are improving modestly. Median survival for patients with glioblastoma treated in a large randomized trial that defined our current standard of care was 15 months [4], while outcomes in patients treated on clinical trials in the 5 years after that study was published was 20 months [5]. Apart from a possible element of patient selection bias, reasons may include improved treatments at the time of disease recurrence or an improvement in the standard of clinical and supportive patient care over time.

Standard treatment of glioblastoma includes maximal safe resection, involved field radiation, and concomitant and adjuvant temozolomide. Large retrospective studies have shown that patients who receive a more extensive resection, defined as 78–98 % of contrast enhancing tumor, have improved survival compared to patients who receive a subtotal resection or biopsy [6, 7], so extensive resection is warranted when feasible. The benefit from radiotherapy was defined by randomized trials, which showed a significant improvement in outcomes with radiotherapy compared to chemotherapy alone or best conventional care [8, 9]. A series of randomized studies established the standard dosing and fractionation of 60 Gy in 30 daily fractions [10–17].

A large randomized study defined the role of adjuvant chemotherapy with temozolomide, and found that patients who received concomitant and adjuvant temozolomide had a significant improvement in median survival compared to patients treated with radiation alone (12.1 vs. 14.6 months) [4] The proportion of patients surviving 5-years after diagnosis was five times higher in the temozolomide group (9.8 % vs. 1.9 %) [18]. This "Stupp protocol" has become the standard of care for initial management of glioblastoma. More recently, non-randomized data suggest that bevacizumab may improve outcomes after disease recurrence, with median progression-free survival of 4–6 months and median overall survival of 8–9 months [19, 20], which compared favorably to historical controls [21, 22].

Prospective, randomized trial data defining the utility of these modalities in anaplastic gliomas (WHO grade III) is lacking. These tumors are more heterogeneous in terms of

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TABLE 6.1. CBTRUS estimates of the number and age-adjusted incidence rates of malignant glial tumors, 2005–2009.

Histology	*N*	% of all tumors	Median age	Rate (95 % CI)
Glioblastoma	49,088	15.8	64	3.19 (3.16–3.22)
Anaplastic astrocytoma	5,374	1.7	54	0.36 (0.35–0.37)
Anaplastic oligodendroglioma	1,687	0.5	49	0.11 (0.11–0.12)
Glioma, malignant, NOS	6,574	2.1	40	0.45 (0.44–0.46)

From Dolecek TA, Propp JM, Stroup NE, Kruchko C: CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. *Neuro Oncol* 2012, 14 Suppl 5:v1-49, with permission

TABLE 6.2. CBTRUS estimates of 1-, 2-, 5-, and 10-year relative survival rates, 1995–2009.

Histology	1-year	2-years	5-years	10-year
Glioblastoma (%)	35.7	13.6	4.7	2.3
Anaplastic astrocytoma (%)	60.1	41.5	25.9	17.6
Anaplastic oligodendroglioma (%)	81.0	66.9	49.4	34.2
Glioma, malignant, NOS	61.9 %	50.4 %	43.3	38.3 %

From Dolecek TA, Propp JM, Stroup NE, Kruchko C: CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. *Neuro Oncol* 2012, 14 Suppl 5:v1-49, with permission

their behavior, genetics, and response to therapy compared to glioblastomas, so the most appropriate up-front treatment has not been established, and may vary depending on the histologic and genetic subtype. Early clinical trials that defined the benefit from radiotherapy included patients with grade III tumors, and based on those data it is generally accepted that radiotherapy improves outcomes in anaplastic glioma, although the number of patients with grade III tumors on those trials was too small to allow a statistically robust subgroup analysis [8, 9]. Most physicians treat patients with anaplastic astrocytoma with radiation and temozolomide per the Stupp protocol [23], and there are retrospective data that suggests a benefit of chemoradiotherapy over radiotherapy alone in patients whose tumors do not harbor a 1p/19q co-deletion [24]. Recent data indicate that in anaplastic oligodendrogliomas, addition of chemotherapy to radiotherapy also benefits patients with non-co-deleted, isocitrate dehydrogenase 1 (IDH1) mutant tumors [25]. The benefit of adjuvant temozolomide, however, has not been confirmed in prospective trials [23, 25–27]. The prospective data that do exist used a more toxic regimen of procarbazine, lomustine, and vincristine, so whether patients with anaplastic tumors benefit from adjuvant temozolomide is still an open question, and large randomized trials in patients with co-deleted [28] and non-co-deleted tumors [29] are ongoing. In order to circumvent the risk of radiation-induced neurocognitive deficits, there is a growing interest in treating selected patients with chemosensitive tumors (e.g., those with 1p/19q co-deletion) with chemotherapy alone based on retrospective [24] and prospective [30] data suggesting outcomes similar to radiation alone.

After more than three decades of clinical trials, it is clear that there is significant heterogeneity in the biology and behavior of these tumors and their response to treatment. A better understanding of the histopathologic, genetic, and epigenetic changes that underlie tumor biology will allow for more tailored treatment of these heterogeneous tumors, and this will be the subject of the rest of this review.

Histopathology

Diffuse gliomas are infiltrative glial tumors characterized by increased cellularity, nuclear atypia, and mitotic activity. They are subclassified according to their cellular morphology as either astrocytic, oligodendroglial, or mixed gliomas.

Astrocytomas are composed of cells with elongated or irregular hyperchromatic nuclei and scant cytoplasm. Cell processes form a loose fibrillary matrix and glial fibrillary acidic protein (GFAP) staining highlights both the cytoplasm and cell processes. Proliferative index, as measured by Ki-67 or MIB-1, is generally between 5 and 10 % but is highly variable. Oligodendrogliomas also exhibit GFAP immunoreactivity, but morphologically the cells have rounded hyperchromatic nuclei, perinuclear halos, and few cellular processes. They have a characteristic branching capillary pattern and focal microcalcifications are common (Fig. 6.1). Oligoastrocytomas display features intermediate between astrocytoma and oligodendroglioma. While a biphasic distribution where distinct areas display astrocytic or oligodendroglial differentiation has been described in the literature, this is extremely rare and when found is of uncertain clinical significance. Most commonly mixed oligoastrocytomas represent an indeterminate diffuse variant where the two phenotypes are intermingled [2]. Recent data suggest that from a biologic perspective, mixed oligoastrocytoma is not a distinct entity and as a category is likely composed of a mix of tumors with "oligodendroglioma" biology (e.g., 1p/19q co-deletion) together with tumors with "astrocytoma" biology (e.g., TP53 mutation). These considerations

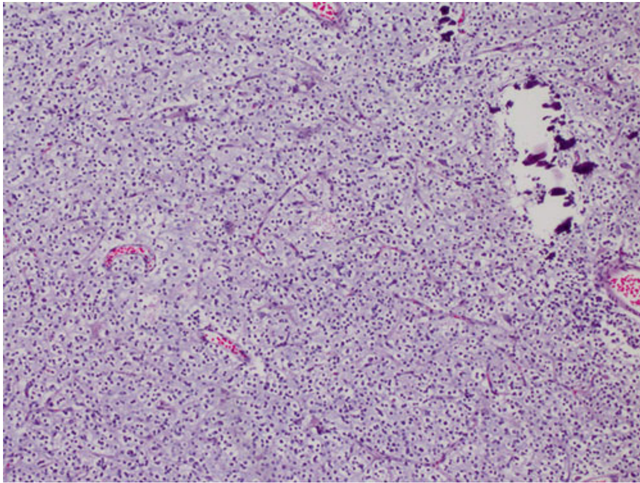

Fig. 6.1. Oligodendroglioma, with characteristic rounded hyperchromatic nuclei, perinuclear halos, and branching capillary pattern.

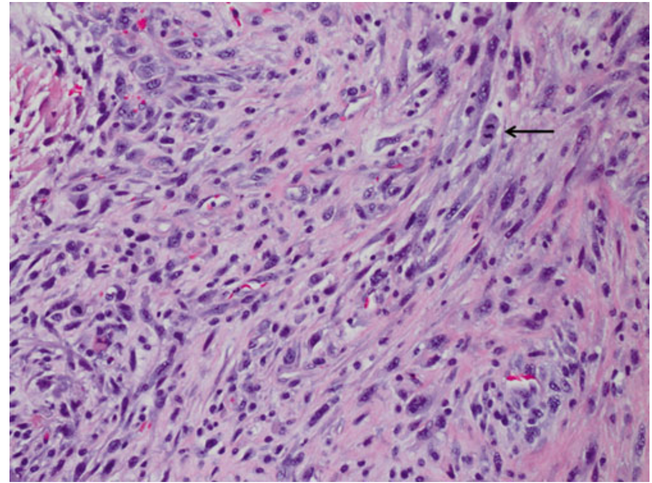

Fig. 6.3. Gliosarcoma, with bundles of elongated spindle cells.

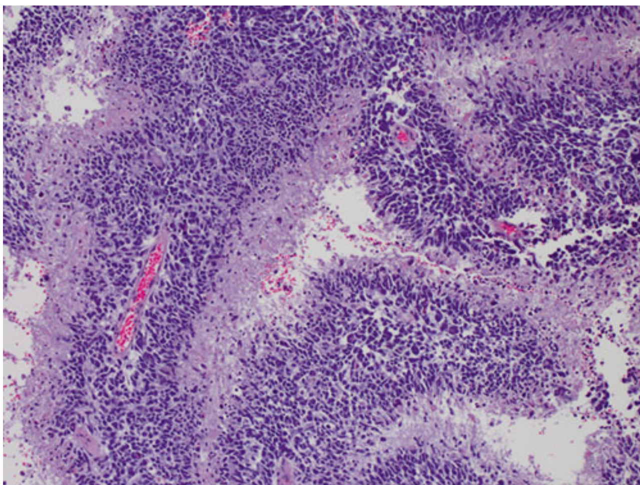

Fig. 6.2. Glioblastoma, with pseudopalisading necrosis.

highlight an important concept likely to be introduced into future classification systems of glioma, where key molecular markers are to be used as an important adjunct to conventional histopathologic analysis.

In addition to the features described above, glioblastomas are defined by the presence of microvascular proliferation and/or necrosis (Fig. 6.2). The cells are poorly differentiated and pleomorphic, and regional heterogeneity is common. Several variants have been described, including small cell glioblastoma, glioblastoma with an oligodendroglioma component, giant cell glioblastoma, and gliosarcoma [2].

Small cell glioblastoma is characterized by a monomorphic population of densely packed small, round cells with a high nuclear:cytoplasmic ratio and modest atypia. Proliferative activity is high, and GFAP immunoreactivity can be minimal. Their outcome is similar to standard GBMs [31]. Glioblastomas with an oligodendroglial component contain foci that resemble oligodendroglioma. The presence of areas with both astrocytic differentiation and necrosis differentiates them from anaplastic oligoastrocytomas. Giant cell glioblastomas have numerous multinucleated giant cells along with smaller fusiform cells. There is some data to suggest that the prognosis of glioblastomas with an oligodendroglial component and giant cell glioblastomas may be better compared to standard glioblastomas [32–34]. Gliosarcomas have a mixture of cells with gliomatous and sarcomatous differentiation, either in distinct geographic areas or intermixed. The gliomatous areas show typical features of glioblastoma. Sarcomatous areas often resemble fibrosarcoma, with bundles of spindle cells, but can also show mesenchymal differentiation with cartilaginous, osteoid, myomatous, or lipomatous features. These areas are GFAP negative [2] (Fig. 6.3). The prognosis of gliosarcoma is similar to standard glioblastoma [35].

Cytogenetics and Molecular Genetics

1p/19q Co-deletion

Loss of heterozygosity (LOH) of 1p and 19q is a common event in oligodendroglial tumors, occurring in 80–90 % of low-grade oligodendrogliomas and 50–70 % of anaplastic oligodendrogliomas [36]. However, since the distinction of oligodendroglioma from astrocytic tumors is subject to interobserver variability, the rate of 1p/19q co-deletion relative to histologic diagnosis is only approximate. Several groups have reported that it is mutually exclusive from genetic changes common to astrocytic tumors, including TP53 mutations [37, 38], EGFR amplification [38, 39], and LOH 10q [39, 40]. It is associated with oligodendroglial morphology [41] and is felt to be a reliable diagnostic biomarker of the oligodendroglial phenotype [37, 42]. Tumors with 1p/19q co-deletion often exhibit several other genetic and epigenetic

changes including IDH mutations [43, 44], methylguanine methyltransferase (MGMT) promoter methylation [44], CpG island methylator phenotype (G-CIMP) [45], and a proneural gene expression profile [46, 47], which will be discussed in greater detail below.

Recent work has identified candidate tumor suppressor genes on 1p and 19q. Mutations in the homolog of the *Drosophila capicua* gene (CIC) on 19q13.2 are present in 50–80 % of 1p/19q co-deleted oligodendrogliomas [48–50]. CIC is a downstream transcriptional repressor of receptor tyrosine kinase (RTK) pathways, including EGFR, Ras, Raf, and MAP kinases [48]. The exact mechanism of tumor pathogenesis remains unclear [42]. Mutations in the far-upstream element (FUSE) binding protein 1 (FUBP1) on 1p31.1 are seen in about 20 % of oligodendrogliomas [48, 50]. FUBP1 binds to the FUSE of Myc, a well-known oncogene [51] and negatively regulates Myc expression [52]. Although these are theoretically attractive genes for being involved in the pathogenesis of oligodendrogliomas, no definitive mechanism has yet been identified.

Chromosome 10 Deletion

LOH of chromosome 10 is the most frequent genetic alteration in GBM, and occurs in 60–80 % of cases [53, 54]. Loss of the entire chromosome is common, but partial deletions in three common regions have been described, suggesting that several tumor suppressor genes may exist in chromosome 10 [2, 55]. LOH 10q occurs in both primary and secondary glioblastomas at similar frequencies [53] while LOH 10p is generally seen in primary glioblastomas [56]. One established tumor suppressor in this region is the PTEN (phosphatase and tensin homology) gene on 10q23.3 [57]. PTEN is a phosphatase which inhibits PIP3 signaling, and thereby downregulates the activity of AKT and mTOR and inhibits cell proliferation [58]. It is mutated in 15–40 % of glioblastomas [59, 60] most often in primary glioblastomas [53, 61].

Chromosome 7 Amplification

The most frequent amplification event in glioblastoma is amplification of 7p12 in the region of the EGFR gene [62]. Like PTEN mutation and LOH 10q, EGFR amplification is common in primary glioblastoma, where it occurs in about 40 % of cases [53, 63]. EGFR amplification is significantly less frequent in secondary glioblastoma [63]. About 50–60 % of tumors with EGFR amplification also express a truncated variant of the receptor, EGFR variant III (EGFRvIII), which is constitutively active and ligand-independent [64, 65]. EGFR amplification and EGFRvIII mutation have been associated with an increased proliferation rate, increased invasiveness, resistance to cytotoxic therapy, and worse patient outcomes [66–68]. When other clinical and genetic variables and known prognostic factors are considered in a multivariant analysis, however, it is less clear that EGFR amplification and particularly the EGFRvIII mutation turn out to be independent prognostic factors. The EGFR signaling pathway will be discussed in further detail below.

IDH1 and IDH2

Mutations in isocitrate dehydrogenase-1 and -2 (IDH1 and -2) were first identified as important driver mutations in a subset of glioblastomas by Parsons et al. in 2008 [69]. IDH catalyzes the oxidative carboxylation of isocitrate to α-ketoglutarate (α-KG) within the citric acid cycle. The IDH family of genes code for enzymes that catalyze the NADP/NAD-dependent oxidative decarboxylation of isocitrate to alpha-ketoglutarate, with subsequent NADPH/NADH release [5]. These enzymes are found both in the cytoplasm (IDH1), and in the mitochondria (IDH2 and IDH3). IDH mutations are present in 50–75 % of anaplastic tumors and 75–85 % secondary glioblastomas, but are uncommon in primary glioblastomas, occurring in about 5 % [43, 44, 69–71]. IDH mutations frequently occur in association with other mutations common to secondary glioblastomas, such as TP53 mutations, 1p/19q co-deletions, and methylated MGMT promoter, and are inversely associated with alterations in PTEN, EGFR, and LOH 10, which are common to primary glioblastomas [70, 72, 73].

IDH1 mutations are point mutations at position 395 (G395A) of the IDH1 gene (codon 132 of the IDH1 binding site), most commonly with replacement of arginine with histidine (IDH1-R132H) [69], which accounts for >90 % of IDH1 mutations [43, 69, 71]. IDH2 mutations are less common, accounting for only 4–5 % of IDH mutations, and appear largely in the setting of 1p/19q co-deleted tumors [43, 71] IDH2 are also point mutations at a homologous codon (172) within the binding site [42]. Collective data suggest that IDH mutation is a very early lesion in the pathogenesis of lower grade (grade II–III) diffuse glioma. Co-deletion of 1p/19q is almost invariably observed in the setting of an IDH mutation. Interestingly, IDH-mutated but non-1p/19q-co-deleted tumors are nearly always TP53-mutated, suggesting that either co-deletion or TP53 mutation occur after IDH mutation and either of these aberrations is required for most cases of glioma pathogenesis. There is a mutant-specific commercially available antibody to the IDH1-R132H protein which is a reliable diagnostic biomarker [74]. From a practical and clinical perspective, this immunohistochemical test has several diagnostic uses, which include mutation detection and distinction of diffuse glioma from entities not associated with IDH mutation (for example circumscribed glioma and ependymoma). In addition, in the setting of a differential diagnosis of diffuse glioma versus reactive conditions (astrogliosis, treatment effects), R132H-specific immunohistochemistry can be very useful when positive. It is important to remember that absence of staining/mutation is not always helpful, since not all diffuse gliomas are IDH-mutated.

The pathogenesis of IDH mutations is still under active investigation. The mutation causes reduced enzymatic activity in the conversion of isocitrate to α-KG [75], but since the remaining allele produces functional enzyme it is likely that it is a gain of function which is pathogenic [76]. The mutated enzyme has been shown to catalyze the reduction of α-KG to 2-hydroxyglutarate (2-HG), and 2-HG levels are elevated in IDH1 mutated tumors, suggesting that 2-HG is an oncometabolite [77]. This is supported by the fact that patients with inborn errors of metabolism leading to accumulation of 2-HG in the brain have an elevated risk of brain tumors [78]. 2-HG is a competitive inhibitor of α-KG-dependent dioxygenases including histone demethylases and the TET family of hydroxylases [79], which are involved in DNA methylation [80]. Recent data shows that IDH mutation causes DNA hypermethylation over serial passages and is sufficient to establish the G-CIMP hypermethylated phenotype [81]. Mutations in IDH genes are not specific to gliomas, and have been described in other neoplasms, including acute myelogenous leukemia (AML) and cartilaginous neoplasms, among others. IDH mutations in these tumors, unlike gliomas, have not been shown to have a more favorable outcome.

The absence or presence of IDH mutation in diffuse gliomas largely accounts for the prior designation of primary and secondary pathways to glioblastoma, respectively. Lower grade diffuse gliomas (grade II–III) are largely IDH mutant, while grade IV gliomas (glioblastomas) are largely IDH wild type. However, a subset of grade II–III gliomas are IDH wild type and likely represent a precursor lesion to glioblastoma. In addition, while IDH-mutant glioblastomas are rare in the setting of a new glioma diagnosis, they likely represent malignant progression from a previously undiagnosed lower grade IDH-mutant glioma. Although indistinguishable histologically, many distinctions exist between IDH-mutant and IDH wild-type diffuse gliomas in terms of methylation pattern, DNA copy number aberrations, gene expression profiles, and somatic mutational profiles. Future progress in the classification and management of diffuse gliomas will likely benefit by treating IDH-mutant and IDH wild-type tumors as separate clinico-pathological entities.

ATRX

Alpha Thalassemia/Mental Retardation Syndrome X-Linked (ATRX) is a component of the SWI/SNF complex of chromatin remodeling proteins and is involved in gene regulation. Jiao et al. have recently described mutations in ATRX in gliomas, occurring in more than one third of astrocytomas (71 %, grades II and III), 57 % of secondary glioblastomas, and 68 % of mixed oligoastrocytomas. The frequency in primary glioblastoma and oligodendroglioma was much lower (4 % and 14 %, respectively). ATRX mutation was almost always seen in the presence of a TP53 mutation (94 %) and IDH mutations (99 %) in adult gliomas [82].

Epigenetics and Gene Expression Profiling

MGMT

O^6-MGMT is a DNA repair protein on 10q26 that removes alkyl groups from the O^6 position of guanine, thereby counteracting the activity of alkylating agents such as temozolomide and nitrosoureas [83]. It can be epigenetically silenced via methylation of 5′-CpG islands within the transcription factor binding sites [84]. MGMT is methylated in about 40 % of primary glioblastomas, 70 % of secondary glioblastomas, and 50 % of anaplastic astrocytomas [85, 86]. MGMT methylation is strongly associated with 1p/19q co-deletion [87, 88] and IDH mutations [44, 86]. MGMT methylation is predictive of response to temozolomide [89] and nitrosoureas [90], consistent with their mechanism of action. There is emerging evidence, however, that MGMT methylation is also an independent prognostic marker and associated with improved outcome in patients treated with radiotherapy alone [30, 72, 91], suggesting that this epigenetic change is associated with biological effects beyond DNA alkylator damage repair [92]. MGMT methylation is also associated with the G-CIMP hypermethylated phenotype [45] and it is possible that these genetic and global epigenetic changes underlie the prognostic effect of MGMT methylation. That said, whether MGMT methylation has the same prognostic significance in patients with IDH-mutant tumors as has been shown in patient cohorts with largely IDH wild-type glioblastomas, is not entirely clear.

Glioma-CpG-Island Methylator Phenotype (G-CIMP)

Noushmehr et al. have identified a subgroup of glioblastomas characterized by a distinct pattern of hypermethylation of CpG islands in a subset of glioma-specific genes, including genes involved in cell adhesion, regulation of transcription, metabolic processes, and nucleic acid synthesis [93]. This glioma-CpG-island methylator phenotype (G-CIMP) leads to transcriptional silencing of specific genes via methylation of promoter regions. It is associated with younger age, the "proneural" gene expression profile, a high frequency of IDH1 and TP53 mutations, and a low frequency of PTEN, NF1, and EGFR mutations. As outlined above, there is evidence to suggest that IDH mutations are sufficient to cause this glioma-specific methylation phenotype.

Gene Expression Subtypes

The Cancer Genome Atlas (TCGA) has identified four gene expression subtypes within glioblastoma including proneural, neural, classical, and mesenchymal subtypes [94]. The frequency of copy number alterations and mutations by subtype in this cohort are described in Tables 6.3 and 6.4.

TABLE 6.3. Copy number alterations in glioblastoma subtypes in TCGA samples.

	Proneural (%)	Neural (%)	Classical (%)	Mesenchymal (%)	Gene(s) in ROI
Low and high level amplified events					
7p11.2	54	96	100	95	EGFR
7q21.2	46	96	92	89	CDK6
7q31.2	54	92	86	91	MET
7q34	52	92	86	91	
High level amplification events					
7p11.2	17	67	95	29	EGFR
4q12	35	13	5	9	PDGFRA
Homozygous and hemizygous deletion events					
17q11.2	6	17	5	38	NF1
10q23	69	96	100	87	PTEN
9p21.3	56	71	95	67	CDKN2A/ CDKN2B
13q14	52	46	16	53	RB1
Homozygous deletion events					
9p21.3	41	54	92	53	CDKN2A/ CDKN2B

From Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP et al.: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 2010, 17(1):98-110, with permission

TABLE 6.4. Distribution of mutated genes in glioblastoma subtypes.

Gene	Proneural (%)	Neural (%)	Classical (%)	Mesenchymal (%)
TP53	54	21	0	32
PTEN	16	21	23	32
NF1	5	16	5	37
EGFR	16	26	32	5
IDH1	30	5	0	0
PIK3R1	19	11	5	0
RB1	3	5	0	13
ERBB2	5	16	5	3
EGFRvIII	3	0	23	3
PIK3CA	8	5	5	3
PDGFRA	11	0	0	0

From Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP et al.: Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell* 2010, 17(1):98-110, with permission

The proneural subtype has an oligodendroglial signature, and is characterized by alterations in PDGFRA and IDH1 as well as TP53 mutations. Copy number changes that are common in classic glioblastoma, such as chromosome 7 amplification and chromosome 10 loss, are significantly less frequent in the proneural subtype. Oligodendrocytic development genes, including PDGFRA, NKX2-2, and Olig-2, are highly expressed. Patients with proneural tumors are younger and have a better prognosis, and secondary glioblastomas are more common in this subtype. It is important to realize that the improved prognosis observed by the proneural subclass is likely due to its inclusion of IDH-mutant tumors, which represent a subset of this transcriptomal class.

The neural subtype is associated with both oligodendrocytic and astrocytic gene signatures, and is also characterized by expression of neuronal markers, including NEFL, GABRA1, SYT1, and SLC12A5. Its expression patterns are most similar to normal brain.

The Classical subtype has an astrocytic signature, and is characterized by chromosome 7 amplification and chromosome 10 loss, EGFR amplification and EGFRvIII mutation, and increased expression of Notch and Sonic hedgehog (SHH) signaling pathway constituents. TP53 mutations are not seen in this subtype.

The mesenchymal subtype has a cultured astroglial signature, and microglial markers such as CD68, PTPRC, and TNF are highly expressed. NF1 mutations and PTEN loss are common in this subtype. Phenotypically, these tumors have more abundant necrosis, perhaps related to high expression of genes in the tumor necrosis factor (TNF) and NF-κB pathways. Both mesenchymal (CHI3L1, MET) and astrocytic markers (CD44, MERTK) are expressed.

While the TCGA classification is the most widely cited, it is important to realize that the TCGA classification system for gene expression analysis has not been validated using an unsupervised analysis from an independent data set. However, examination of additional analyses in the literature would suggest that, with some variation in nomenclature, the mesenchymal and proneural subtypes appear to be reproducibly identified in gene expression datasets [95, 96]. In addition, while the mesenchymal subtype is generally restricted to IDH wild-type glioblastoma, the proneural subtype is observed in diffuse gliomas of all three WHO grades. In addition, IDH-mutated gliomas are almost invariably proneural, but a subset of IDH wild-type tumors can also cluster in the proneural class.

Prognostic Stratification

Traditionally, prognostic stratification for malignant gliomas has been based on clinical and histologic features of the tumors. Regressive partitioning analysis has identified age, histologic grade, performance status, and extent of resection as important prognostic factors [97]. Outcomes stratified by these variables are markedly different, and vary from 5 months in the worst prognostic group to almost 60 months in the best prognostic group [97]. The significance of these prognostic markers has been born out in multiple studies [3, 6, 7, 21, 98–100], and they continue to be the foundation for estimating prognosis.

There is evidence that several of the genetic and molecular features of these tumors discussed above have prognostic significance, and they are increasingly being incorporated into prognostic schema. It has long been recognized, for

instance, that 1p/19q loss is associated with a better response to chemotherapy [101, 102] and radiotherapy [25], as well as with longer survival [25, 27, 101]. As discussed above, there is also evidence that MGMT methylation is both a predictive [89, 90] and prognostic [30, 72, 91] biomarker. Both tests are now commercially available, and are routinely used as part of the diagnostic evaluation of these tumors.

More recently IDH1 and 2 mutations have emerged as another important prognostic factor. Several groups have established that gliomas with IDH mutations have a relatively favorable prognosis [43, 69], and that IDH mutations are more prognostic than grade [73, 103] As outlined above, current evidence suggests that IDH status defines two biologically and clinically distinct types of diffuse glioma, and IDH-mutant tumors are less aggressive than IDH wild-type gliomas when matched for histological grade. In contrast, EGFR overexpression is associated with worse prognosis and therapeutic resistance [104, 105]. Although TP53 mutations have emerged as a useful diagnostic biomarker given their higher incidence in secondary glioblastoma compared to primary glioblastoma [63], they do not appear to have prognostic significance in glioblastoma [106].

Molecular Signaling Pathways

The PI3K kinase pathway is altered in the majority of glioblastomas through a variety of mechanisms. Activating mutations or amplifications of multiple RTKs which activate this pathway, including EGFR, Her2, PDGFRα, or MET have been described [107]. PI3K is activated by RTKs and leads to increased cell proliferation and survival via activation of multiple downstream effectors including Akt and mTOR [58, 108]. Activating mutations of the pathway components Ras, PI3K, and Akt are often identified [107]. Inactivating mutations or deletions of NF1, a negative regulator of Ras, or PTEN, a negative regulator of PI3K, are also common [53, 107, 109]. Constituents in this pathway and the frequency of alterations in glioblastoma are summarized in Fig. 6.4a.

TCGA identified alterations in the TP53 tumor suppressor pathway in 87 % of glioblastomas [107]. Disruption of this pathway leads to clonal expansion and genetic instability, as outlined above [110, 111]. Alterations in this pathway can occur via inactivating mutations or deletions in TP53 itself, or amplification of negative regulators of TP53, MDM2, and MDM4. These two events are generally mutually exclusive [107, 112]. Homozygous deletion of the CDKN2A gene, which leads to loss of $p14^{ARF}$, an inhibitor of MDM2, also leads to functional loss of p53 activity [113]. A summary of this pathway and frequency of alterations in glioblastoma is provided in Fig. 6.4b.

The Rb pathway is altered in 78 % of glioblastomas [107]. Rb is a tumor suppressor that controls cell cycle progression from G1 to S phase [113]. Alterations in this pathway are most often due to deletions or mutations of Rb, amplification of its negative regulator CDK4, or deletions of $p16^{INK4a}$ or CDKN2B, which are in turn inhibitors of CDK4 [113]. Of note, $p16^{INK4a}$ is also translated from the CDKN2A gene, underscoring the connectedness of the TP53 and Rb pathways [114]. This pathway is summarized in Fig. 6.4c.

There has been increasing interest in stem-like cell (GSCs) signaling pathways in recent years. GSCs are characterized by their ability for self-renewal, their ability to differentiate into multiple lineages, and their tumorgenicity [115, 116]. GSCs have been identified as an important cause of treatment resistance in malignant gliomas [117, 118]. Two main stem cell signaling pathways that have inconsistently been implicated in GSC biology are the Notch pathway and the SHH pathway. Jagged and Delta-like ligands bind to Notch, which leads to γ-secretase-mediated cleavage of the intracellular domain of the Notch receptor [119]. Notch-IC then translocates to the nucleus where it functions as a transcriptional activator of several physiologic processes, including angiogenesis, specification of cell fate, and regulation of differentiation [119, 120]. SHH binds to the patched homolog receptor, and thereby releases the membrane protein Smoothened homolog, resulting in the activation of Gli proteins [121]. Gli proteins are transcription factors that modulate several target genes including MYC and CCND1 [121]. Different studies have implicated one, both or neither of these pathways in GSC biology. It is therefore likely that these pathways have variable activities and roles in different GSC lines from different tumors. These two pathways are summarized in Fig. 6.5.

Finally, it has long been recognized that gliomas are highly angiogenic tumors, so angiogenic signaling pathways represent a major area of glioma research. The vascular endothelial growth factor (VEGF) signaling pathway is pivotal to the development of tumor-associated blood vessels [120]. VEGF is secreted by tumor cells and binds to the VEGF receptor (VEGFR) on tumor-associated endothelium. This stimulates a signaling cascade through the PI3K/Ras/MAPK pathway, leading to endothelial cell proliferation and migration [120, 122]. Other pro-angiogenic factors and their receptors can stimulate these pathways, including platelet-derived growth factor (PDGF), fibroblast growth factor 2 (FGF-2), hypoxia-inducible factor 1-alpha (HIF-1α), hepatocyte growth factor (HGF), and angiopoetin-1 and -2, via their receptors PDGFR, FGFR, Met, and Tie2 and may be important causes of resistance to VEGF targeted therapy [120, 123, 124]. These pathways are summarized in Fig. 6.5.

Molecular Targeted Therapies

The elucidation of these important signaling pathways in malignant glioma has stimulated extensive research in the use of molecular targeted therapies. The most successful of the targeted therapies to date is bevacizumab, a humanized

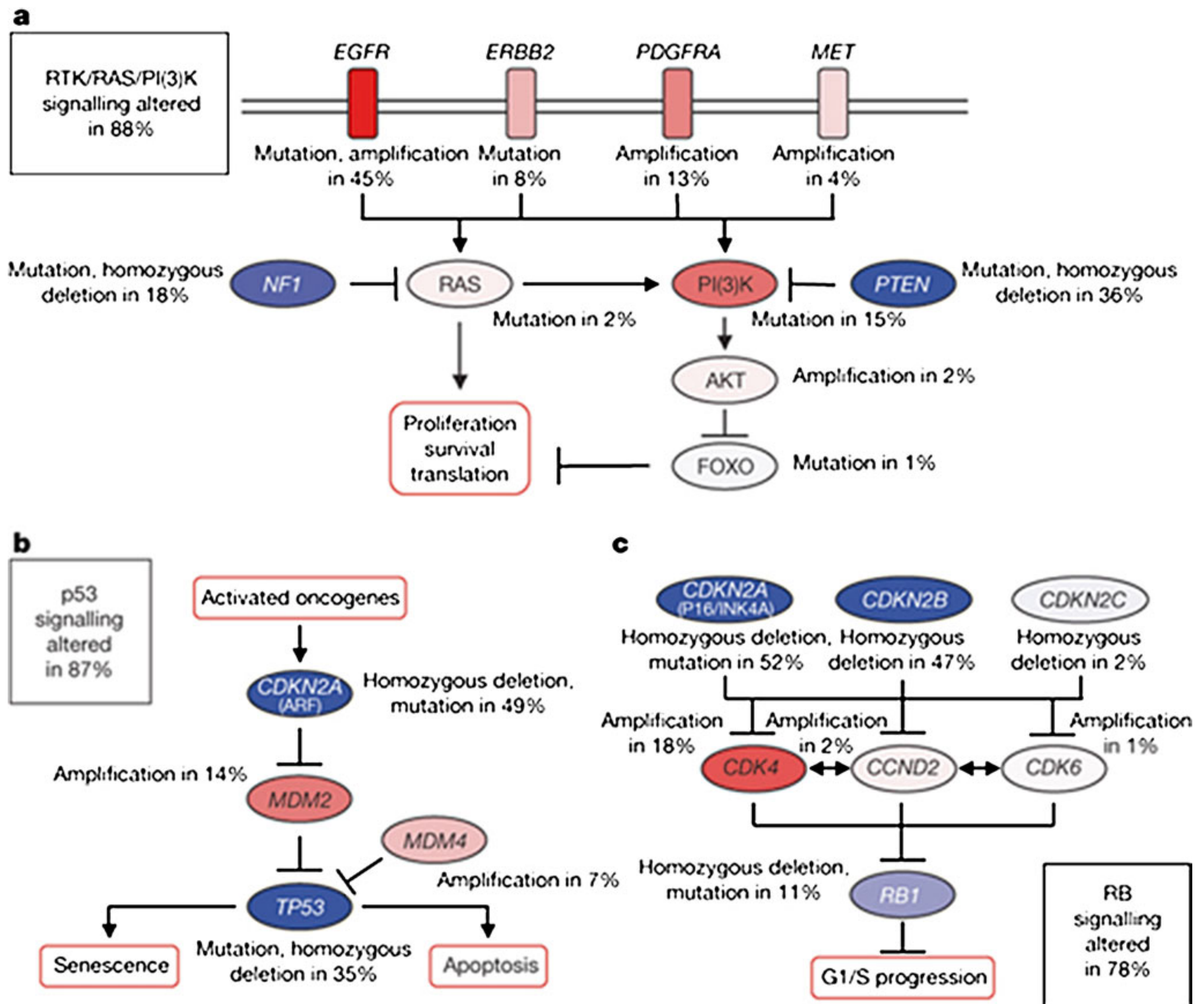

Fig. 6.4 Common signaling pathway alterations in glioblastoma (From Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008, 455(7216):1061-1068, with permission)

monoclonal antibody against VEGF-A ligand, which was approved by the FDA in 2009 for the treatment of recurrent glioblastoma [125]. Treatment with bevacizumab in phase II trials resulted in radiographic responses in 28–57 % of patients, median progression-free survival of 4–6 months, and median overall survival of 8–10 months [19, 20, 126], which compared favorably to historical controls [21, 22].

Two recent phase III trials examined the utility of bevacizumab added to chemoradiation as initial therapy for newly diagnosed glioblastoma. They showed an improvement in progression-free survival, but not overall survival, compared to controls, many of whom received bevacizumab at recurrence [127–129]. These data suggest that bevacizumab is not indicated as part of initial therapy. It should be noted that overall survival in both arms of these two trials was 16–17 months, which was a modest improvement compared to the 14.6 months seen in earlier phase III studies with radiation and temozolomide alone [4].

The success of bevacizumab has spurred interest in other VEGF targeted therapies, including decoy receptors, pan-VEGFR tyrosine kinase inhibitors (TKIs), multitargeted TKIs, integrin inhibitors, protein kinase C-β inhibitors, and inhibitors of other pro-angiogenic pathways. Although some have shown modest antitumor activity [130, 131], many have proven ineffective alone [132–135], or in combination with other targeted agents [136, 137], and none has proven more efficacious than bevacizumab to date. Ongoing clinical trials are exploring the activity of anti-angiogenic agents in combination with other targeted agents [138, 139], and in combination with bevacizumab [140]. Anti-angiogenic agents tested in clinical trials are summarized in Table 6.5.

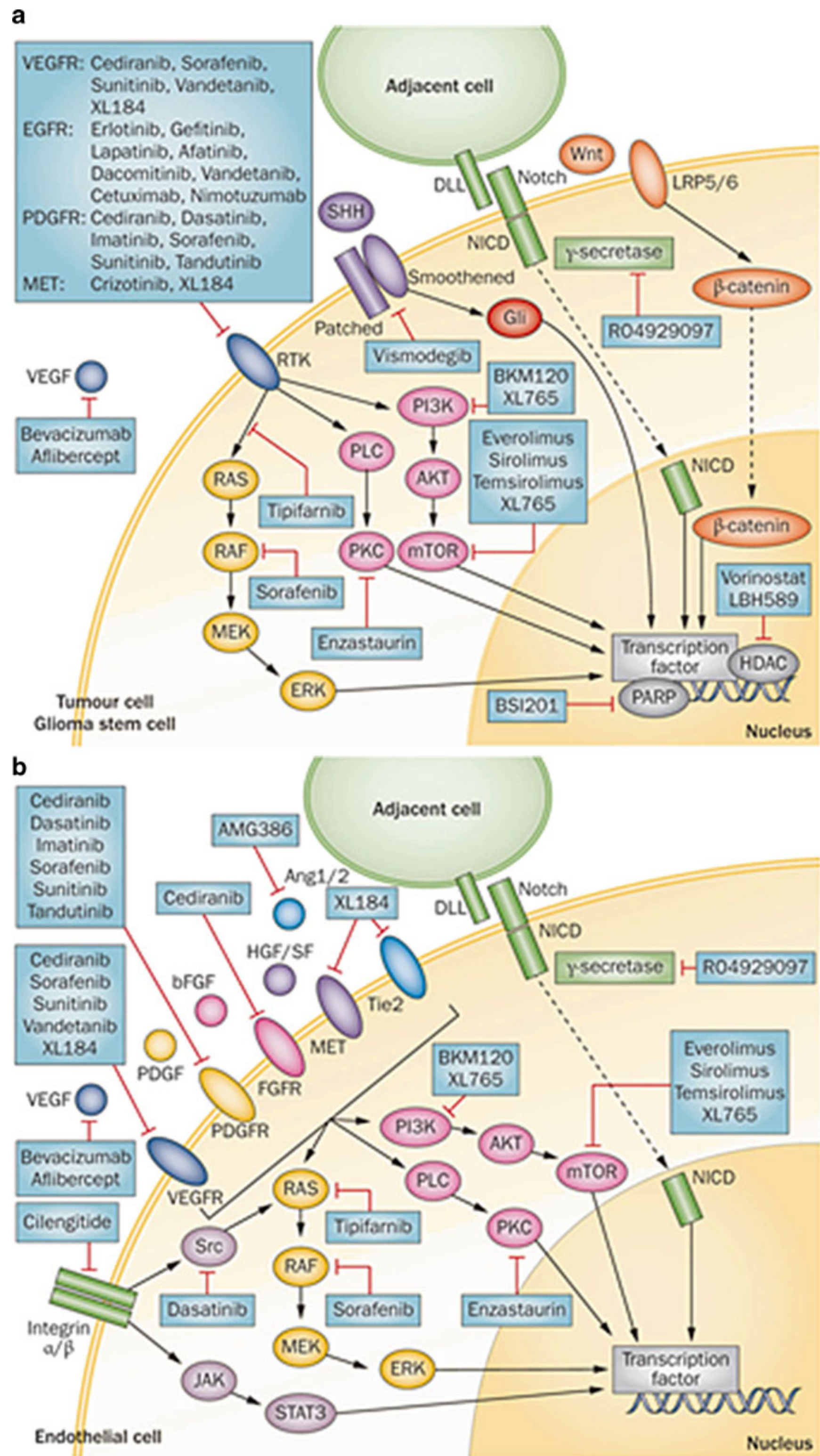

Fig. 6.5 Cell signaling pathways in malignant glioma cells (**a**) and tumor-associated endothelial cells (**b**), and agents under investigation to target these pathways (From Tanaka S, Louis DN, Curry WT, Batchelor TT, Dietrich J: Diagnostic and therapeutic avenues for glioblastoma: no longer a dead end? *Nat Rev Clin Oncol* 2013, 10(1):14-26, with permission)

Another major area of interest is EGFR targeted therapy. Although EGFR alterations are common in glioblastoma, first generation EGFR TKIs including erlotinib and gefitinib, as well as cetuximab, a monoclonal antibody against EGFR, had limited activity [141–147]. This may be due to amplification of multiple RTKs within tumors, which maintain downstream signaling in the setting of EGFR inhibition [148–151]. Vandetanib, an EGFR and VEGFR-2 inhibitor, and lapatanib, a HER-1 and HER-2 inhibitor also had limited activity [132, 152, 153]. A second generation of

TABLE 6.5. Anti-angiogenic agents under investigation.

Agent	Target	References
Bevacizumab	VEGF-A	[19, 20, 126]
Aflibercept	VEGF-A, VEGF-B, PlGF	[135]
Cediranib	VEGFR-1,2,3, PDGFR-α,β, FGFR-1, c-Kit	[130, 161]
Sorafenib	VEGFR-2,3, PDGFR-α,β, BRAF, c-Kit, Ras	[136, 137, 162–164]
Sunitinib	VEGFR-2, PDGFR-β, c-Kit, RET, Flt3	[134, 165, 166]
Vandetanib	VEGFR-2, EGFR, RET	[132, 152]
Cabozantinib	VEGFR-2, Met, RET, c-Kit, Flt3, Tie-2	[133]
Cilengitide	αvβ3, αvβ5 integrins	[131, 167]
Enzastaurin	PKC-β, Akt	[168, 169]
AMG386	Ang-1, Ang-2	[170, 171]

TABLE 6.6. Molecular targeted agents under investigation.

Agent	Target	References
Temsirolimus	mTOR	[172, 173]
Everolimus	mTOR	[174–176]
BKM-120	PI3K	[177–179]
XL-765	PI3K, mTOR	[180]
Sorafenib	VEGFR-2,3, PDGFR-α,β, BRAF, c-Kit, Ras	[136, 137, 162–164]
Imatinib	PDGFR-α,β, c-Kit, Bcr-Abl	[181–183]
Tandutinib	PDGFR-α,β, c-Kit, Flt3	[184, 185]
Dasatinib	PDGFR-α,β, Src, Bcr-Abl, c-Kit, EphA2	[186–190]
PD-0332991	CDK4, CDK6	[191]
RO492097	γ-secretase	[192–195]
Vismodegib	Smoothened homolog	[196]

EGFR TKIs which cause irreversible target inhibition are currently being studied, and may prove more beneficial [154–157]. Several immunotherapy approaches targeting EGFRvIII have also been evaluated. An EGFRvIII peptide vaccine, CDX-110, was evaluated in a phase II trial in patients with newly diagnosed glioblastoma, and showed a significant improvement in overall survival compared to a matched cohort [158]. A phase III trial in newly diagnosed patients is ongoing [159]. A phase I/II trial is also ongoing using T-cells genetically modified to express an anti-EGFRvIII chimeric antigen receptor [160].

A number of other molecular targets have been explored in the past decade. These include inhibitors of other RTKs such as PDGFR and MET, and inhibitors of downstream signaling molecules such as mTOR, PI3K, RAS, RAF, Src, and PKC. Recently there has also been interest in targeting signaling pathways specifically involved in stem cell biology, such as γ-secretase inhibitors, which are involved in Notch signaling, and SHH signaling inhibitors. A schematic of these pathways, and drugs under investigation to target these pathways, is provided in Fig. 6.5. Current clinical trials investigating these agents are summarized in Table 6.6.

Conclusion

The last decade has seen an explosion in our knowledge of the genetic and molecular biology of high-grade gliomas. New data support the great biological heterogeneity that underlies what was previously thought to represent just a few tumor types affirming the heterogeneity of observed clinical endpoints such as response to specific treatments and overall survival. The discovery of discrete mutational events in subsets of high-grade gliomas yield hopes of targeted therapies directed at those genetic and epigenetic aberrations, as well as more rationale clinical trials selected for subgroups of patients enriched for tumors most likely to respond to a particular targeted therapy. Nevertheless, the heterogeneity of genomic and epigenomic profiles from multiple gliomas, as well as the relatively disappointing results of targeted treatments to date, raises the realistic possibility that the future of targeted treatment will not be matching a drug against its specific mutated gene, but rather the need for a more complete understanding of the complexity of the gene regulatory network hardwired into each of those tumors as defined by their specific mutational and epigenetic profiles. Understanding this network may then allow us to identify points of network vulnerability for which future targeted therapies may be developed. Thus, although our understanding of the biology and molecular signatures of diffuse glioma has increased, the pace of clinical improvements for this disease has unfortunately lagged behind and much work to successfully match tumor-specific alterations with specific and efficacious therapeutics is yet to be done.

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7 Pediatric High-Grade Gliomas and DIPG

Oren J. Becher, Kelly L. Barton, Kyle G. Halvorson, and Roger McLendon

The central nervous system (CNS) is the second most common location for tumorigenesis in children with the majority of tumors being benign. Pediatric high-grade gliomas (pHGGs) only account for approximately 8–12 % of all childhood CNS tumors but are a leading cause of mortality in children [1, 2]. pHGG histologies include anaplastic astrocytomas (World Health Organization or WHO grade III), anaplastic oligodendrogliomas (WHO grade III), and glioblastomas/gliosarcomas (WHO grade IV). pHGGs can arise anywhere in the CNS and those that arise in the brainstem are also called diffuse intrinsic pontine glioma or DIPG. Unfortunately, little therapeutic progress has been made over the last few decades, and clinical outcomes for these patients remain dismal. To date the only effective modality is focal radiation, which provides only temporary tumor control in most patients. One of the reasons for this lack of clinical progress has been our limited understanding of the molecular genetics and tumor biology of pHGGs. Very recently, next generation sequencing studies have led to the discovery of a fundamentally novel class of genetic alterations, implicating epigenetic reprogramming as a central component of pHGG pathogenesis and opening potential avenues for new, biology-based treatment approaches.

Additional key insights from recent genomic studies of pHGG include the appreciation of molecular genetic heterogeneity not only between patients, but also within the tumor tissue of the same patients, distinct biological differences compared to adult high-grade gliomas, and the recognition of differences in tumorigenesis based on age and tumor location within the CNS. Clearly, these insights will need to be taken into account in developing novel therapeutic strategies that will hopefully lead to improved outcomes of patients afflicted with these highly aggressive and lethal tumors. In this chapter, we will briefly describe the histopathology, cytogenetic, molecular genetic and epigenetic alterations, as well as gene expression profiling of pHGGs. We will also discuss key signaling pathways pertinent to the advancement of molecular targeted therapies in pHGGs. Lastly, we will review current clinical trials of molecular targeted therapies for pHGGs and emerging future avenues of clinical investigation.

Histopathology

High-grade gliomas consist of the gliomas graded by the World Health Organization as Grades III and IV. As such, the following tumors fall into this group: anaplastic astrocytoma, glioblastoma multiforme (GBM), anaplastic oligodendroglioma, and anaplastic oligo-astrocytoma. While not a strictly histogenetic type, anaplastic glioma has been assigned to those cases that do not clearly fall into either anaplastic astrocytoma or anaplastic oligodendroglioma due to small sample size, therapeutic effect, or some other histologic artifact that renders specific classification difficult.

High-grade gliomas of all types share common features of high mitotic activity, vascular endothelial proliferation and, quite commonly, focal necrosis. Oligodendrogliomas share features with the myelinating cells of the CNS, the oligodendrocytes. These include round, regular nuclei and clear cytoplasm that ring the nuclei producing a fried-egg appearance. Anaplastic oligodendrogliomas exhibit dense hypercellularity, frequent mitotic figures, vascular endothelial proliferation, and foci of necrosis. These foci may or may not be ringed by cells tightly surrounding the focus, defined as pseudopalisading necrosis (Fig. 7.1).

Anaplastic astrocytomas differ from anaplastic oligodendrogliomas by having hyperchromatic nuclei that are often elongated, irregular, or smudged. Mitotic figures may not be common, but can be found by immunohistochemical markers of proliferation such as the MIB-1 index. While the tumor cells are not as densely packed as in the oligodendroglioma, they are nonetheless, obviously more hypercellular than the white matter by a factor of three- to tenfold. Vascular changes are frequent in anaplastic astrocytoma, but the defining element of vascular proliferation as found in the Grade IV

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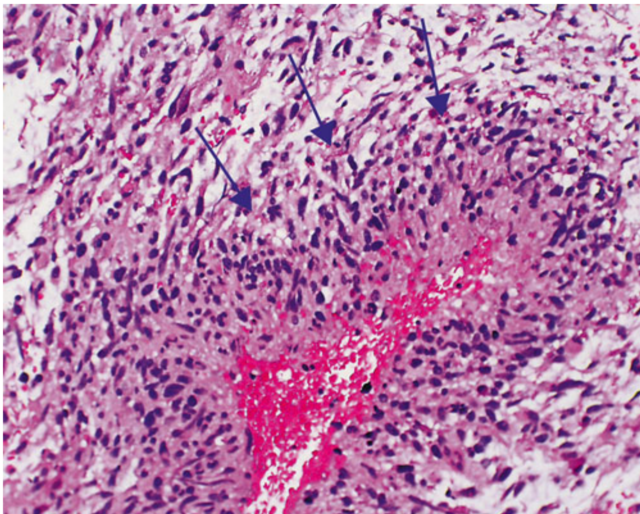


Fig. 7.1 Pediatric glioblastoma exhibiting pseudopalisading necrosis. Hematoxylin and eosin staining of a Grade IV GBM (40× magnification). *Arrows* point to pseudopalisading necrosis

glioblastoma, is debated. While most brain tumor neuropathologists agree that endothelial duplication circumferentially ringing the vessel is a feature of glioblastomas, it is unclear where glomeruloid vessels and vascular garlands or loops of vessels fall in astrocytoma grading. Among astrocytic tumors, foci of necrosis are reserved for glioblastomas.

Glioblastoma multiforme, the most malignant and most common of the high-grade gliomas, is an astrocytic neoplasm that has been found to most commonly arise de novo without a preceding history of glioma, with only 5 % of glioblastomas occurring as a result of progression from a lower grade astrocytoma. Both de novo and progressive GBMs exhibit nuclear pleomorphism, mitotic activity, vascular proliferation, and/or necrosis. While densely cellular in some foci of the tumor, there is commonly an infiltrative component that can be found distant from the central mass. This infiltrative capability currently makes GBMs impossible to cure by surgery or local–regional radiation therapy.

Epigenetics

With the advent of next generation sequencing, our understanding of the genetic alterations in pHGGs has been turned upside down. This technological advance has led to the discovery of heterozygous K27M or G34R/V mutations in the tail of histone 3.3 or K27M mutations in the tail of histone 3.1 and loss of function mutations in the chromatin remodelers ATRX (α-thalassaemia/mental retardation syndrome X-linked) and DAXX (death-domain associated protein) in pHGGs (Fig. 7.2; [3, 4]). As a brief review, the fundamental repeating unit of chromatin is the nucleosome, which consists of approximately 147 bp of superhelical DNA wrapped around the radial surface of an octamer of highly conserved core histone proteins (two copies of each H2A, H2B, H3, and H4). Histone proteins are subject to a wide array of covalent modifications that occur primarily at amino (N^-) and carboxy (C^-) termini. The tail regions of core histones contain flexible and highly basic amino acid sequences that are highly conserved and serve as substrates for several post-translational modifications such as acetylation, methylation, ADP-ribosylation, ubiquitylation, and phosphorylation. These modifications impact gene transcription, DNA replication, and chromatin assembly. The histone code states that distinct patterns of histone modifications act in concert with DNA methylation, noncoding RNAs, and transcription factors to generate "histone-epigenetic codes" that are read by effector proteins [5]. Lastly, another level of complexity is histone variants (e.g., H3.3 vs. H3.1), which are relevant to pHGGs. Although the difference between H3.3 and H3.1 is only four amino acids, H3.1 (also called canonical core H3) is only incorporated into nucleosomes during the S-phase of the cell cycle while H3.3 incorporation into nucleosomes is cell-cycle independent. Furthermore, ATRX and DAXX are both H3.3 chaperones and together they facilitate the deposition and remodeling of H3.3 containing nucleosomes [6].

The initial two manuscripts describing these mutations noted, in the brainstem there are K27M mutations in either H3.3 or H3.1 in up to 80 % of DIPGs, while the G34R/V mutations are found only in H3.3 and were primarily found in pHGGs located in the cerebral cortex. In addition, G34R/V mutations co-occur with loss of function ATRX or DAXX mutations and are associated with the ALT (alternative lengthening of telomeres) phenotype [3, 4, 7, 8]. In a follow-up paper, Sturm and colleagues [9] pursue an integrative approach based on epigenetic, copy number, expression, and genetic analyses on over 200 adult and pediatric GBMs to identify six distinct DNA methylation clusters which were labeled as "IDH," "K27," "G34," "receptor tyrosine kinase (RTK) I (platelet-derived growth factor receptor A or PDGFR-A)," "mesenchymal," and "RTK II (Classic)." A key finding of this analysis was that H3F3A K27 and H3F3A G34 mutations were exclusively distributed to the K27 and G34 clusters, respectively, and these were mutually exclusive of the isocitrate dehydrogenase (IDH1) mutations. The RTK I (PDGFR-A) group had a high frequency of PDGFR-A amplification and the RTK II Classic group had a very high frequency of whole chromosome seven gain, whole chromosome ten loss, frequent deletion of cyclin-dependent kinase inhibitor 2a (CDKN2A), and amplification of epidermal growth factor receptor (EGFR). This RTK II Classic subgroup is completely devoid of pediatric patients. Remarkably, the clusters are associated with patient age, with K27M patients being the youngest (median age 10.5; range 5–23) and G34 patients being the second youngest (median age 18 years; range 9–42). The RTK I "PDGFR-A" cluster (median age 36 years, range 8–74 years) and the IDH cluster (median age 40 years, range 13–71 years) mostly comprised young

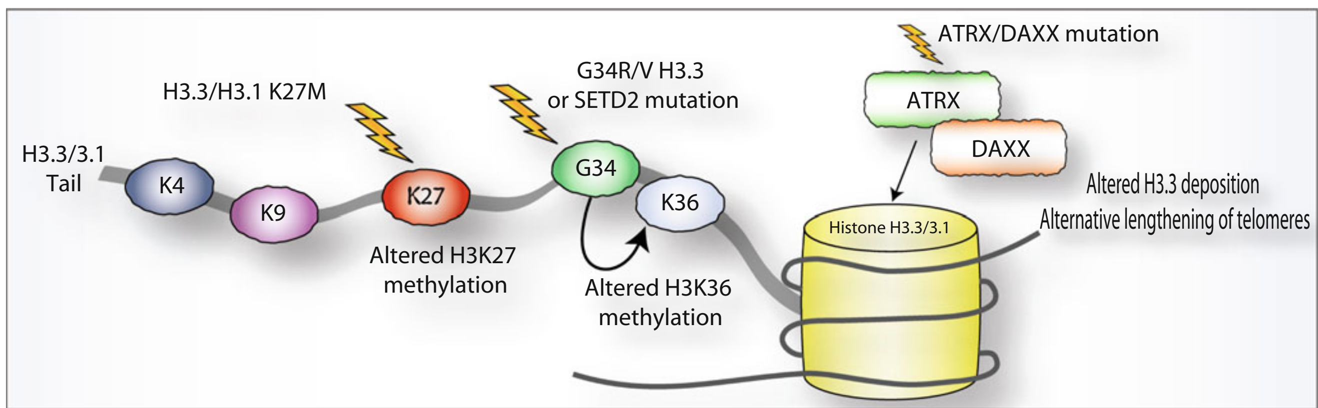


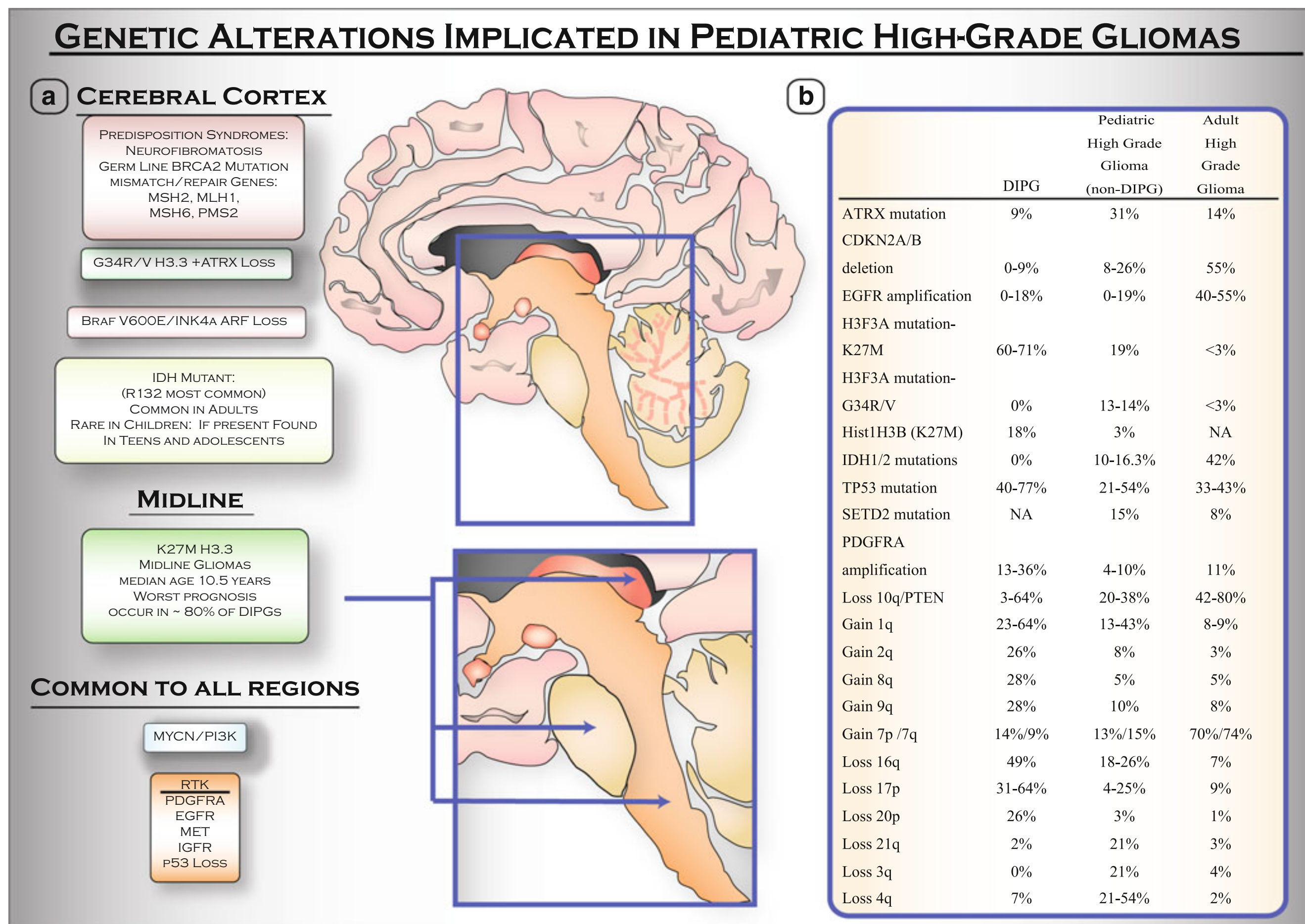
Fig. 7.2 Epigenetic alterations associated with pediatric high-grade gliomagenesis. This schematic of the H3.3/H3.1 tail illustrates the three types of epigenetic mutations seen in pHGG: (1) K27M mutations which impact H3K27 methylation, (2) G34R/V or SETD2 mutations, both of which impact H3K36 methylation, and (3) ATRX/DAXX mutations which likely impact H3.3 deposition and alternative lengthening of telomeres

adults, while the oldest cluster, the RTK II "Classic" cluster, comprised older adults (median age 58, range 36–81 years). The other remarkable finding was that the epigenetic GBM subgroups showed region-specific predilection within the CNS whereby the K27-mutant tumors were predominantly seen in midline locations such as the thalamus, pons, and the spinal cord while the tumors in the other five subgroups almost exclusively arose in the cerebral hemispheres. This remarkable observation clearly suggests that the mechanism of gliomagenesis is distinct in different regions of the CNS. Lastly, these subgroups also correlated with survival with the K27 subgroup having the shortest survival, the IDH subgroup with the longest survival, and the other subgroups in between. Interestingly, both the IDH and H3F3A mutations co-occur with p53 mutations, suggesting that p53 mutations do not have independent prognostic significance [9].

How histone mutations contribute to pHGG pathogenesis is subject to current investigations, but Lewis and colleagues recently reported an initial glimpse into the mechanism. They reported that the K27M H3.3/H3.1 mutations inhibit polycomb repressive complex 2 (PRC2), the enzyme complex that adds methyl groups to H3 lysine 27. Under normal circumstances, this histone mark is repressive, and inhibition of PRC2 results in global loss of H3 lysine 27 trimethylation. The mechanism of the G34R/V histone mutations is less well understood, but results in a local decrease in H3 lysine 36 trimethylation [10]. In addition to the epigenetic mutations described above, pHGGs recently have also been reported to harbor loss of function mutations in SETD2, an H3K36 trimethyltransferase. Perhaps not surprisingly, SET2D mutations were mutually exclusive with H3F3A mutations, but they did overlap with IDH1 mutations [11]. High-grade gliomas with SET2D mutations were found exclusively in tumors arising in the cerebral hemispheres, reinforcing the notion that H3K36 is important for gliomagenesis in the cerebral hemispheres, while H3K27 is central in the etiology of tumors arising in midline structures of the CNS. Lastly, while IDH mutations are primarily found in adult gliomas, adolescents 13 years old and older can also harbor activating mutations in IDH1 in amino acid 132 [12, 13]. These IDH1 mutations have also been reported to impact histone marks, but through a completely different mechanism [14].

Cytogenetics

Several studies have investigated the spectrum of copy number aberrations in pHGGs [15–22]. Copy number changes in pHGGs are best subdivided between broad chromosomal gains and losses and focal gains and losses. Broad low-amplitude gains of chromosome 1q were identified as well as broad losses of 10q, 13q, and 14q. Focal gains have been reported in PDGFR-A, cyclin D1-3 (CCND1-3), cyclin-dependent kinase 4 (CDK4), cyclin-dependent kinase 6 (CDK6), MYC, v-myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog (MYCN), EGFR, V-Erb-A Avian Erythroblastic Leukemia Viral Oncogene Homolog-Like 4 (ERBB4), MET, hepatocyte growth factor (HGF), insulin-like growth factor-1 receptor (IGF1R), insulin-like growth factor 2 (IGF2), platelet-derived growth factor B (PDGFB), Neuregulin 1 (NRG1), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), PIK3C2B, PIK3C2G, PIK3R5, Kirsten rat sarcoma viral oncogene homolog (KRAS), v-akt murine thymoma viral oncogene homolog 1 (AKT1), AKT3, S6K1, and murine double minute 4 (MDM4). The most common homozygous focal loss was at CDKN2A/CDKN2B. Other homozygous focal losses included the following genes: CDKN2C, neurofibromin-1 (NF1), PTEN



	DIPG	Pediatric High Grade Glioma (non-DIPG)	Adult High Grade Glioma
ATRX mutation	9%	31%	14%
CDKN2A/B deletion	0-9%	8-26%	55%
EGFR amplification	0-18%	0-19%	40-55%
H3F3A mutation-K27M	60-71%	19%	<3%
H3F3A mutation-G34R/V	0%	13-14%	<3%
Hist1H3B (K27M)	18%	3%	NA
IDH1/2 mutations	0%	10-16.3%	42%
TP53 mutation	40-77%	21-54%	33-43%
SETD2 mutation	NA	15%	8%
PDGFRA amplification	13-36%	4-10%	11%
Loss 10q/PTEN	3-64%	20-38%	42-80%
Gain 1q	23-64%	13-43%	8-9%
Gain 2q	26%	8%	3%
Gain 8q	28%	5%	5%
Gain 9q	28%	10%	8%
Gain 7p /7q	14%/9%	13%/15%	70%/74%
Loss 16q	49%	18-26%	7%
Loss 17p	31-64%	4-25%	9%
Loss 20p	26%	3%	1%
Loss 21q	2%	21%	3%
Loss 3q	0%	21%	4%
Loss 4q	7%	21-54%	2%

Fig. 7.3 Genetic alterations implicated in pHGGs. Genetic alterations vary by location and age. (**a**) Genetic alterations observed in pHGGs located in the cerebral cortex, or in midline areas of the CNS, or common to all pHGGs. Arrows pointing to midline areas from top to bottom: thalamus, pons, and spinal cord. (**b**) Distinct molecular genetics of DIPG as compared to pHGGs (non-DIPG) and adult high-grade gliomas. Table is adapted from: Kristin M Schroeder, Christine M Hoeman, and Oren J Becher, Children are not just little adults: recent advances in understanding of diffuse intrinsic pontine glioma biology, *Pediatric Research*, 2014 [57]

(phosphatase and tensin homolog), retinoblastoma (RB1), TP53, TP73, and protein tyrosine phosphatase receptor type D (PTPRD). Interestingly, copy number alterations are also age- and region specific. PDGFR-A is the most common amplified receptor tyrosine kinase (RTK) in pHGGs while EGFR amplification is the most common amplified RTK in adult high-grade glioma. Gains of chromosomes 2q, 8q, and 9q and losses of 16q, 17p, and 20p were significantly more frequent in DIPG than in non-brainstem pHGGs. Furthermore, focal deletions of CDKN2A are extremely rare in DIPGs and are found in 26 % of non-brainstem pHGGs [23]. Lastly, Barrow et al. described homozygous loss of ADAM3A in 16 % of pHGGs, although the function of this gene and how its loss contributes to pediatric gliomagenesis is not known [17]. Figure 7.3 includes a summary figure of the genetic alterations in pHGGs, as well as a table, which lists the genetic alterations in DIPG, non-brainstem pHGG, and adult HGG.

Gene Expression

Gene expression profiling, a method to analyze the mRNA expression of all genes in the tumor, is another useful technique to study the complex biology of cancer. In fact, mRNA analysis of a select number of genes is used to make clinical decisions in some types of breast cancer. In pHGGs, unsupervised hierarchical clustering identified three main tumor subgroups: HC1/proliferative, HC2/proneural, and HC3/mesenchymal [15]. Gene ontology analysis across the groups revealed that HC1 is most associated with cell-cycle genes; HC2 is most associated with neuronal differentiation, while HC3 is most associated with extracellular matrix–receptor interactions and cell adhesion. Interestingly, HC1 is most associated with amplifications targeting the PDGFR signaling cascade, which ties together PDGF signaling with cell growth. If one were to compare the expression profiling

of pHGGs to adult high-grade gliomas, PDGFR-A mRNA is significantly overexpressed in pHGGs relative to adult high-grade gliomas while EGFR mRNA is significantly repressed in pHGGs relative to adult high-grade gliomas. With regard to the HC3/mesenchymal group, immune response genes were also enriched and more specifically associated with microglia/macrophages and monocytes [24]. Lastly, using principal component analysis (PCA), two independent groups noted that the expression profiling of DIPGs consists of a distinct cluster separate from non-brainstem gliomas, reinforcing the notion of region-specific differences in pediatric CNS gliomagenesis [23, 25].

Prognostic Stratification

Until recently, the concept that HGGs comprise several, biologically distinct diseases associated with age and location was not fully appreciated. As a consequence, current research efforts center on developing a better understanding of tumor biology and accordingly, devise appropriate classification schemes. Similar to the increased stratification of leukemias in children, molecular stratification of pHGG will continue to become increasingly refined, in parallel with advances in our understanding of disease biology. The ongoing challenge is how to best incorporate new molecular prognostic factors with well-established prognostic factors, such as extent of resection and tumor grade [2]. For over a decade, p53 overexpression has been recognized as a poor prognostic factor in pHGGs. Most importantly, this association was found to be independent of age, histologic features, the extent of resection, or tumor location [26]. Overexpression of p53 as determined by immunohistochemistry, however, is an imperfect proxy for oncogenic p53 mutations, and taken in context with our current knowledge of the molecular genetics of HGG, a clearer picture emerges. For example, as previously mentioned in the epigenetics section of this chapter, IDH mutations frequently co-occur with p53 mutations, and these tumors currently have the best prognosis. However, p53 mutations also overlap with K27M mutations, which appear to have the worst prognosis. According to retrospective studies, K27M is a poor prognostic factor in pediatric GBM, although it is unclear whether this is due to a different biology versus the midline location of these tumors, which limits surgical options [9, 27]. Within the K27M subgroup, DIPGs have the worst prognosis, with a median survival time of 9–12 months and greater than 90 % of children dying within 2 years [28]. Lastly, PTEN mutations or loss of PTEN expression by IHC have been reported to be significantly associated with decreased survival in pHGGs, but this has so far only been reported in small cohorts and will require further validation [29, 30].

Molecular Signaling Pathways

Three pathways that are most implicated in pHGG pathogenesis are the p53, retinoblastoma protein (Rb), and RTK/Ras/phosphoinositide 3-kinase (PI3K) signaling pathways. These pathways are dysregulated in both pediatric and adult high-grade gliomas, and the importance of these three pathways in adult gliomagenesis was recently underscored by the mutual exclusivity of alterations affecting these pathways [31]. Evolving knowledge of precisely how these pathways contribute to tumor initiation and growth is expected to lead to better-informed molecular targeted therapeutic approaches. With regard to activation of the RTK/Ras/PI3K pathway, 80 % of pHGGs activate this pathway through amplification of RTKs, and/or activating mutations in PI3K, and/or loss of PTEN either through deletion or promoter methylation [32]. Below is a brief summary of the key molecular signaling pathways.

RTK/Ras/PI3K Pathway

The axis of PI3K signaling in cancer begins with engagement of growth factors by RTKs such as PDGFR, MET, EGFR, and IGF-1R (Fig. 7.4). PI3K, a lipid kinase, is then recruited to plasma membrane-anchored receptors, is activated, and phosphorylates PIP2 to generate PIP3. Through its pleckstrin homology (PH) domain, the nodal kinase AKT (also known as PKB) binds to PIP3, where it is activated by two phosphorylation events, and triggers a complex cascade of signals that regulate growth, proliferation, survival, and motility. The lipid phosphatase, PTEN, antagonizes this process by dephosphorylating PIP3 to inhibit activation of AKT. PI3K is activated downstream of numerous RTKs that directly, or through adaptor proteins, bind and activate PI3K. PI3K activity is thus carefully regulated by growth factor–receptor interactions. In fact, the vast majority of PI3K remains inactive in the cytoplasm, removed from its plasma membrane-associated substrates, and only a small percentage of PI3K becomes activated upon growth factor stimulation. Therefore, even slight modulations in receptor activity can lead to manyfold increases in PI3K activity [33].

In addition to copy number alterations, pHGGs can also harbor additional alterations in the RTK/Ras/PI3K pathways through somatic mutations or alternative splicing. The most commonly mutated RTK in pHGGs is PDGFR-A where mutations in the extracellular domain as well as in the tyrosine kinase domain have been described in approximately 10 % of these tumors [3, 25, 34]. By contrast, the most commonly mutated RTK in adult high-grade glioma is EGFR. EGFRvIII (an EGFR lacking exons 2–7 resulting in ligand-independent signaling) is an alternatively spliced EGFR variant found in 19 % of adult HGGs, but has also been reported in 17 % of pHGGs [31,

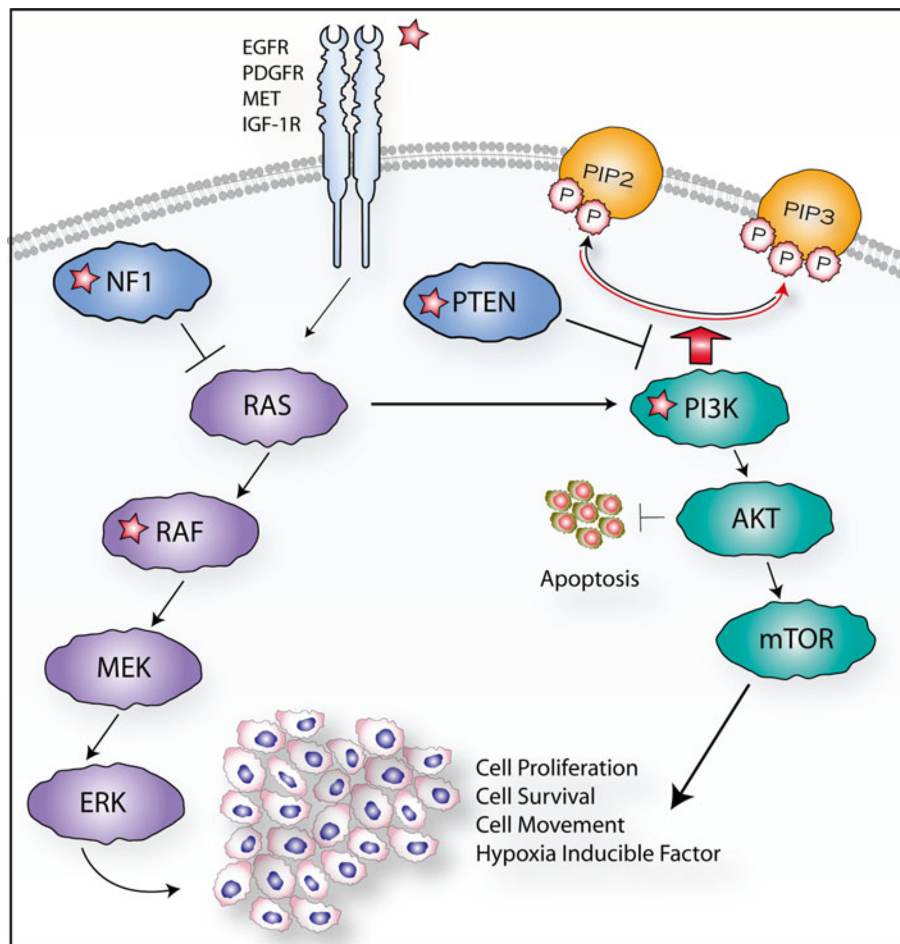


Fig. 7.4 RTK/Ras/PI3K pathway. Growth factors such as EGF, PDGF, HGF, and IGF2 engage with their respective RTKs leading to PI3K activation. PI3K activation phosphorylates PIP2 to generate PIP3. AKT binds to PIP3, becomes activated, and triggers a complex cascade of signals that regulate growth, proliferation, survival, and motility. PTEN, a naturally occurring antagonist of this pathway, dephosphorylates PIP3 inhibiting activation of AKT. Growth factors and RTK interactions also regulate cell proliferation and survival through activation of Ras followed by sequential activation of Raf, Mek, and Erk. Starred (*asterisk*) factors mutated in RTK/Ras/PI3K pathway are prevalent in pHGGs

35]. Downstream of RTKs, activating mutations in PIK3CA have been described in a subset of pHGGs both inside and outside of the brainstem [36, 37]. While activating Ras mutations have rarely been described in pHGGs (G12V Kras reported by Schiffman et al. [16]), loss of function NF1 (neurofibromatosis type 1 which negatively regulates the Ras pathway) mutations has been identified in a subset of pHGGs outside of the brainstem [3]. Furthermore, activating V600E Braf mutations have also been described in pHGGs [11, 16, 38, 39]. V600E Braf mutations can also be found in low-grade gliomas, but usually in isolation, suggesting that cooperating mutations (such as CDKN2A) may be required for a high-grade phenotype in V600E Braf mutant tumors [16].

RB Pathway

The retinoblastoma protein (RB) is a tumor suppressor protein and key regulator of cell-cycle control. The RB pathway consists of five families of proteins: CDKN (e.g., Ink4a), D-type cyclins, D-cyclin-dependent protein kinases (cdk4, cdk6), RB-family of pocket proteins (RB, p107, p130), and the E2F-family of transcription factors. Each Ink4-protein (p16Ink4a, p15Ink4b, and p18Ink4c) can bind to and inhibit the activity of cdk4 and cdk6. Each D-cyclin protein can associate with cdk4 or cdk6 to form the active kinase complex. Therefore, Ink4 proteins compete with the D-cyclins for cdk4/6 to prevent the formation of the active kinase complex [40]. Importantly, proteins in this pathway are com-

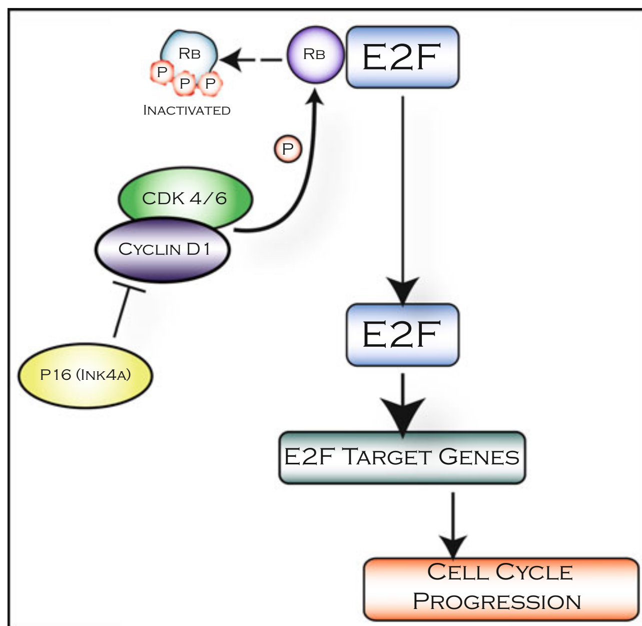


Fig. 7.5 RB pathway. The RB pathway is an important regulator of cell-cycle control. Cyclin D1 and cdk4/6 bind to form a complex that phosphorylates RB. In its active form RB is bound to E2F transcription factors. Hyperphosphorylation of RB renders it inactive and allows for the release of E2F transcription factors. E2F transcription factors activate E2F target genes, leading to initiation of S-phase and cell-cycle progression. CDKN (e.g., INK4a) is a tumor suppressor gene that inactivates the cyclin D1/cdk4/6 complex. Cdkn2a deletions and/or amplification of cdk4, cdk6, cyclin D1, D2, or D3 are common in pHGGs

monly altered in pHGGs, primarily through copy number aberrations: cdkn2a deletions and/or amplification of cdk4, cdk6, cyclin d1, d2, and d3 [15, 19, 23].

The RB pathway regulates cell proliferation as the proteins in this pathway are activated and/or inhibited by growth-promoting, as well as growth-suppressing signals (Fig. 7.5). During regulated cell proliferation, as cells respond to mitogenic signals and commit to cell-cycle entry the complex of D-cyclin/cdk4/6 is activated. The primary cellular targets of the D-cyclin/cdk4/6 complex are the RB-family of pocket proteins (henceforward referred to as RB), which inhibit transcription by directly inhibiting the activity of E2F. Hyperphosphorylation of RB by activated D-cyclin/cdk4/6 complexes renders RB inactive and in turn allows for the release of E2F transcription factors, leading to the activation of E2F target genes involved in cell-cycle progression [40]. Recently, a preclinical trial using genetically engineered mouse models identified a population of pHGG patients that may be sensitive to treatment with a highly selective cdk4/6 inhibitor [41]. Inhibition of cdk4/6 in murine high-grade gliomas harboring CDKN2A loss provided a significant survival benefit and holds promise for translation into clinical trials.

P53 Pathway

The p53 protein plays a key role in eliciting cellular responses to a variety of stress signals, such as DNA damage, hypoxia, and aberrant proliferative signals such as oncogene activation. Following cellular stresses, p53 is stabilized and binds to DNA as a tetramer, in a sequence-specific manner that results in the transcriptional regulation of genes involved in DNA repair, cell-cycle arrest, senescence, and apoptosis [42]. The critical role of this gene in tumor suppression in pHGG is clear as evidenced by the abundant, inactivating somatic mutations, which were recently reported in as many as 77 % of DIPGs [27]. Besides p53 mutations, other mechanisms to inactivate the p53 pathway include amplification of mouse double minute 2 homolog (MDM2) and MDM4. MDM2 is an important negative regulator of p53 working through two mechanisms: It is an E3 ubiquitin ligase that targets p53 for proteosomal degradation and it can also inhibit p53 transcriptional activation. MDM4 is a homolog of MDM2 and can also inhibit p53 transcriptional activity. In pHGGs, MDM2 is overexpressed but it is not amplified while MDM4 amplifications have been observed [15, 19, 43]. Interestingly, p53 mutations were reported to occur significantly more often in pediatric GBM relative to adult GBM [3]. This may be related to the fact that CDKN2A deletions are significantly more common in adult GBMs. CDKN2A encodes two transcripts: Ink4a (an endogenous cdk4/6 inhibitor discussed in the RB section) and alternative reading frame (ARF). ARF inhibits p53 degradation by sequestering MDM2 to the nucleolus and rendering it inactive. In summary, the p53 pathway is inactivated in the majority of pHGGs primarily through p53 mutations but also through MDM2 overexpression, MDM4 amplification, and CDKN2A loss, preventing the tumor cells from responding appropriately to cellular stresses.

Molecular Targeted Therapies

Despite recent advancements in our knowledge of key molecular alterations in pHGGs, this new depth of understanding has not translated into improved clinical therapies thus far. There have been numerous clinical trials with molecular targeted therapies for children with high-grade gliomas, but none have been demonstrated to significantly prolong survival. Most of the targeted therapy trials to date have focused on targeting RTKs (the upstream part of the RTK/Ras/PI3K pathway), and angiogenesis (VEGF or αv integrins). The lack of efficacy is likely due to activation of feedback loops, redundant activation of RTK pathways in glioma [44], intratumoral heterogeneity [15], and potentially inadequate drug delivery across the blood–brain barrier [45]. The following is not an exhaustive list of all targeted therapies that have been evaluated in pHGGs, but a brief description of some of the most relevant studies.

There have been multiple studies evaluating EGFR inhibitors (erlotinib, lapatinib, gefitinib) in pHGGs and none of them have demonstrated significant efficacy, even though the target has been demonstrated as present in a subset of pHGGs ([13, 45–49]. Other studies evaluating molecular targeted therapies in pHGGs include evaluation of inhibitors of PDGFR (imatinib), mTOR (temsirolimus), Ras (tipifarnib), VEGF (bevacizumab), αv integrin antagonist (cilengitide), Notch (MK-0752), and VEGFR2 (vandetanib) without success [47, 50–55]. Recently, a combination study of vandetanib and dasatinib (PDGFR inhibitor) was also reported with limited success [56]. Interestingly, the authors noted a 2 % cerebrospinal fluid to plasma exposure in two of the patients in the study, suggesting that inadequate drug delivery may explain the lack of response. Adequate drug delivery across the blood–brain barrier remains an obstacle in pHGG, and particularly in DIPG.

Future Directions

It is our hope that advances in our understanding of the genetic alterations of pHGGs will eventually translate into improved therapies. There is a great deal of excitement surrounding the discovery of highly specific histone mutations in pHGGs, and it remains to be seen how one can target such genetic alterations therapeutically. So far, there are two classes of epigenetic drugs that have been FDA approved for cancer: histone deacetylase (HDAC) inhibitors for cutaneous T-cell lymphoma and DNA methyltransferase (DNMT) inhibitors for myelodysplastic syndrome. In addition, there are numerous new classes of epigenetic drugs that have shown promise in preclinical trials and have recently entered clinical trials such as bromodomain inhibitors (a bromodomain is a protein domain that can bind an acetylated lysine) and enhancer of zeste 2 (EZH2) inhibitors. Furthermore, there are new therapeutic targets that are currently being evaluated in clinical trials for children with high-grade gliomas such as inhibitors of the enzyme poly (ADP-ribose) polymerase (PARP), inhibitors of telomerase (Imetelstat), and V600E Braf inhibitors. In summary, there are numerous new promising therapeutic targets in pHGGs, and the challenge is how to prioritize the translation of novel agents into clinical trials in children with high-grade gliomas and how to combine these novel agents synergistically. Undoubtedly, deeper insights into the biology of pHGGs will continue to emerge over the next years, opening new therapeutic avenues. The inter-patient heterogeneity of the genetic alterations in pHGGs implies that more personalized approaches may be needed, and a current V600E Braf inhibitor pediatric study with dabrafenib (ClinicalTrials.gov NCT01677741) is one example in the right direction, as only patients whose tumors harbor a V600E Braf mutation are allowed to enroll.

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8 Oligodendroglial Tumors

Stephen Yip and Jaishri Blakeley

Oligodendroglioma: An Infiltrating Glioma

The broad category of gliomas encompasses tumors comprised of neoplastic astrocytes and oligodendrocytes. Traditionally, these are classified based on morphologic pathology and clinical behavior. The most widely applied classification system was developed (and updated) by the World Health Organization (WHO) [1]. In this framework, gliomas are divided into astrocytomas, oligodendrogliomas (ODG), and mixed oligoastrocytomas (MOA) (Table 8.1). Oligodendroglial tumors are the least common of all gliomas, accounting for only 3–20 % of all glial tumors [2]. Of these, roughly 70 % are WHO grade II low-grade oligodendrogliomas (LGO) and 30 % are WHO grade III anaplastic oligodendrogliomas (AO) [3]. Given that ODG are a subtype of infiltrating glioma, they share many of the features common to this group of tumors, such as invasive growth often involving expansive regions of brain parenchyma, increasing aggressiveness with progression from low-grade to high-grade histology over time and ultimately, causing death, with the exception of small and localized tumors that are amenable to radical surgical resection. However, OGDs have several unique molecular genetic and clinical features that distinguish them from other infiltrating gliomas.

Specifically, ODG tend to be less diffuse than the other gliomas. Although there are certainly widely infiltrative ODG, these tumors are often confined to the superficial rather than deep portions of the cerebral hemispheres and in some cases, are relatively well demarcated, allowing for more radical surgical debulking compared to other infiltrative gliomas [4, 5] (Fig. 8.1). Another highly favorable property is the chemosensitivity of ODG, particularly those with 1p19q co-deletion. This was first noted more than 20 years ago when AO were shown to be responsive to cytotoxic chemotherapy [6]. These initial observations have matured into multiple randomized controlled clinical trials for AO that have had encouraging results relative to other diffuse or high-grade glioma subtypes [7, 8]. Unfortunately, despite these favorable properties, both LGO and AO remain almost always incurable and inevitably progress to an increasingly aggressive and treatment-resistant disease. This sobering reality is the major driver of the ongoing research into the unique features of ODG that is hoped to result in improved therapies and outcomes for patients with ODG.

Histopathology

ODG is characterized by tumor cells that morphologically and phenotypically resemble oligodendrocytes, which produce myelin that serves to optimize nerve transmission via the insulation of axons. This helps to facilitate saltatory transmission of action potential that greatly expedites nerve impulse speed. They are located within the white matter of the brain, an area that is rich in myelin due to the concentration of axons in this region. ODG display classical histological appearance marked by "back-to-back" tumor cells with regular, round hyperchromatic nuclei in association with clearing of the cytoplasm and close proximity to fine branching vasculature [1]. The former is whimsically referred to as "fried-egg appearance" and the latter as "chicken-wire vasculature." Nonetheless, these terms accurately describe the classical histological appearance of ODG (Fig. 8.2a). Note that perinuclear clearing is secondary to the "washing out" of cytoplasmic content by organic solvent during the process of formalin-fixed paraffin-embedded (FFPE) specimen preparation and is absent in frozen sections. ODG can also present with subpopulations of gliofibrillary oligodendrocytes and minigemistocytes. Other associated histological features include the frequent presence of microscopic calcospherites which often coalesce into grossly observable calcific deposits that lend themselves to stereotypical radio-dense appearance in computer tomography imaging (Fig. 8.2b).

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TABLE 8.1. 2007 World Health Organization (WHO) classification of gliomas.

Grade I	Grade II	Grade III	Grade IV
Angiocentric glioma	Diffuse astrocytoma	Anaplastic astrocytoma	Glioblastoma
Pilocytic astrocytoma	Oligodendroglioma	Anaplastic oligodendroglioma	Giant cell glioblastoma
Subependymal giant cell astrocytoma (SEGA)	Oligoastrocytoma	Anaplastic oligoastrocytoma	Gliosarcoma
	Pilomyxoid astrocytoma		Small cell glioblastoma
	Pleomorphic xanthoastrocytoma		Glioblastoma with oligodendroglioma features

From WHO Classification of Tumours of the Central Nervous System (IARC WHO Classification of Tumours) (v. 1), 2007, with permission of the World Health Organization

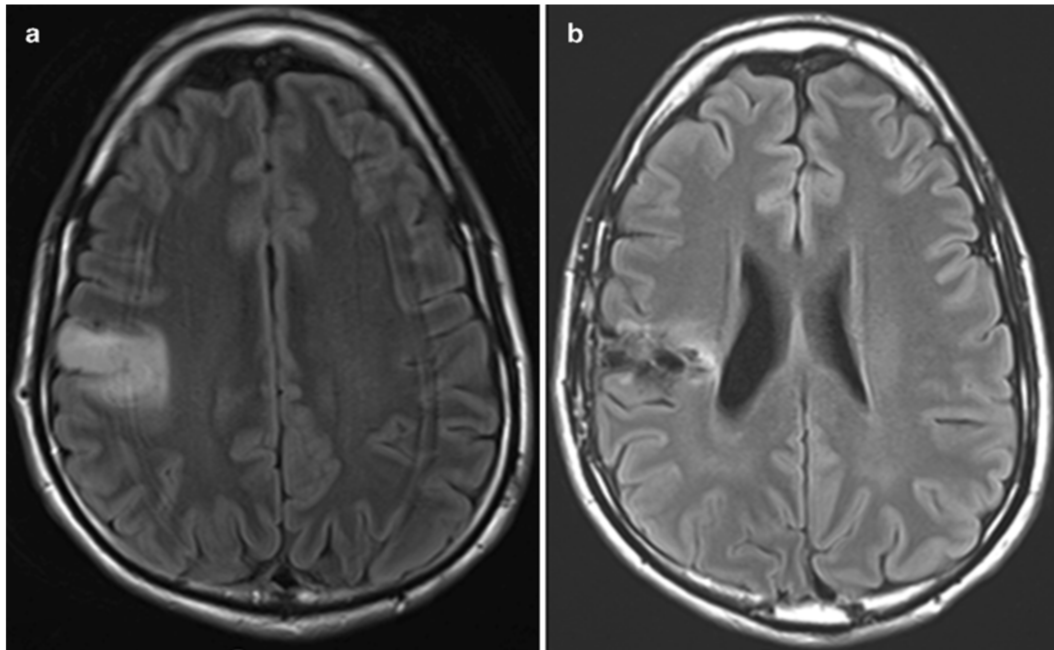

FIG. 8.1. Low-grade oligodendroglioma of the right frontal lobe as depicted in FLAIR brain MRI sequences before (**a**) and after (**b**) surgical resection. This is a classic presentation with the predominant involvement being in the cortical rather than deep regions.

Other histological features include foci of microcystic change and nuclear palisading, which historically was referred to as spongioblastic pattern usually associated with anaplastic ODG (WHO III) [9] (Fig. 8.2c). A feature shared by most ODG is the rather benign nuclear morphology which is frequently lost during malignant transformation to higher grade glioma. However, a focus with low-grade histological feature is sometimes present within glioblastoma suggestive of transformation. ODG tumor cells exhibit a strong physical affinity for non-neoplastic elements of the adjacent neuropil, a common characteristic shared with other infiltrating gliomas. These histological entities, including perineuronal and perivascular satellitoses, as well as subpial and intrafascicular spread, are collectively known as secondary structures of Scherer [10] (Fig. 8.2d).

Immunohistochemically, ODG tumor cells, especially those exhibiting classical morphology, are negative for glial fibrillary acidic protein (GFAP) expression. Morphological variants such as gliofibrillary oligodendrocytes and minigemistocytes display GFAP immunopositive cytoplasm and processes. Nonetheless, there is frequent background of GFAP reactivity due to the infiltrative nature of the tumor. Also, one must be cognizant of the reactive astrocytes admixed with tumor cells (Fig. 8.3a). Ki67 specific antibody highlights the proliferative fraction of the tumor. Immunoexpression of P53 is not very informative given *TP53* mutation is a rare event in ODG (Fig. 8.3b) [11]. The clinical introduction of IDH1 R132H mutant specific antibody (clone H-09) has dramatically altered the daily practice of neuropathology (Fig. 8.3c) [12]. Since a majority of WHO II/III infiltrating gliomas exhibit *IDH1* mutations, and R132H variant is the dominant form, the antibody is very useful in pinpointing tumor cells, even in small and challenging specimens.

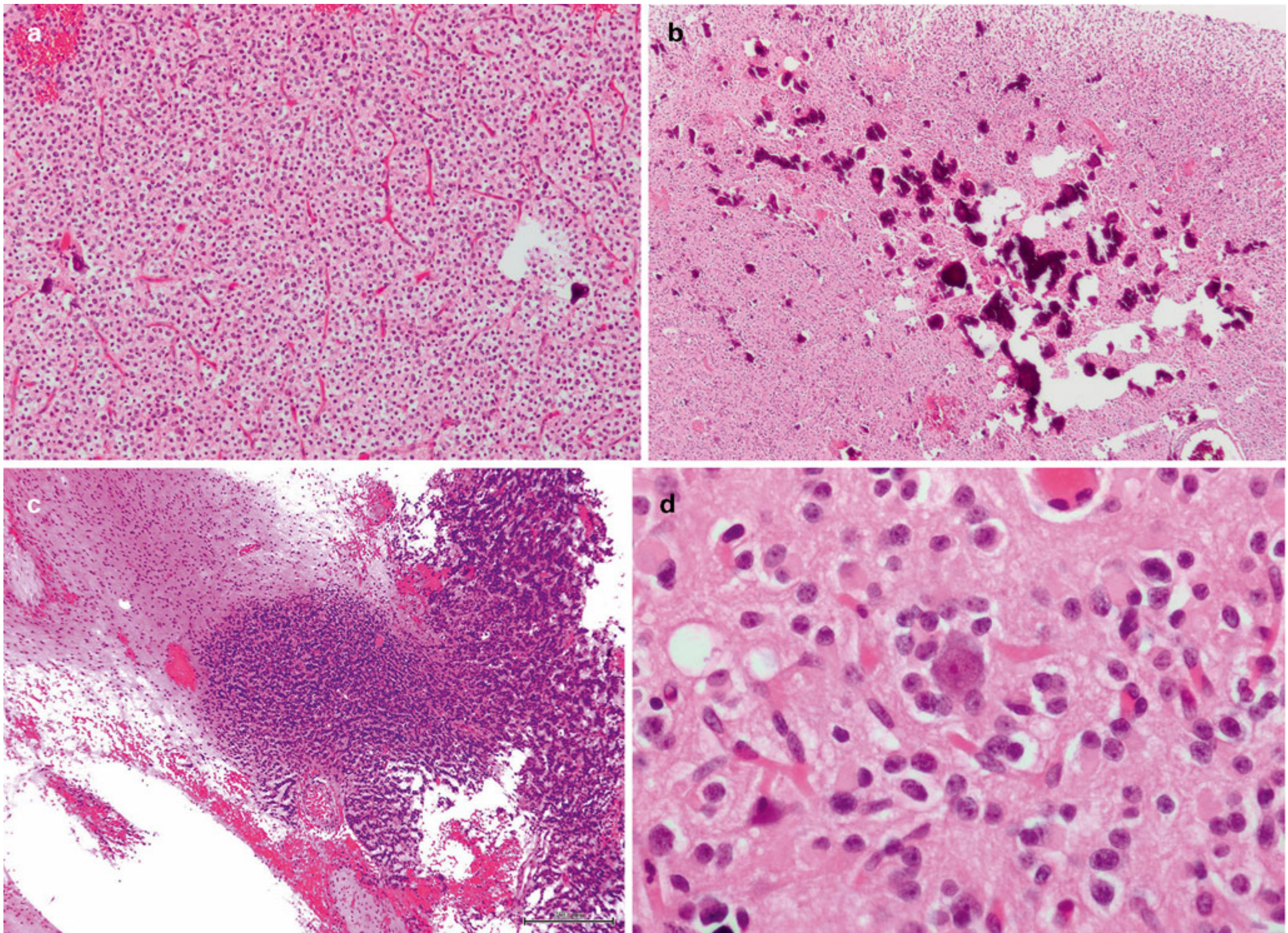

Fig. 8.2 (**a**) ODG displays classical appearance of perinuclear clearing and fine branching vasculatures, (**b**) frequent deposits of microcalcifications are common in ODG, (**c**) nuclear palisading is sometimes seen in ODG and especially associated with WHO III anaplastic ODG, (**d**) perineuronal satellitoses by ODG tumor cells is a feature shared with other infiltrating gliomas

As mentioned previously, the WHO grading scheme divides ODG with predominantly malignant oligodendroglial morphology into grades II and III, with the latter known as anaplastic oligodendroglioma (AO). The principal features of WHO III AO include predominance of hypercellular foci, worsening cytological pleomorphism, tumor necrosis, conspicuous microvascular proliferation, and significantly elevated mitotic activity. The latter two are particularly associated with aggressive behavior [13]. Presence of necrosis, even of the pseudopalisading variant, might not portend a worse prognosis as long as there is a predominance of tumor cells with classic ODG morphology [14]. Conversely, necrosis in tumors with mixed oligoastrocytic populations is associated with poor outcome.

Given the considerable subjectivity in the histological differentiation of WHO grade II and III oligodendroglial neoplasms, there is an even greater challenge in differentiating between pure ODG and mixed oligoastrocytomas (WHO grade II and III). Recently, this conundrum has extended further to clinical diagnosis of glioblastoma with oligodendroglioma component, or GBMO, vs. glioblastoma, or GBM—both of which are WHO grade IV malignant gliomas with poor prognosis. GBMO, when using strict diagnostic criteria, certainly presents distinctly from GBM under the microscope. Recent studies have also highlighted the uniqueness of the molecular underpinnings in GBMO [15]. It remains contentious whether GBMO is associated with a more favorable outcome compared to GBM, and this is exacerbated by the relatively loose definition of this entity along with the absence of agreed upon molecular biomarkers [16].

Inclusion of subjective histomorphological criteria presents significant problems in ensuring diagnostic uniformity among neuropathologists. This is especially problematic in accounting for the malignant oligodendroglial component in an infiltrating glioma and whether to diagnose a tumor as pure ODG or a mixed oligoastrocytoma. However, the most important issue arises from the vagaries of glioma cell morphology, which can lead to over-diagnosis of

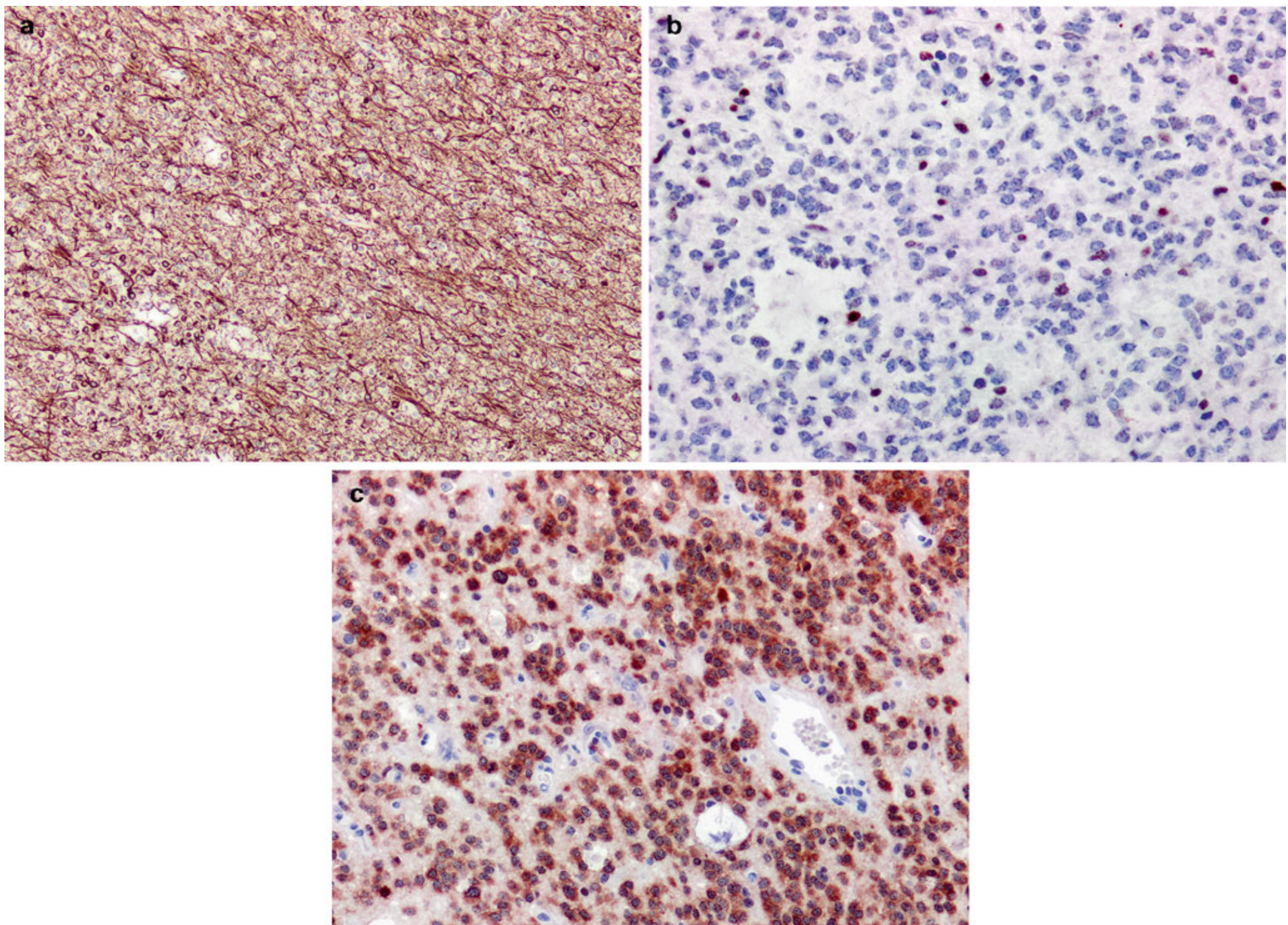

Fig. 8.3 (**a**) ODG is generally immunonegative for GFAP except for tumor cells with gliofibrillary oligodendrocytic and minigemistocytic morphology. There is strong background immunopositivity consistent with infiltrative nature of tumor cells, (**b**) ODG is typically negative for mutations in *TP53* and is reflected in "non-neoplastic" pattern of TP53 reactivity, (**c**) ODG tumor cells display strong cytoplasmic reactivity for IDH1 R132H mutant protein

oligodendroglial neoplasms, pure or mixed. Strong correlation between 1p19q co-deletions and the classical ODG morphology described above highlights association between underlying glioma genetics and histopathology [17]. This is an especially powerful finding given the survival benefits observed in 1p19q co-deleted WHO grade II and III oligodendroglial neoplasms [18, 19].

Clinical Behavior of Oligodendroglial Tumors

The clinical behavior of ODG is characterized by slow progression. LGO have a peak incidence commiserate with all low-grade gliomas, in the third decade of life. There is no clear gender or race distribution. The most common clinical presentation is seizures. Seizures, rather than focal neurologic deficits such as hemiplegia, are thought to be the presenting symptom due to the slow growth rate and infiltrative pattern of LGO, leading to brain irritation rather than to mass effect (source?). However, in some cases of AO where the tumor may grow more rapidly, patients may present with focal neurologic symptoms such as weakness, sensory alteration, vision change or personality change depending on the specific location of the tumor. Alternatively, patients with rapidly growing AO may present with more general symptoms of elevated intracranial pressure such as new and persistent headache or confusion. However, as above, new onset seizure is the most common presentation for ODG (50–90 % of patients) [2, 20].

The event of a first time seizure in an adult often leads to urgent medical evaluation including a computed tomography (CT) of the head or a magnetic resonance imaging (MRI) of the brain. Head CT will commonly show an area of hypodensity and in the case of more chronic tumors, may show punctate hyperdensities consistent with calcification or

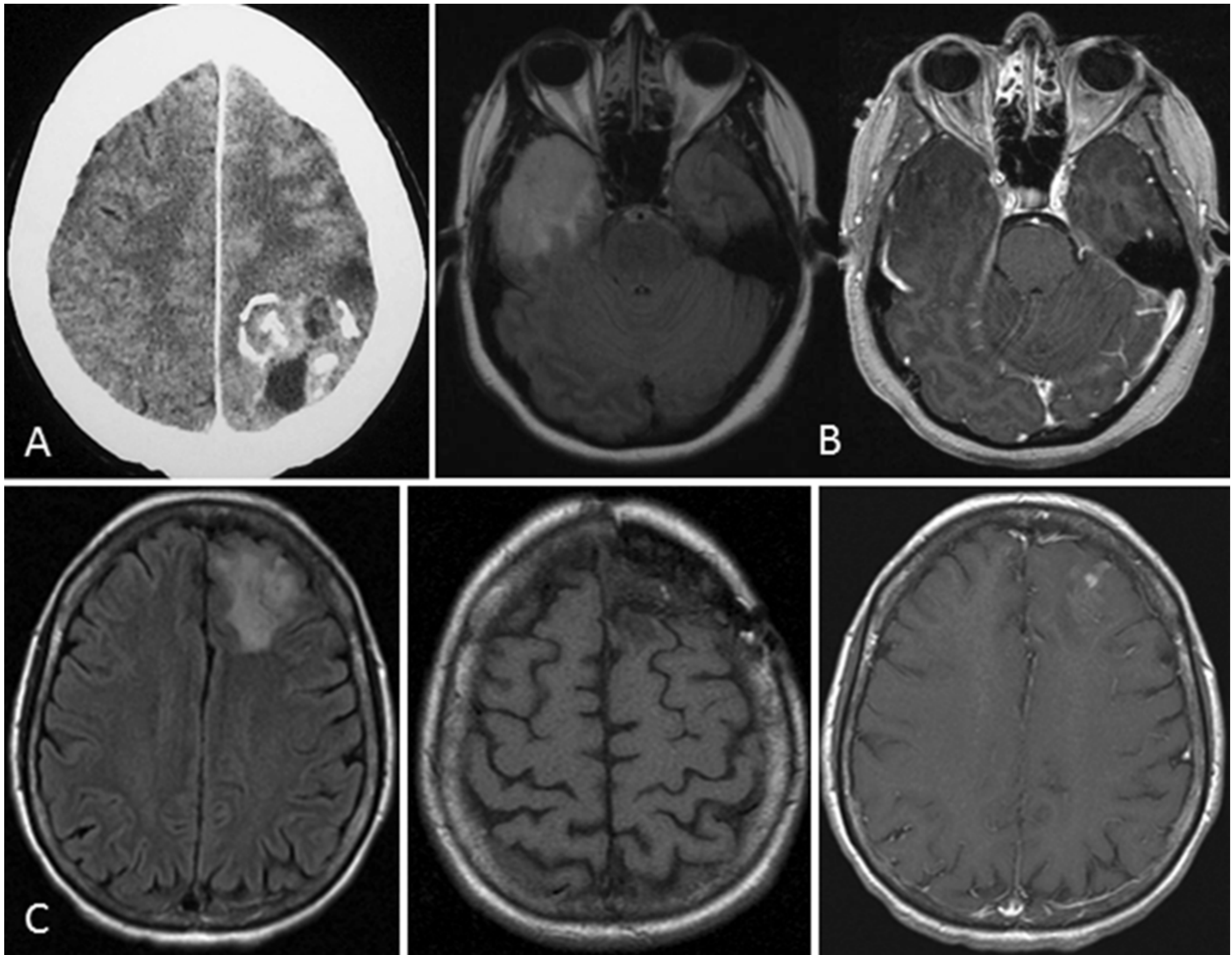

Fig. 8.4 Classic imaging features of ODG. (**a**) computed tomography scan showing hyperdensities consistent with calcification and possibly, microhemorrhages; magnetic resonance imaging (MRI) of AO with (**b**) FLAIR, (**c**) T1w, and (**d**) T1wGd sequences

microhemorrhages (Fig. 8.4a). The typical MRI features for a LGO include diffuse hyperintensity on T2-weighted or fluid attenuated inverse recovery (FLAIR) imaging sequences, a lack of gadolinium contrast enhancement, and normal diffusion weighted imaging (Fig. 8.4b). In comparison, the characteristic MRI features for AO include a mixed-intensity central core with diffuse surrounding signal hyperintensity on T2-weighted or FLAIR sequences and hypointensity on T1-weighted sequences, with some scattered hyperintensity due to calcification or microhemorrhage, as well as heterogeneous enhancement after administration of gadolinium [21–23] (Fig. 8.4c). Importantly, the presence of contrast enhancement does not uniformly predict a higher histological grade. In fact, in a recent study of MRI features of ODG, 28 % of AO did not have gadolinium (Gd) enhancement whereas 20 % of LGO did demonstrate Gd enhancement [24].

Functional MRI has recently been applied to ODG for the purpose of predicting grade as well as allele status (1p19q co-deletion vs. intact), since 1p19q status has been confirmed as both a prognostic and predictive marker [25, 26]. For example, perfusion-weighted imaging and proton MR spectroscopy added to standard anatomical imaging result in 72 % accuracy (83 % sensitivity and 65 % specificity) for distinguishing tumors with intact vs. co-deleted 1p/19q [25]. Although applying functional MRI and metabolic imaging remains experimental, interesting observations have been made that may offer insights into the molecular behavior of ODG subtypes in vivo. Specifically, when the metabolic radiolabelled tracers ^{201}Thallium (^{201}Tl) and ^{18F}fluorodeoxyglucose (^{18F}F-FDG) were assessed in patients with LGO, AO, and mixed oligoastrocytoma (with and without 1p19q co-deletion), 80 % of high-grade tumor had markers of elevated metabolism as might be expected [27].

Interestingly, LGO with 1p19q co-deletion also had an elevated metabolism profile compared to tumors without this molecular marker. In summary, the unique features of ODG tumors are increasingly being explored with specialized imaging techniques in the context of molecular discoveries, with the goal to better understand disease biology of these tumors and aid in the clinical management.

The time of disease progression for both LGO and AO is highly variable. Traditionally, factors such as age at diagnosis, degree of surgical resection, and tumor grade were considered the major predictors. Recently, based on data from the European Organization for Research and Treatment of Cancer (EORTC) study 26951 for AO [28], nine variables including adjuvant treatment received, age, tumor location (frontal or other), extent of resection, WHO performance status, the presence of endothelial abnormalities or necrosis, and finally, 1p/19q co-deletion and *IDH1* mutation status, were shown to be important prognostic factors. Based on these factors, they divided patients into low, intermediate, and high risk groups, with the best prognosis being associated with patients with age of diagnosis <40 years, a good performance status, tumor location in the frontal lobe, confirmed extensive resection without residual tumor on imaging, absence of endothelial abnormalities or necrosis, and 1p/19q co-deletion with *IDH1* mutation. For patients with AO who met all of these criteria, the median overall survival (OS) was 127 months (95 % CI: 95 months—not reached).

Treatment of Oligodendroglial Tumors

The first step in establishing a treatment plan for any patient with a suspected diagnosis of glioma is surgery. Surgery serves the role of both obtaining tissue to confirm the histology and molecular subtype and offering an opportunity for cytoreduction. Although the advanced imaging techniques discussed above may help in distinguishing tumor subtypes with increasing confidence, tissue is required for diagnostic confirmation. As discussed below, it is now widely accepted that initial molecular studies in any tumor suspected of being ODG should include analysis of 1p19q status. Studies such as *IDH1*/2 status and *MGMT* promoter methylation are also commonly obtained at the time of initial diagnosis, as these allow for molecular subclassification and have impact on both overall prognosis and treatment decisions.

The first therapeutic intervention for all ODG is maximum safe surgical resection. There are a series of single center studies that all support the conclusion that gross total resection of the entire area of tumor involvement based on abnormal MRI signal is optimal as long as this can be accomplished safely without causing significant postoperative neurologic deficits [5, 29–31]. If significant tumor debulking is not feasible due to tumor location and risk for neurologic injury, the options are biopsy to confirm diagnosis, or if LGO is suspected, it may be reasonable to proceed with cautious surveillance in select patients until clinical or radiologic evidence of tumor progression. In widely infiltrative tumors where there is no clearly optimal location for diagnostic sampling, recent studies suggest that functional imaging techniques (diffusion tensor imaging, MR spectroscopy, perfusion imaging) may help determine the optimal location of tissue sampling [32, 33].

When the diagnosis of an AO is confirmed histologically, the next question to address is 1p19q status. For patients with 1p19q co-deleted AO, there is level one evidence in support of the use of procarbazine, carmustine (CCNU), vincristine (PCV) either before or after radiation therapy [7, 8]. This is based on two independent studies, EORTC 26951 and RTOG 9402, that had very similar conclusions despite slightly different designs. In EORTC 26951, patients with newly diagnosed AO were treated with RT alone vs. RT followed by up to six cycles of PCV [8]. At median 5-year follow-up, there was a significant difference in progression free survival (PFS), but not OS between the RT+PVC group and the RT alone group regardless of 1p19q status. However, at median 140 months follow-up, in patients with 1p19q co-deleted AO treated with RT+PCV median OS had not been reached vs. median OS of 9 years in patients treated with RT alone. In the RTOG 9402 trial, patients with AO were randomized to either four cycles of PCV followed by RT or RT alone. Similar to the EORTC study, there was no statistically significant difference in median OS at 5 years or at 12 years when all AO patients are analyzed in aggregate. However, at 12 year follow-up, the subpopulation of patients with 1p19q co-deleted AO treated with PCV+RT had a median OS of 14.7 vs. 7.3 months for those treated with RT alone [7] In contrast, there was no statistically significant difference in median OS for patients without 1p19q co-deletion treated with RT alone vs. RT+PCV. In summary, for patients with 1p19q co-deleted AO, there is strong evidence in support of a treatment paradigm that incorporates PCV and RT, however, there is no clear data to support a specific sequence (Table 8.2).

Table 8.2. Evidence for PCV chemotherapy for 1p19q co-deleted anaplastic oligodendrogliomas.

Study	Tumor grade	Treatment	Molecular subtype	mPFS (years)	mOS (years)
RTOG 9402 [7]	AO	PCV+RT	1p/19q co-deleted (*n*=59)	8.4	14.7
			All others (*n*=89)	1.2	2.6
		RT	1p/19q co-deleted (*n*=67)	2.9	7.3
			All others (*n*=76)	1.0	2.7
EORTC 26951 [8]	AO	PCV+RT	1p/19q co-deleted (*n*=43)	13.1	Not reached
			All others (*n*=114)	1.2	2.1
		RT	1p/19q co-deleted (*n*=37)	4.1	9.3
			All others (*n*=122)	0.73	1.8

Despite the compelling data using PCV, many neuro-oncologists advocate for consideration of RT and temozolomide (TMZ) as first line therapy for patients with newly diagnosed AO. This practice is based on the fact that as alkylator-based chemotherapies, PCV and TMZ have similar mechanisms of action and that PCV is much more toxic, resulting in only 42 % of the patients in RTOG 9402 and 30 % of the patients in EORTC 26951 receiving all prescribed chemotherapy [7, 8]. When the initial reports of these studies were published in 2006, the high rates of toxicity and lack of OS benefit led many neuro-oncologists to apply the regimen of RT + TMZ followed by six cycles of TMZ to AO based on the proven benefit of this regimen in glioblastoma [34–36]. However, despite evidence that TMZ does have activity in AO (newly diagnosed and recurrent), it remains to be proven that TMZ plus RT has similar efficacy to PCV plus RT in patients with newly diagnosed AO [37–41]. Ongoing studies that assess the benefit of RT + TMZ in patients with anaplastic gliomas segregated based on 1p19q status will help further clarify this issue. Specifically, the Chemoradiation and Adjuvant Temozolomide in Non-deleted anaplastic glial tumors (CATNON, NCT00626990) trial is addressing the question of whether adding TMZ to RT improves OS for non-deleted anaplastic gliomas. A concurrent study, a randomized trial of Chemoradiation vs. Radiation vs. Temozolomide in 1p/19q Co-deleted anaplastic gliomas (CODEL, NCT 00887146) was initially designed to assess the impact of RT alone, vs. RT + TMZ, vs. TMZ alone on OS in the setting of 1p19q co-deleted anaplastic gliomas. However, in the wake of the long-term results of the RTOG 9402 and EORTC 26951 studies, it was amended to include three comparator arms: RT + PCV, RT + TMZ and adjuvant TMZ, and TMZ alone. In summary, to date the only level 1 evidence to guide treatment recommendations is for the use of RT + PCV for patients with newly diagnosed 1p19q co-deleted AO, while there remains no agreed upon optimal therapy for non-deleted AO.

There is also no agreed upon optimal therapy for LGO. Even more so than for high-grade tumors, the extent of resection has been associated with better PFS and OS [42]. After maximal safe surgical resection, the treatment options include surveillance, RT, or for patients with 1p19q co-deletion, possibly chemotherapy. RT has been the traditional first line therapy for all low-grade gliomas, including LGO. However, data shows that there is no difference in OS between patients who received RT at the time of initial diagnosis or at the time of progression [43]. To help clarify which patients may benefit from early RT treatment, the definition of high and low risk groups for low-grade glioma has been proposed [44]. The high risk group is defined as having tumor dimension ≥6 cm and astrocytoma histology. Low risk gliomas were defined as tumor <6 cm with oligodendroglioma histology. Additional prognostic factors include 1p19q status, Mini Mental Status Examination score and extent of resection. Another unanswered question is whether there is benefit to adding chemotherapy to RT for LGO. RTOG 98-02 assessed the effect of RT + PCV vs. RT alone on OS. There was no statistically significant difference between the RT + PCV and RT groups, although there was an improvement for the combined therapy group in PFS (5-year PFS 63 % RT + PCV vs. 46 % RT) alone (HR, 0.6; 95 % CI, 0.41–0.86; $p = 0.06$; log-rank $p = 0.005$) [45]. The ongoing Eastern Oncology Group E3F05 study: Radiation Therapy with or without Temozolomide in Treating Patients With Low-Grade Glioma (NCT 00978458) is assessing the benefit of RT alone vs. RT + TMZ as first line therapy in patients with symptomatic or progressive low-grade gliomas. Finally, there remains the question about whether RT should be deferred for LGO with chemotherapy being offered as first line therapy. A series of studies have suggested that TMZ shows significant activity in patients with LGO, with the most robust effects seen in patients with 1p19q co-deletion, *IDH1* mutation status, and *MGMT* methylation [46–49].

Molecular Genetics of ODG: Predictive, Prognostic, and the Impact on Treatment Decisions

The discovery of associations between ODG histomorphology, 1p19q co-deletion, and clinical outcome resulting from slow and predictable natural history and chemosensitivity remains a landmark in modern neuro-oncology and pathology [50]. Initial observations of a favorable response to the PCV regimen have been extended to TMZ [48, 51]. Due to its strong association with chemoresponsiveness, molecular testing for 1p19q co-deletion, either via fluorescence in situ hybridization (FISH) or loss of heterozygosity (LOH) assays, in gliomas with oligodendroglial features on histology has become a standard diagnostic adjunct in modern neuro-oncology (Fig. 8.5) [52, 53]. The unique yet simple histology

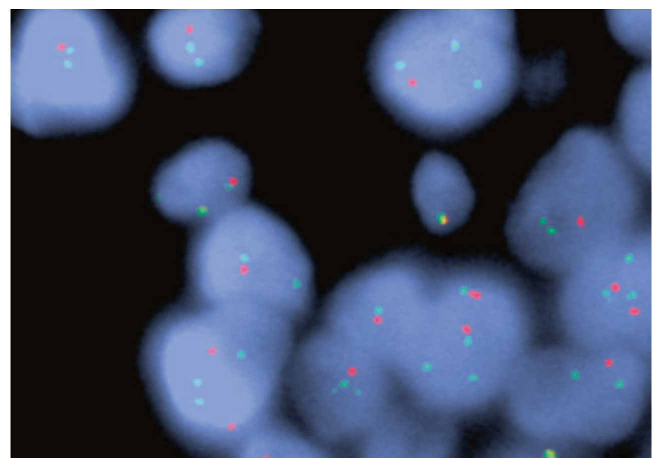

Fig. 8.5. 1p FISH shows individual ODG tumor cells contain relatively more 1q chromosomes (*green* probe) compared to 1p chromosomes (*red* probe) consistent with 1p deletion.

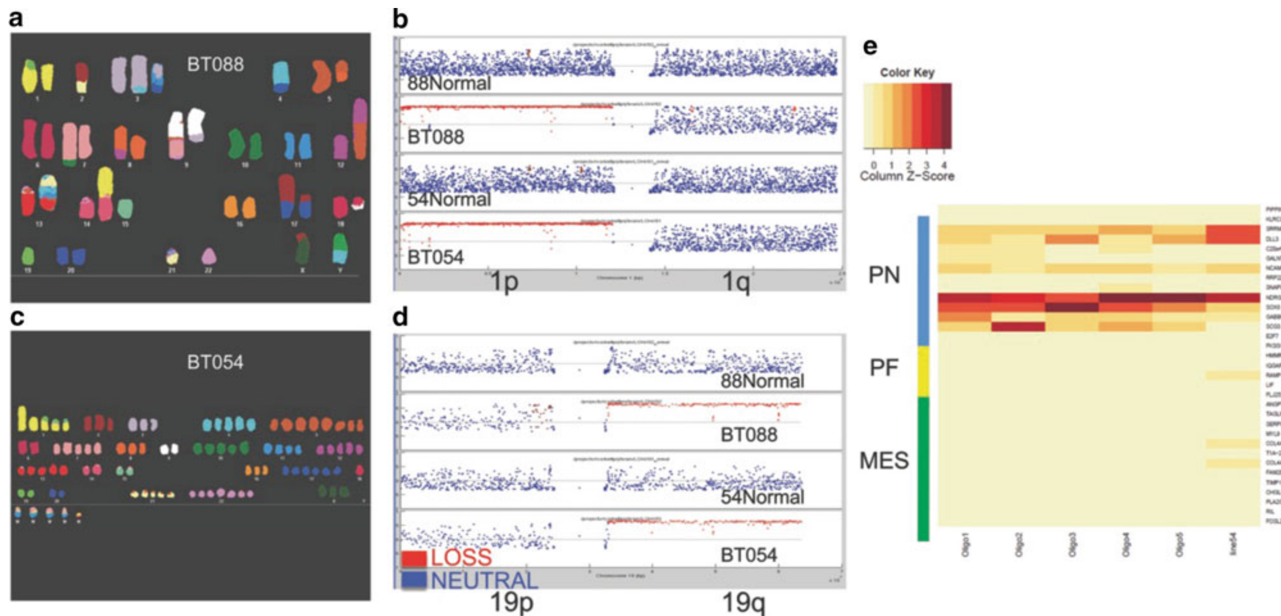

Fig. 8.6. Next generation sequencing of two 1p19q co-deleted brain tumor initiating cell lines derived from ODG patients (**a**, **c**) confirmed copy number losses of 1p and 19q (**b**, **d**) as well as proneural gene expression signature compatible with ODG (**e**).

of ODG also belies other distinctive molecular features that span the genomic, transcriptomic, and epigenomic realms, which will be explored in detail below [54–58]. A recent report highlighted how a genetic variant rs55705857 in 8q24.21 is associated with ODG development in a background of mutant *IDH1/2* [59]. This study suggests that germline variants may be important factors in acquiring OGD, and perhaps other gliomas as well.

Fundamentally, ODG is defined by 1p19q co-deletion, or more specifically, unbalanced translocations of chromosomes 1p and 19q [60, 61]. This essentially leads to loss of one copy each of 1p and 19q, resulting in loss of heterozygosity (LOH) affecting the regions of numerical loss. Moreover, 1p19q co-deleted anaplastic ODG that exhibits polysomy for chromosomes 1p and 19q has intermediate survival between 1p19q retained tumors and 1p19q co-deleted ODGs in a euploidy background [62, 63]. This suggests that accumulation of additional genetic aberrations, such as those regulating cell cycling and homologous recombination that contribute to the polysomy state, might contribute to earlier recurrence. An issue raised by these data is the importance of the specific assay used for determination of 1p19q status, since only FISH (and not LOH PCR) permits the enumeration of absolute numbers of chromosomes and determination of ploidy status.

In addition to 1p19q co-deletion as a unique biomarker of ODG chemoresponsiveness, early studies have identified somatic mutations in *TP53* as virtually restricted to astrocytomas and not occurring in ODG [11, 64]. Whole exome sequencing has confirmed this early finding in untreated ODG [65]. Another significant molecular feature of OGD is the strong association with mutations of *IDH1/2* [66]. In the same study, 99 of 107 WHO II/III pure ODG tumors showed 1p19q co-deletion or loss of heterozygosity of which 90 contain *IDH1* and three contain *IDH2* mutations [67]. In a review of published studies by Kloosterhof and colleagues, they found 76 % and 67 % *IDH1* and 4 % and 5 % *IDH2* mutations in LGO (WHO II) and AO (WHO III), respectively [68]. These percentages are similar to WHO II/III infiltrating astrocytomas and mixed oligoastrocytomas. Heterozygous neomorphic mutations in *IDH* genes result in the generation of the onco-metabolite 2-hydroxyglutarate or 2HG [69, 70]. 2HG has significant pleiotropic effects on the tumor epigenome, leading to aberrant histone regulation and development of the glioma hypermethylator phenotype [71–73]. This important discovery has generated leads for practical applications in improved diagnosis and treatment of *IDH* mutated gliomas [74–76]. Prior to the discovery of recurrent mutations in the *IDH* genes, the sole focus was on uncovering genetic candidates within the 1p and 19q LOH regions and particular effort on the former given its stronger association with the ODG phenotype and superior clinical performance [77]. Obvious candidates such as *NOTCH2*, located at 1p11 have been extensively investigated [78, 79]. Identification of recurrent somatic mutations in *CIC*, a gene that resides in chromosome 19q, is 69 % of ODG with 1p19q co-deletions and *IDH* gene mutation using next generation sequencing (NGS) [66, 80]. NGS excels at the unprecedented depth and breadth of profiling of the transcriptome, exome, and genome of cancer. NGS has refined many long-standing molecular findings of ODG at basepair resolution (Fig. 8.6). Data from subsequent studies have

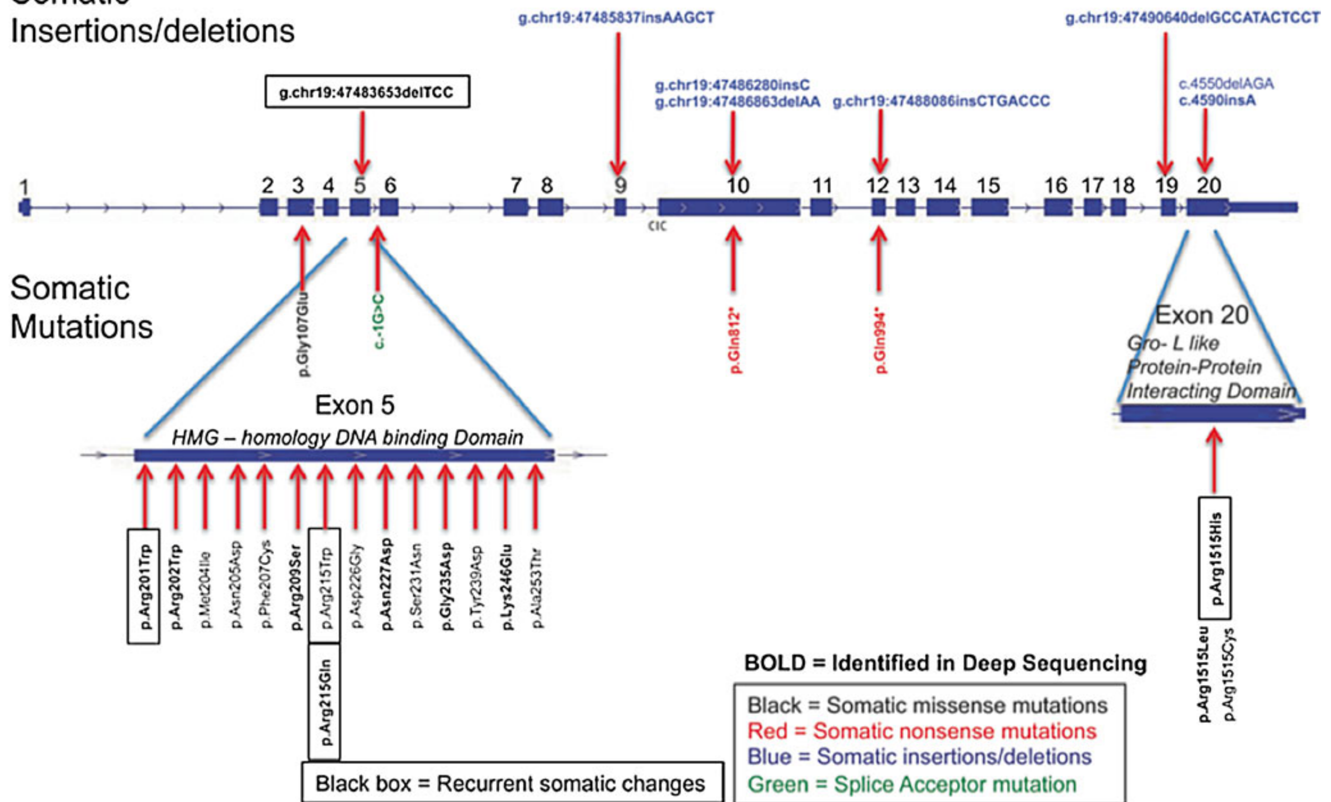

FIG. 8.7. Somatic mutations in the remaining allele of *CIC* in 19q are distributed across the gene but cluster in exons 5 and 20, which code for functionally crucial domains.

added to these initial findings [81, 82]. The *CIC* gene encodes the mammalian homolog of *Capicua*, a transcriptional repressor in *Drosophila* that is essential for inhibition of RAS/MAPK signaling [83–85]. There is a high level of cross-species homology of the *CIC* gene, especially in the functionally critical DNA-binding and protein-interacting domains. Somatic *CIC* mutations in oligodendrogliomas most commonly cluster in the HMG-homology DNA-binding domain in exon 5, and recurrent mutations affecting codons 201, 202, and 215 have been identified (Fig. 8.7). The second most common recurrent mutations are clustered within the exon 20 protein-interacting Gro-L homology domain. A slightly lesser number of *CIC* mutations are scattered throughout the gene. Strikingly, somatic *CIC* mutations are very strongly associated with 1p19q co-deleted and *IDH* mutated OGD, and only found in 2 % of astrocytomas not associated with 1p19q co-deletion. Importantly, the majority of the *CIC* mutations in this cohort are located outside of exons 5 and 20. This highlights potential selective pressure on the development of *CIC* mutations in ODG and also the functional significance of these domains. At this time, very little is known about the mechanistic role of CIC in brain tumor development, and hence the impetus to pursue ongoing impetus to pursue ongoing functional investigations into its role functional investigations into its role in the development of this tumor [86]. The close association of CIC mutations with *IDH* mutations in 1p19q co-deleted ODG suggests that both cooperate in tumorigenesis, which may require a pro-oncogenic environment facilitated by the presence of 2HG. However, it remains unclear whether *CIC* mutations represent loss-of-function or gain-of-function, and how they contribute mechanistically to the development of ODG. Somatic mutations in *FUBP1*, located in 1p, also appear to cluster in 1p19q co-deleted ODG [80, 81]. Inactivating mutations of *ATRX*, which code for a chromatin remodeler, are virtually exclusive to astrocytomas and mixed oligoastrocytomas and rarely found in ODG, whereas *CIC* and *FUBP1* mutations are preferentially associated with ODG and only discovered in 10 % of the former [82]. Therefore, ODG biology and clinical behavior are governed

by the complex interactions of the presence and absence of unique genetic alterations. These emerging data highlight the need to develop a molecular classification for these tumors that goes beyond histology and accurately reflects entities with distinct biology and clinical behavior.

Gene Expression in OGD

ODG with 1p19q co-deletion exhibits a proneural gene expression signature that is associated with a more favorable prognosis [54, 57], in keeping with similar findings in glioblastoma [87]. One of the differentially expressed genes dependent on 1p19q status, *α-internexin* [88], can be easily interrogated with economical, automated immunohistochemical (IHC) testing in lieu of molecular genetic testing. Another example of a biomarker associated with clinical tumor behavior that can be assessed by IHC is EGFR protein expression in ODG [89].

Epigenetics of ODG

In addition to somatic DNA aberrations, the epigenome of brain tumors, including OGD, has emerged as a key driver of tumor biology, as illustrated by the broad epigenetic consequences of *IDH* mutations. LOH can be mediated genetically via the acquisition of mutations in the remaining allele or epigenetic silencing of regulatory regions of a gene in the remaining allele [90]. Therefore, epigenetic modification of genes located within 1p and 19q, such as *CIC* and *FUBP1*, could perhaps provide for an alternative mechanism of gene silencing. Transcriptome studies of 1p19q co-deleted ODG have confirmed the selective down-regulation of *SLC9A1* gene expression in the absence of somatic mutation. This gene, located in chr1p36.1, codes for a sodium/hydrogen exchanger essential for the maintenance of intracellular pH [91]. Attenutation of *SLC9A1* gene expression in 1p loss tumors is a result of promoter hypermethylation and reversible upon the introduction of 5-azacytidine. Aberrant expression of this protein results in significantly reduced intracellular pH which affects acid load recovery in ODG that may partly explain the biological and phenotypic difference between ODG and other infiltrating gliomas. Hypermethylation of the *MGMT* promoter is associated with improved outcome in glioblastoma patients receiving concurrent TMZ and ionizing irradiation [92]. However, in AO a hypermethylated phenotype is a stronger predictor of survival than selective *MGMT* methylation [93, 94]. This so-called G-CIMP (glioma—CpG island methylator phenotype) signature is tightly associated with IDH-mutant low-grade gliomas with proneural gene expression signature [95]. Therefore, one can start to appreciate the interconnectivity between the genomic, transcriptomic, and epigenomic spaces of ODG and the significance of an integrative approach to studying this tumor.

Future Directions

Evolving concepts of ODG molecular pathology are directly impacting the way this tumor is managed clinically. Traditional reliance on subjective descriptive terminology is slowly being replaced by objective molecular findings, with a significantly stronger association with outcome and response to specific targeted therapeutics [96, 97]. Introduction of advanced diagnostic platforms in routine laboratory workflow has provided the opportunity to profile large cohorts of ODG at an unprecedented depth and breadth. Both the Ion Torrent PGM and Illumina MiSeq sequencing platforms accommodate deep amplicon sequencing suitable for DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissues [98]. Since FFPE is the common currency of pathology labs, the ability to extract potentially informative genetic material for multiplex deep-sequencing assays from archival samples will dramatically alter the landscape of genetic-clinical outcome correlative studies by increasing the number of eligible study cases (Fig. 8.8) [98, 99]. Similarly, introduction of the Nanostring nCounter platform that can perform multiplex gene expression analysis of FFPE-derived mRNA has opened up limitless research opportunities as illustrated in several recent publications [100, 101]. A custom designed codeset was able to distinguish between ODG and other brain tumors (Fig. 8.9).

Infiltrating glioma is an ideal disease model that lends itself to deeper interrogation of spatial and temporal heterogeneity of cancer. Given the diverse histology of tumor cells within GBM, the discovery of substantial underlying genomic heterogeneity is perhaps not surprising. For example, a recent landmark study has demonstrated mosaic amplification of growth factor genes in GBM [102]. This raises the fundamental question whether tumors with more homogeneous and regular histology, such as ODG, also exhibit underlying genomic heterogeneity. One of the authors (SY) has embarked on a study to address this question via deep sequencing and gene expression profiling of F-DOPA-PET-guided biopsy specimens of WHO II/III infiltrating gliomas including ODG. Tumor from a patient may yield several spatially unique tissue samples based on F-DOPA signatures—these are subjected to NGS to reveal allelic frequencies of mutations and to enumerate expression of selected genes. Clinical follow-up and repeated metabolic imaging permits for the association of metabolic signatures, genomic and transcriptomic profiles, and location of disease recurrence to generate a “radio-metabolo-genomic” signature of glioma recurrence. This epitomizes the emerging concept of “integrative diagnostics” which amalgamate traditional and functional imaging with pathology and advanced molecular diagnostics. This may not only direct the use of currently available therapies, but lead to

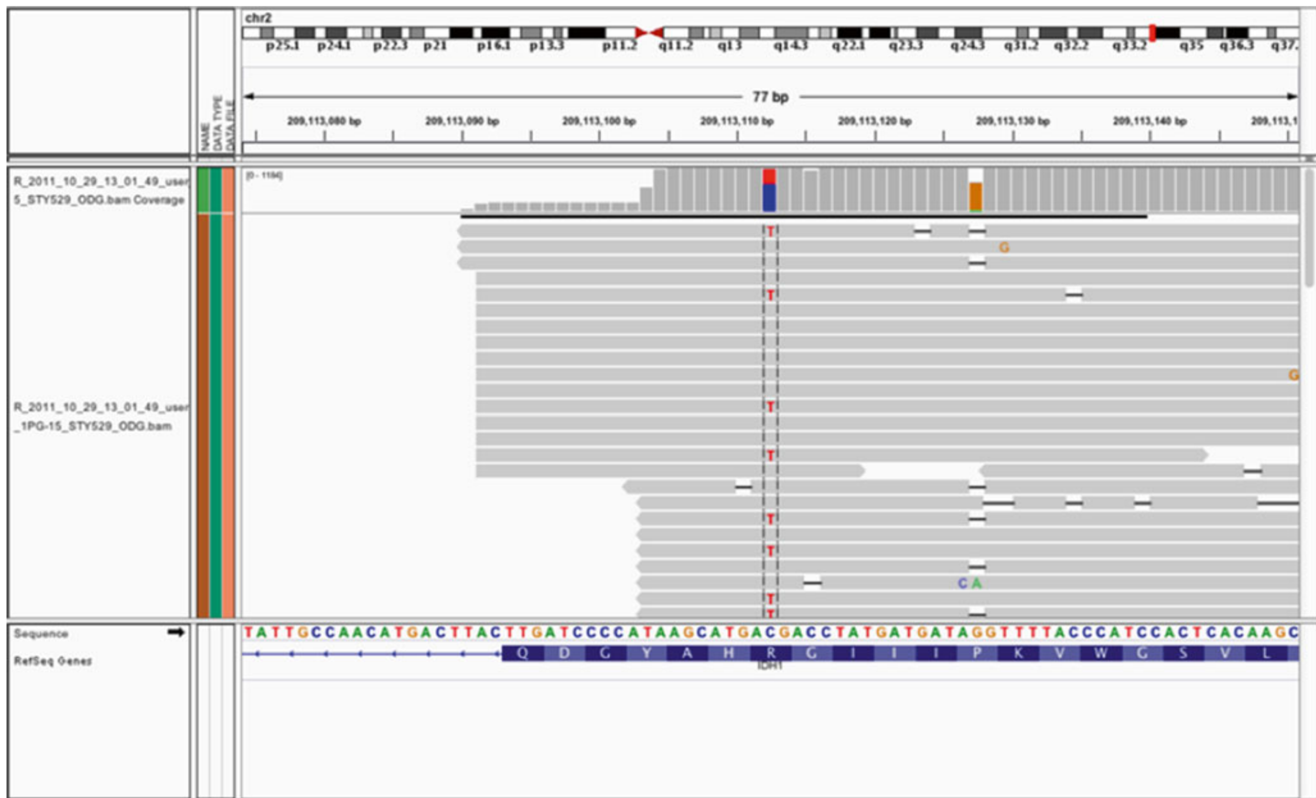

Fig. 8.8. Deep amplicon sequencing of 10 ng of FFPE DNA from an ODG using the AmpliSeq cancer panel on the Ion Torrent PGM sequencing platform reveals heterozygous mutation leading to R132H change in *IDH1*.

the identification of new targetable nodes and therefore, the development of new therapies.

Such scientifically driven advances are desperately needed, as although the last 5–10 years have been full of great discovery for ODG tumors, they remain an enigmatic entity and a cure is still out of reach today. However, there is promise for further advancement in the near future. Historical observation of good clinical behavior was supplemented by discovery of strong association with 1p19q co-deletions, that later transcended into unique gene expression and epigenetic features of ODG. NGS has allowed for unbiased and genome-wide interrogation of ODG which resulted in the discoveries of *CIC* and *FUBP1* mutations. However, these are unlikely to be the sole molecular drivers and not all mutations can be readily converted to therapeutic targets. Hence, ongoing investigation into the wide range of genetic and epigenetic components in addition to a permissive metabolic milieu permitted by *IDH* mutations, for example, is required. Ongoing efforts by the TCGA low-grade glioma consortium will significantly augment our knowledge of this enigmatic tumor. Fortunately, the field is well versed in the practice of clinical-translational research allowing close collaboration between laboratory and clinical scientists that will yield the next treatment breakthrough.

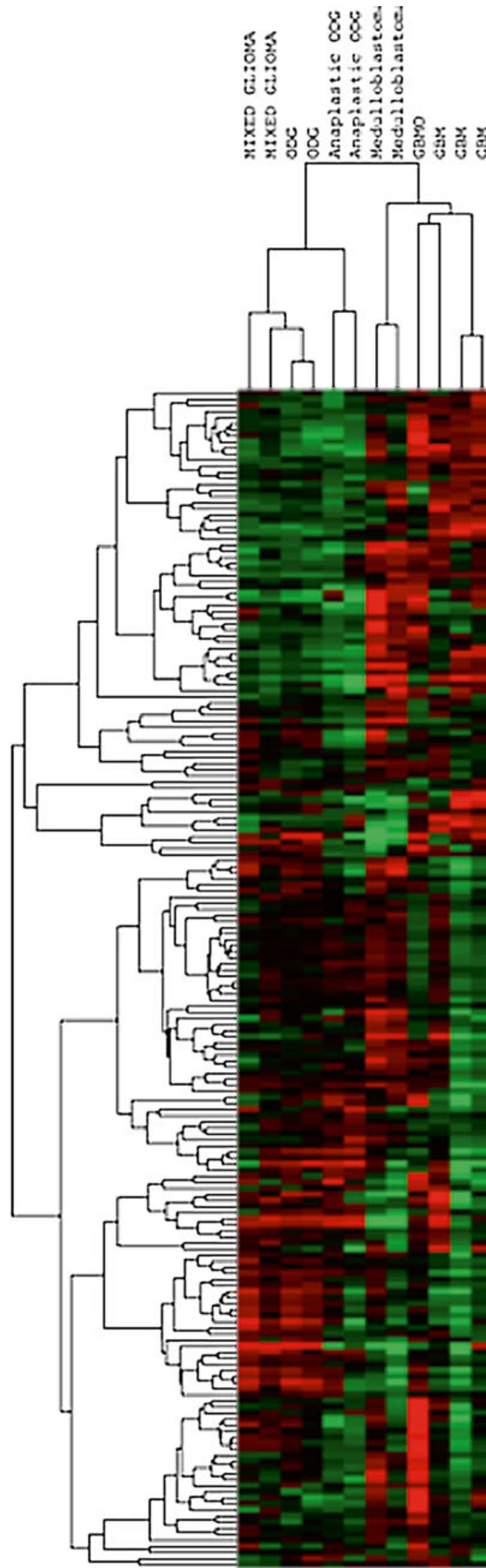

Fig. 8.9. Unsupervised hierarchical clustering of gene expression data generated from 250 ng mRNA extracted from FFPE blocks of various brain tumors show distinctive clustering pattern.

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9 Medulloblastoma and CNS Primitive Neuroectodermal Tumors

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Medulloblastoma

Medulloblastoma is a malignant (WHO Grade IV) small round blue cell tumor of the cerebellum that constitutes one of the most common malignant brain tumors in children [1]. The disease has a peak age of onset of ~7–8 years, but is also diagnosed in young infants and well into adulthood. Males are more commonly affected than females, with a male:female ratio of ~1.5:1. Current treatment strategies for medulloblastoma include a combination of surgical resection, craniospinal radiation (in children older than 3 years), and cytotoxic chemotherapy. Five-year overall survival rates for standard-risk patients (i.e., patients older than 3 years with less than 1.5 cm^2 residual disease and no evidence of metastasis) are in the range of 70–80 %. In contrast, patients currently stratified as high-risk, including those that are less than 3 years old, patients with greater than 1.5 cm^2 residual disease, and/or those with metastatic dissemination, typically have inferior outcomes. Patients that are effectively cured of their disease currently face significant challenges and treatment-related sequelae, including pronounced developmental, neurological, and psychosocial deficits.

The WHO currently recognizes five distinct histological subtypes of medulloblastoma: classic, desmoplastic, medulloblastoma with extensive nodularity (MBEN), large cell, and anaplastic (Fig. 9.1) [1]. The classic medulloblastoma subtype accounts for the majority of cases, followed by the desmoplastic subtype, collectively accounting for ~80 % of cases, and the other subtypes comprising the remainder. Numerous studies have demonstrated improved survival rates for desmoplastic/nodular medulloblastoma, which is contrasted by an inferior prognosis usually encountered with large cell/anaplastic disease [2–7]. Varying degrees of desmoplasia, anaplasia, and intratumoral heterogeneity can make the diagnosis of these histological subtypes difficult and subjective, confounding consistency in diagnoses.

Understanding the molecular biology underlying medulloblastoma is currently an area of intense interest among the pediatric neuro-oncology community. It is anticipated that knowledge gained from genomic and biological studies will translate to more accurate and consistent diagnoses, improved risk-stratification schemes, and the development and implementation of molecularly targeted therapies that are more effective and less toxic.

Molecular Genetics of Medulloblastoma: A Historical Perspective

Recurrent cytogenetic aberrations have been observed in medulloblastoma specimens for several decades [8]. The presence of an isochromosome 17q (i[17]q), essentially resulting in the net loss of one copy of the chromosome 17 p-arm and a net gain of one copy of the q-arm, is a signature event in medulloblastoma, found in up to 50 % of patient samples (Fig. 9.2a). Other chromosomal abnormalities that are commonly encountered include gains of chromosomes 1q and 7 and losses of chromosomes 6, 8p, 9q, 10q, 11, 16q, and X.

High-level amplification of the *MYC* proto-oncogene in the form of double-minute chromosomes has been observed for more than two decades, reported to occur in 5–10 % of patients (Fig. 9.2b). Historically, amplification of *MYC* has been recognized as a marker of poor patient outcome, often occurring in patients with a particularly aggressive form of the disease [3, 9–16].

Rare familial tumor syndromes, namely Gorlin and Turcot Syndromes, have provided considerable insight into the genetics underlying specific subsets of medulloblastoma patients [17]. Gorlin Syndrome (also referred to as Nevoid Basal Cell Carcinoma Syndrome, NBCCS) is an autosomal dominant disorder that results in abnormal facial and skeletal phenotypes, with affected individuals prone to the

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Fig. 9.1. Histological subtypes of medulloblastoma. (**a**) Classic histology; (**b**) desmoplastic histology; (**c**) medulloblastoma with extensive nodularity (MBEN); (**d**) large cell/anaplastic (LC/A) histology.

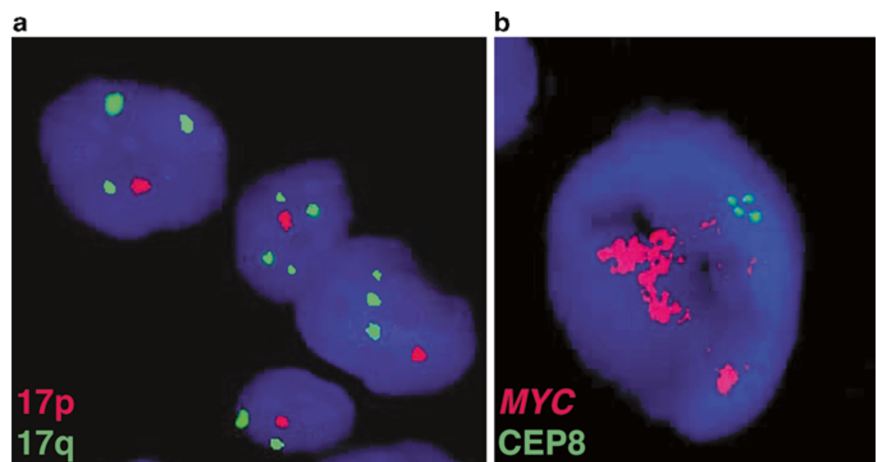

Fig. 9.2. Historical cytogenetic aberrations of medulloblastoma. (**a**) Interphase FISH performed on a medulloblastoma sample exhibiting copy number imbalance on chromosome 17, characterized by deletion of 17p and duplication of 17q (i[17]q). (**b**) A medulloblastoma sample with high-level amplification of the *MYC* proto-oncogene on chromosome 8q24 as demonstrated by FISH.

development of numerous basal cell carcinomas and predisposed to medulloblastoma. Germline loss-of-function mutations in the *PTCH1* tumor suppressor gene on chromosome 9q are responsible for this disorder [18–20]. PTCH1 is a negative regulator of the Sonic Hedgehog (SHH) signal transduction pathway, an important developmental signaling cascade. As will be discussed in detail below, aberrant activation of the SHH pathway is found in ~25–30 % of all

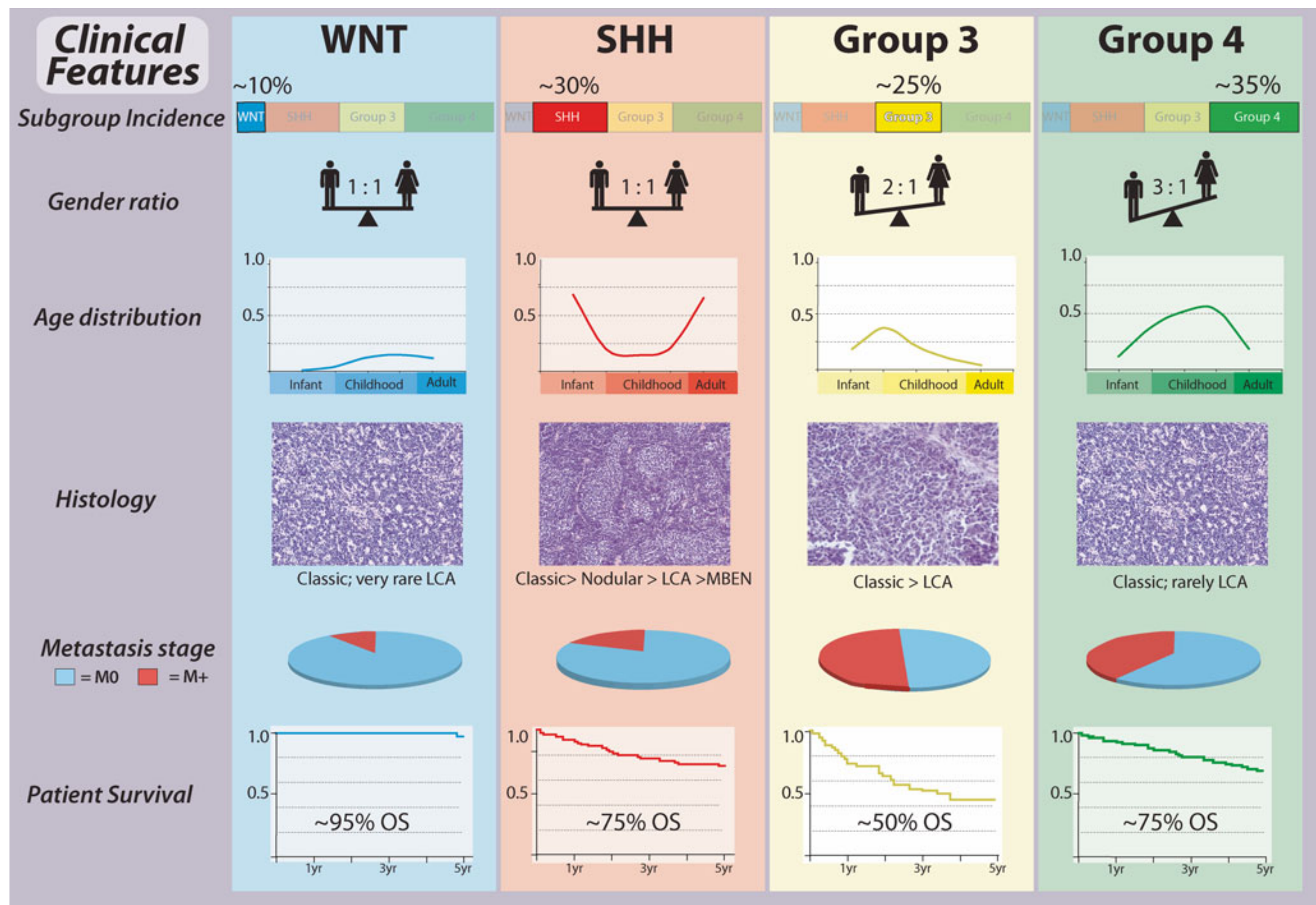

FIG. 9.3. Clinical features of medulloblastoma subgroups. General summary of the clinical characteristics intrinsic to the core molecular subgroups of medulloblastoma. *LCA* large cell and anaplastic, *MBEN* medulloblastoma with extensive nodularity, *OS* overall survival.

medulloblastoma patients and is perhaps the most well characterized molecular pathway involved in medulloblastoma development.

Turcot Syndrome is also an autosomal dominant condition; attributed to germline mutations in either *APC* or the DNA mismatch repair genes *MLH1* or *PMS2*. Affected individuals are predisposed to either medulloblastoma (linked with *APC* mutation) or glioblastoma multiforme (linked with *MLH1* or *PMS2* mutations) [21]. The APC protein functions to control the activity of β-Catenin, the central molecule of the Wingless (WNT) signaling pathway. Activation of WNT signaling is observed in ~10–15 % of medulloblastomas, a distinct subset of patients with a highly favorable outcome, as will be discussed in detail below.

Molecular Subgroups of Medulloblastoma: Discovery and Initial Characterization

The Molecular Subgroup Concept

Clinical outcome of patients with medulloblastoma can be highly variable, irrespective of parameters routinely used in the clinic to predict patient risk (i.e., patient age, extent of resection, and metastatic stage). This is often exemplified by the very disparate therapeutic responses observed among patients with histologically identical disease. Furthermore, survival patterns reported for different age groups of medulloblastoma patients have strongly suggested underlying biological differences that must account for the distinct age of onset and therapeutic response of these patient subgroups [6, 22]. Much of this biological and clinical heterogeneity observed in medulloblastoma is now being explained by the recognition of unique molecular subgroups of the disease, a concept that has been supported for more than a decade now [23].

Hybridization of moderate-to-large series of RNAs isolated from primary medulloblastoma specimens to gene expression microarrays has revolutionized our concept of medulloblastoma as a disease. Based primarily on expression array profiling, numerous studies performed by independent laboratories have reported the existence of discrete molecular subgroups of medulloblastoma [24–28], each characterized by distinct genetics, cytogenetics and transcriptional profiles, as well as patient demographics, tumor phenotype, and clinical behavior (Figs. 9.3 and 9.4) [29].

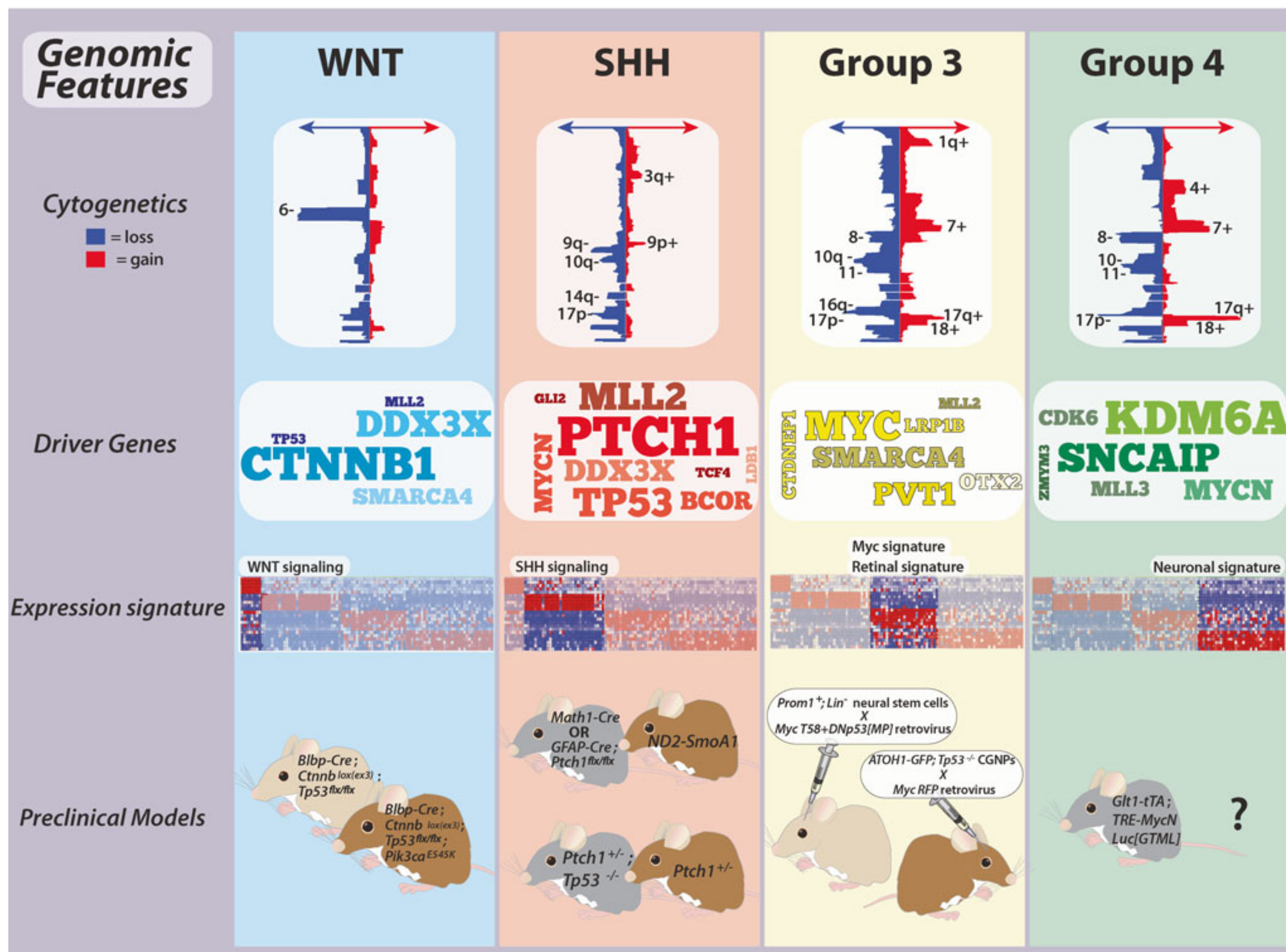

Fig. 9.4. Genomic features of medulloblastoma subgroups. Summary of the genomics of medulloblastoma subgroups, including cytogenetics, prominent driver genes, transcriptional signatures, and available preclinical models.

In late 2010, a consensus meeting attended by leading groups in the medulloblastoma community was held where it was proposed that medulloblastoma should be regarded as consisting of a four-subgroup structure: WNT, SHH, Group 3, and Group 4 [30]. The impact of this consensus report and the studies that precipitated it have changed the way medulloblastoma is both viewed and studied in the basic research setting. Moreover, the implications for the development of clinical trials, revamping patient risk-stratification, and the administration of targeted therapy have been dramatic, all of which are now beginning to take molecular subgroup status into consideration.

WNT Medulloblastoma

The least common of the four consensus medulloblastoma subgroups are those belonging to the WNT subgroup, accounting for just 10–15 % of cases. This subgroup affects a higher than expected proportion of female patients (male:female ratio of ~1:1 compared to an expected ratio of ~1.5:1 for all medulloblastomas) and is predominantly diagnosed in childhood and adolescence, almost never encountered in infants. WNT medulloblastomas are nearly without exception of classic histology and non-metastatic, currently having the best overall prognosis of any patient subgroup with cure rates of almost 100 % [4, 24, 27, 28, 31, 32].

Somatic missense mutations in exon 3 of *CTNNB1* (which encodes β-Catenin) are present in >90 % of WNT medulloblastomas [33]. These mutations constitutively activate β-Catenin and prevent it from being degraded, resulting in its nuclear accumulation and consequent deregulation of WNT target genes. Monosomy 6 is another characteristic genetic feature of WNT subgroup medulloblastomas, highly enriched in this subgroup and found in the vast majority of cases [32, 34]. Immunohistochemistry (IHC) for β-Catenin and fluorescence in situ hybridization (FISH) for chromosome 6 constitute two commonly used “readouts” for WNT subgroup assignment, with either nucleo-positivity for β-Catenin or monosomy 6 acting as capable surrogates for identifying WNT subgroup patients (Fig. 9.5) [35]. More specific assays

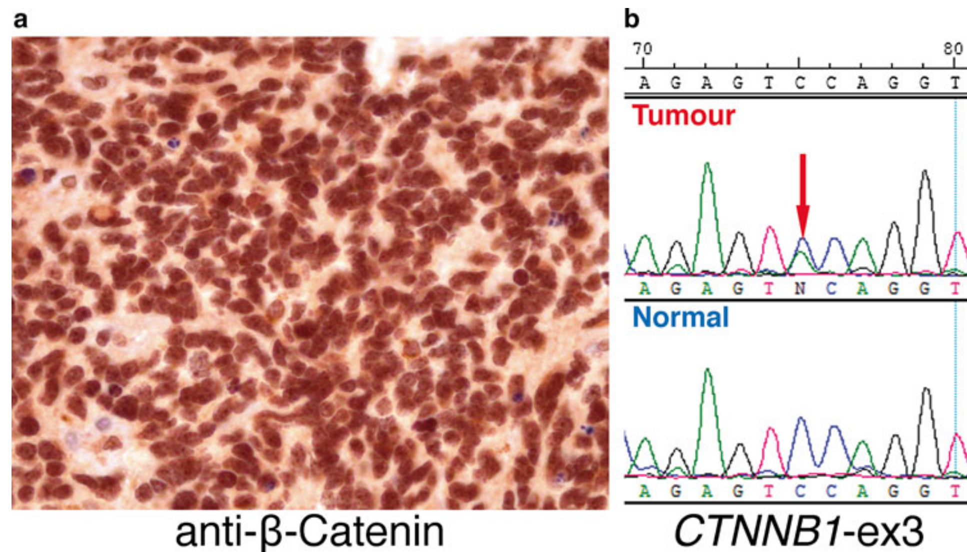

Fig. 9.5. Molecular assays for assignment of WNT medulloblastomas. (**a**) IHC shows strong nucleo-positivity typical of a WNT subgroup medulloblastoma. (**b**) DNA sequencing electropherogram showing a heterozygous *CTNNB1* exon 3 mutation (*red arrow*) present in the tumor DNA of a WNT medulloblastoma (labeled "Tumor") that is absent in matched normal germline control DNA (labeled "Normal") from the same patient.

such as re-sequencing of *CTNNB1* exon 3 performed in combination with additional state-of-the-art molecular profiling methods (discussed below) are now being recommended as more specific and sensitive methods for identifying this important subgroup of medulloblastoma patients.

SHH Medulloblastoma

Medulloblastomas exhibiting aberrant activation of the SHH signaling pathway account for ~25–30 % of cases [32]. In infants (age 3 years or less) and older patients (age 16 years and up), SHH-activated cases predominate, accounting for up to 70 % of patients in these age groups [36]. The gender distribution for SHH subgroup medulloblastomas is comparable to what is seen among all medulloblastoma patients, with an observed male:female ratio of ~1.5:1. True desmoplastic and MBEN histologies appear to be largely restricted to this subgroup [35]; however, large cell/anaplastic (LCA) disease is also encountered, particularly in childhood cases. Metastatic disease (M+) is also observed but relatively uncommon. With respect to patient outcome, SHH cases appear to represent an intermediate prognosis class of patients [32], although specific patient subsets may have highly favorable or disparate clinical outcomes, depending on their underlying genotypes (as will be discussed later) [22, 37].

As was first verified in Gorlin Syndrome patients that develop medulloblastoma, *PTCH1* is the prototypical tumor suppressor in the SHH subgroup, with somatic mutations inactivating PTCH1 confined to this subgroup. *SUFU*, another tumor suppressor functioning as a regulatory component of the SHH signaling pathway, is likewise mutated in this subgroup, both in the germline and somatically [38–40]. Loss of heterozygosity (LOH) on chromosomes 9q and 10q is enriched in SHH medulloblastomas [27, 34], presumably as a mechanism for inactivating the remaining wild-type allele in cases exhibiting *PTCH1* or *SUFU* mutations, respectively. Similar to the use of β-Catenin nucleo-positivity as a biomarker for WNT medulloblastoma, comparable IHC-based methods have been proposed and implemented with modest success for the identification of SHH medulloblastomas, including SFRP1, GLI1, and GAB1, all of which exhibit apparent specificity for this subgroup [27, 28, 35, 41, 42].

Group 3 Medulloblastoma

The generically named Group 3 medulloblastomas account for ~25 % of all cases and appear to be restricted to pediatric patients, with only extremely rare instances reported in adults [42]. There is a male gender bias in Group 3, with an observed male:female ratio of ~2:1. M+ disease is common in Group 3 and has been documented in up to half of these cases. Group 3 subgroup affiliation currently carries with it the most dismal overall survival of the four subgroups, with only ~50 % of these patients or less alive at 5 years from the time of initial diagnosis [32].

Amplification of *MYC* is a characteristic oncogenic event observed in this subgroup, an event that is by and large restricted to Group 3 and found in 15–20 % of cases [34]. Cytogenetic aberrations such as gain of chromosomes 1q and 7, and loss of chromosomes 8p, 11, 16q and i[17]q are

commonly observed in Group 3. Although preliminary biomarkers for Group 3 have been suggested (i.e., *MYC* amplification), there are currently no gold-standard single gene/marker assays for assigning medulloblastomas to this subgroup.

Group 4 Medulloblastoma

Group 4 medulloblastomas represent the most common patient subgroup, accounting for 35–40 % of all cases [32]. These tumors occur across all age groups but constitute the most predominant form of the disease in childhood and adolescence. There is a strong gender bias in Group 4, with an observed male:female ratio of ~3:1. Metastatic disease is observed in approximately one-third of Group 4 patients. Similar to the SHH subgroup, Group 4 patients tend to comprise an intermediate outcome subgroup [32], although there is growing evidence for clinical heterogeneity within this large fraction of patients [43].

Cytogenetically, Group 4 medulloblastomas share some commonalities with Group 3, most notably being the highly prevalent i[17]q which is noted in up to 70–80 % of Group 4's. Chromosome X loss in female Group 4 patients is also a frequent occurrence. Compared to other subgroups, Group 4 medulloblastomas remain the least well understood with regards to oncogenic driver genes, although a few interesting novel candidates have emerged from recent genomic studies, as will be discussed in detail below.

Next-Generation Genomics of Medulloblastoma: Assigning Driver Genes to Medulloblastoma Subgroups

Recent technological breakthroughs in the field of genomics have dramatically improved the resolution at which the cancer genome is studied [44, 45]. Application of high-density microarray platforms and next-generation sequencing (NGS) to medulloblastoma has led to the identification of a host of novel candidate genes that are recurrently affected by somatic copy number alterations (SCNAs) or mutation, and sometimes both (Figs. 9.4, 9.6, and 9.7) [33]. Many of these events appear to be enriched or restricted to a particular subgroup and are thus likely playing an integral role in the biology driving the initiation, maintenance, and progression of the subgroup(s) harboring the mutational event. Genes and pathways emerging as important oncogenic drivers in medulloblastoma, including how they are distributed within the subgroups, are described below.

Known Cancer Genes

A number of genes previously implicated in medulloblastoma have now been accurately placed in the context of the molecular subgroups. Several of these candidates were once thought to be rarely affected when studying medulloblastoma as a single entity, but are now considered of higher relevance given their subgroup-specificity and increased frequency within a particular subgroup.

The *TP53* tumor suppressor, classically reported as being somatically mutated in only ~5 % of medulloblastomas, has now been observed to be almost exclusively mutated in WNT and SHH subgroup cases, affecting ~10–15 % of cases from each subgroup [37, 46]. Moreover, germline mutations in *TP53*, the hallmark genetic event causing Li-Fraumeni Syndrome (LFS) [47, 48], a condition predisposing to the development of a variety of different cancers including medulloblastoma, have now been confirmed to be restricted to the SHH subgroup [49], especially in childhood and adolescent patients.

In contrast to the *MYC* proto-oncogene which is amplified exclusively in Group 3, high-level amplifications of *MYCN* are found in both SHH and Group 4 but rarely in Group 3 and never in WNT medulloblastomas [34, 43]. Likewise, copy number gains of *OTX2*, a developmental transcription factor previously implicated in medulloblastoma pathogenesis [50–54], appear to be restricted to Groups 3 and 4 [34, 55], suggesting it plays an important role in the biology of these tumors.

Additional oncogenic copy number alterations showing enrichment in SHH-driven medulloblastoma now include (but are not limited to) amplification of *GLI2*, *MYCL1*, *PPM1D*, *YAP1*, *IGF1R*, *IRS2*, *MDM4*, and *miR-17/92* and focal homozygous deletion of *PTEN* and *PTCH1* [34, 56, 57]. These events collectively suggest that at least three main pathways contribute to the majority of SHH-driven medulloblastomas: SHH signaling, RTK/PI3K signaling, and TP53 signaling [34].

DDX3X

Identified as a common target of recurrent mutation in three parallel NGS studies of medulloblastoma [58–60], *DDX3X* is among the newest candidates to be implicated as an important medulloblastoma driver gene. DDX3X is a DEAD-box RNA helicase that has been shown to play a role in a variety of cellular processes, ranging from chromosome segregation to transcription and translation [61–64]. Mutations in *DDX3X* are confined to either of its two helicase domains and are always non-truncating variants [33], suggesting that these mutations alter the function of DDX3X rather than causing loss-of-function [59, 60]. Approximately half of all WNT cases harbor a *DDX3X* mutation, whereas 10–15 % of SHH cases are likewise mutated. Recent NGS of adult SHH medulloblastomas has revealed a high proportion of *DDX3X* SNVs, suggesting this candidate is particularly important in the biology of adult SHH cases. In contrast, mutations in *DDX3X* are seldomly observed in Groups 3 and 4.

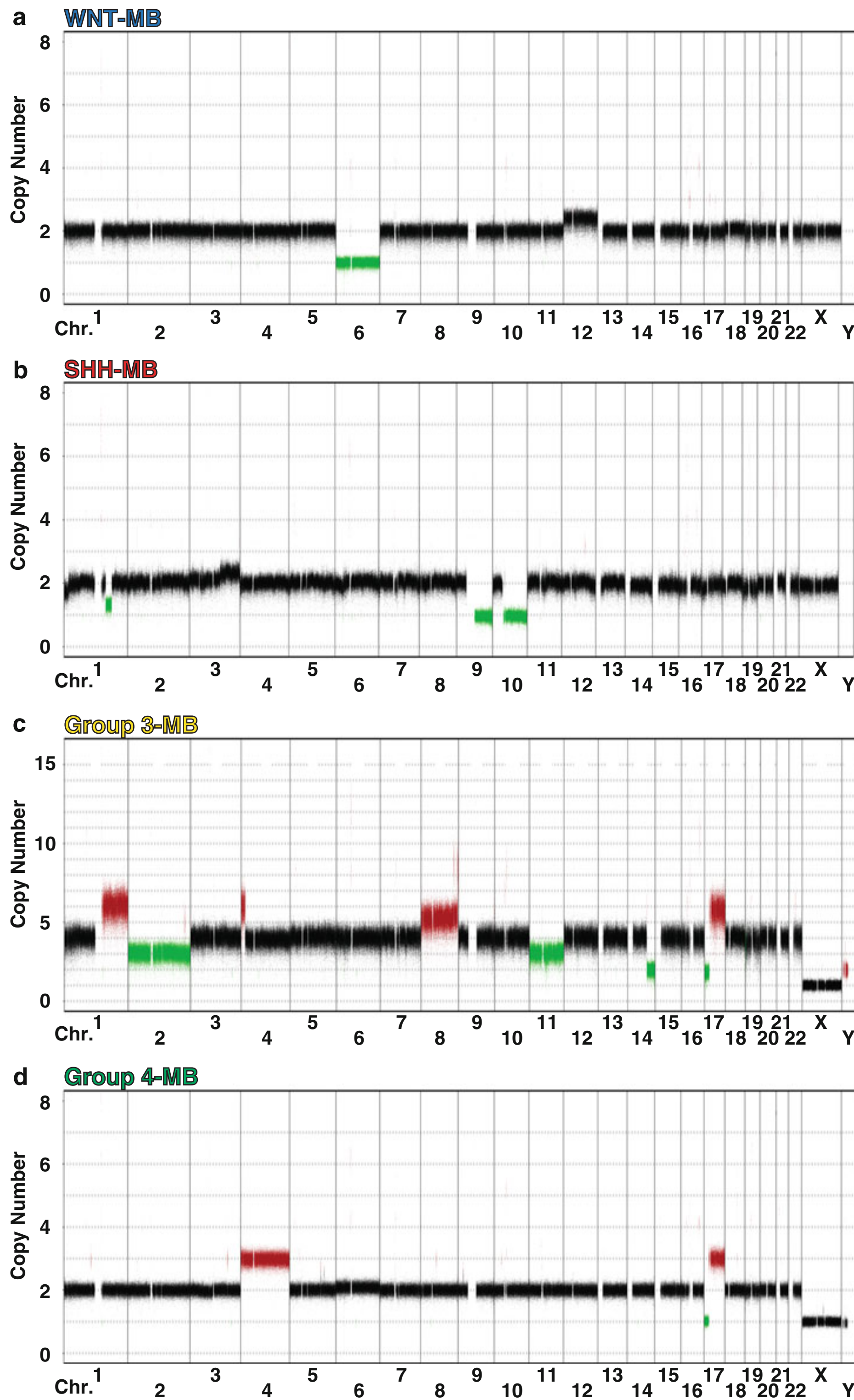

Fig. 9.6. Characteristic cytogenetics of medulloblastoma subgroups. Genome-wide copy number profiles highlighting chromosomal gains and losses typical of each of the four subgroups. (**a**) A WNT subgroup medulloblastoma exhibiting monosomy 6 and an otherwise balanced genome. (**b**) A SHH subgroup medulloblastoma characterized by signature deletions of chromosomes 9q and 10q. (**c**) A Group 3 medulloblastoma with prototypical gains of chromosome 1q and chromosome 8 (including *MYC* amplification), as well as an isochromosome 17q (i[17]q). (**d**) A Group 4 medulloblastoma exhibiting gain of chromosome 4 and i[17]q.

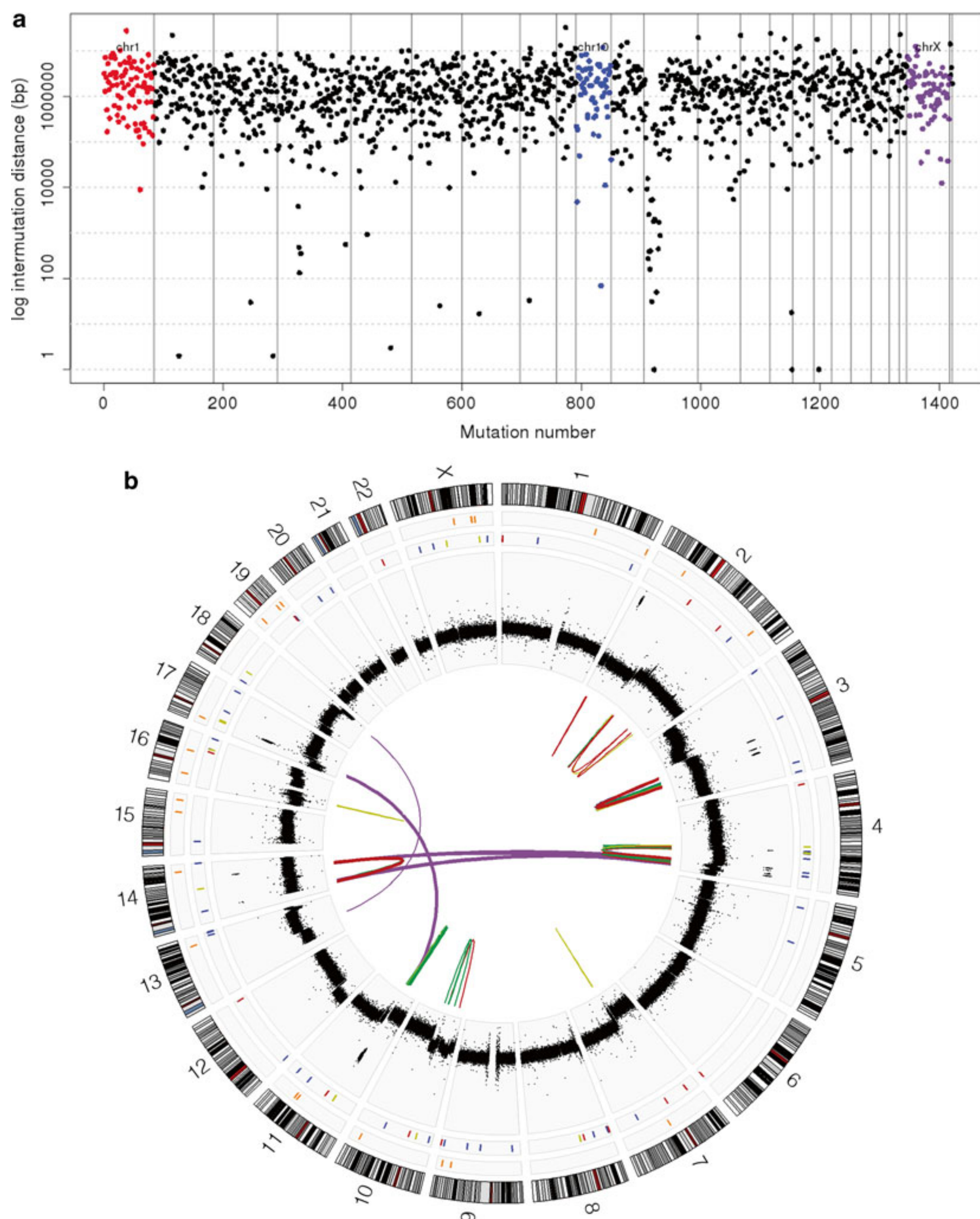

Fig. 9.7. Next-generation sequencing of medulloblastoma. (**a**) Rainfall plot depicting the distribution of somatic mutations (single nucleotide variants; SNVs) across the genome of a SHH subgroup medulloblastoma as determined by NGS. (**b**) Circos plot of NGS data for a SHH subgroup medulloblastoma exhibiting excessive genomic rearrangements and somatic copy number alterations (SCNAs).

Chromatin Modifiers

One of the most unexpected findings disclosed from recent genomic studies of medulloblastoma concerns the high frequency of mutations and copy number alterations affecting chromatin modifiers [33, 65, 66]. In 2009, Northcott and colleagues reported a series of infrequent but recurrent SCNAs targeting histone lysine methyltransferases, histone lysine demethylases, histone acetyltransferases, and chromatin remodelers [67]. A subsequent landmark exon re-sequencing study performed by Parsons et al. identified recurrent and mutually exclusive mutations in histone 3, lysine 4 (H3K4) methyltransferases, *MLL2* and *MLL3*, collectively mutated in ~16 % of surveyed cases [68]. Since these two initial reports implicating deregulation of the chromatin machinery in

medulloblastoma, this theme has been further substantiated in all recent medulloblastoma NGS studies, now in the context of molecular subgroups [33, 58–60]. Interestingly, *MLL2* mutations have been confirmed to be more common in WNT and SHH tumors, whereas *MLL3* mutations are more prevalent in Groups 3 and 4. The SWI/SNF family gene *SMARCA4* that encodes BRG1, a component of a multi-protein chromatin-remodeling complex, is recurrently inactivated in WNT and Group 3 medulloblastomas. Similarly, chromatin-modifying genes *LDB1*, *BCOR*, and *LMO4* are targeted either by mutations, copy number alterations, or both exclusively in SHH-driven cases. Finally, *KDM6A*, a histone 3, lysine 27 (H3K27) demethylase, appears to be inactivated by either somatic mutation or focal deletion specifically in Group 4. Moreover, EZH2, which imposes the opposite function of KDM6A, catalyzing the trimethylation of H3K27 (H3K27me[3]) is aberrantly over-expressed in Groups 3 and 4, suggesting a propensity for an aberrant H3K27 methylation state in these subgroups [60, 69]. Collectively, this series of recent observations strongly supports the notion that deregulation of the histone code is a key event in medulloblastoma pathogenesis, with somatic alterations occurring in a high proportion of cases across the four subgroups.

Atypical Structural Variation in Medulloblastoma

In addition to the spectrum of genes described above as recurrently mutated or affected by SCNAs, more complex mechanisms of deregulation, including both gene-specific and genome-wide structural rearrangements have been uncovered during the genomics era of medulloblastoma (Fig. 9.8). These recurrent structural variants appear to be common to medulloblastoma and may play an even bigger

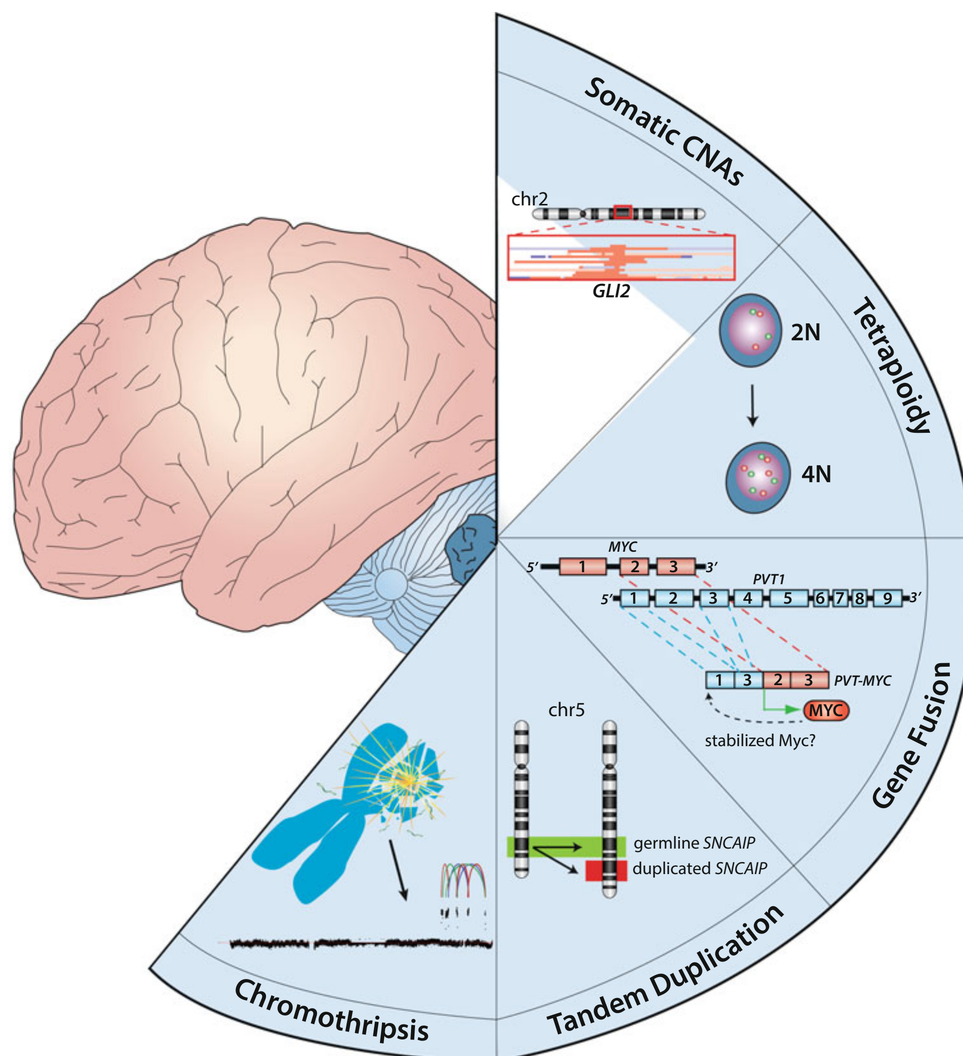

Fig. 9.8. Mechanisms of recurrent structural variation in medulloblastoma. Cartoon showing the different mechanisms responsible for the prominent structural variation reported in medulloblastoma. Depicted types of structural variation include SCNAs, tetraploidy, gene fusions, tandem duplications, and chromothripsis.

role than standard SNVs and SCNAs affecting protein-coding sequences.

NGS of a series of LFS medulloblastomas recently identified massive chromosomal rearrangements known as chromothripsis (Fig. 9.7b) [49]—excessive genomic rearrangements (i.e., inversions, amplifications, deletions) clustered on one or a few chromosomes presumed to have arisen from a single catastrophic DNA breakage/repair event during tumorigenesis [70–72]. This phenomenon was observed in the majority of LFS medulloblastomas analyzed, was specifically enriched in SHH medulloblastoma, and co-occurred with cases harboring either germline or somatic *TP53* mutations [49]. Importantly, chromothripsis observed in these cases often resulted in amplification of known medulloblastoma oncogenes (i.e., *MYCN* and *GLI2*), providing a mechanism for the activation of these critical driver genes.

Using a combination of single nucleotide polymorphism (SNP) arrays and RNA sequencing (RNASeq), Northcott et al. reported *PVT1-MYC* fusion genes as being highly recurrent and specific to *MYC*-amplified Group 3 medulloblastoma [34]. This is the first example of a recurrent fusion gene identified in medulloblastoma and is suspected to potentiate MYC activity by yet an unknown mechanism. In the same study, tandem duplications affecting the *SNCAIP* gene on chromosome 5q were reported in up to ~25 % of a particular Group 4 subtype. *SNCAIP* is a neuronal gene implicated in Parkinson's disease [73, 74]; how these alterations contribute to Group 4 medulloblastoma biology currently remains unclear.

Whole genome duplication (i.e., tetraploidy) has been observed in medulloblastoma karyotypes for more than two decades [75, 76]. Now as a result of NGS, tumor cell ploidy can be readily estimated based on allele frequencies of SNPs and SNVs present in the genome. In a recent report by Jones et al., up to one-third of investigated medulloblastoma genomes were shown to be tetraploid, with higher frequencies noted in Groups 3 and 4 [58]. The significance of the high frequency of tetraploidy observed in medulloblastoma, particularly in Groups 3 and 4, will require further investigation but is thought to contribute to the overall genomic instability (i.e., gains and losses of whole chromosomes or chromosome arms) often noted in these tumors.

Preclinical Models of Medulloblastoma: Validating the Genetics of the Human Disease

Understanding the biological consequences of the genetic and epigenetic events observed in medulloblastoma and its subgroups requires accurate and faithful preclinical models that allow for comprehensive in vitro and in vivo functional studies. A series of established, immortalized medulloblastoma cell lines derived from human patient samples have been in use for the past 20–30 years [77–81]. These models have served as convenient systems for a variety of purposes including, evaluating candidate gene function, investigating genetic, epigenetic, and transcriptional alterations, and testing novel therapies, all in the context of medulloblastoma. Now with the recognition of medulloblastoma subgroups and increasing knowledge of the genetics underlying these subgroups, the validity of these "workhorse" medulloblastoma models has been put into question. Ongoing genomic analysis of these lines suggests they do not faithfully recapitulate the four medulloblastoma subgroups and harbor events not observed in primary medulloblastoma counterparts as a result of their continued evolution during long-term passage in culture [34]. Novel, low-passage lines and medulloblastoma xenograft models that require passaging in the mouse have recently emerged as possible solutions to the caveats associated with immortalized, high-passage cell lines and will likely be more heavily relied upon in the future [82, 83].

An immense amount of knowledge regarding the developmental biology of medulloblastoma has been gained from the use of genetically engineered mouse models [84]. The majority of such murine models generated and studied to date have been driven by activation of the SHH pathway in neuronal progenitor and stem cell populations [85]. Germline inactivation of one copy of the *Ptch1* gene (often referred to as $Ptc^{+/-}$ mice) results in 15–20 % of mice developing cerebellar tumors that are histologically similar to human medulloblastomas and exhibit aberrant SHH pathway activation suggesting they are accurate models of this subgroup [86, 87]. Combining loss of Ptch1 with inactivation of Trp53 (i.e., $Ptc^{+/-}$; $Trp53^{-/-}$) dramatically increases tumor incidence and reduces latency, with up to ~95 % of mice developing medulloblastoma within 12 weeks [88]. Several other SHH-activated mouse models have been generated, including those driven by an activated Smoothened transgene [89–91], homozygous germline deletion of *Ptch1* in specific cell types [92], those driven by administration of SHH ligand with cooperating oncogenes [93–95], and others [96–99]. These models have led to a better understanding of the genetics underlying SHH-driven medulloblastoma, the probable cells-of-origin for this subgroup, and provided the research community with tools for asking an array of questions related to medulloblastoma biology.

Representative models for the remaining subgroups have also been published, providing important clues regarding their differing biologies. Gibson et al. expressed a constitutively active form of β-Catenin (i.e., $Ctnnb1\Delta^{ex3}$) in progenitor cells of the developing hindbrain, successfully generating the first WNT-driven medulloblastoma mouse model [100]. More recently, complementary studies by Pei et al. and Kawauchi et al. combined over-expression of Myc with loss of wild-type Trp53 in orthotopic transplantation models to generate tumors resembling human Group 3 medulloblastoma [101, 102]. Finally, a model relying on transgenic over-expression of Mycn is believed to represent the lone Group 4 medulloblastoma model currently available [103].

As a plethora of new candidate medulloblastoma genes have recently been discovered, it is anticipated that many novel models based on these genes and rational gene combinations will be generated and introduced during the next few years. These models will serve not only to functionally validate events observed in the human disease but also to further progress our understanding of their role in disease biology and evaluate their relevance and utility as potential targets for molecularly informed therapy.

Translational Significance of the Medulloblastoma Genomics Era

Molecular Classification and Risk-Stratification

Medulloblastoma subgroups exhibit highly disparate molecular genetics and clinical characteristics, suggesting they should be treated as different diseases in the clinic. Before such a concept is put into general practice, robust, highly accurate, and efficient methods that are accessible to treating physicians for establishing subgroup assignments are necessary. Novel assays that are gaining an appreciation in this arena include the use of DNA methylation arrays and platforms for measuring the expression of custom gene panels. Both Schwalbe et al. and Hovestadt et al. have recently demonstrated that DNA methylation arrays can be used to assign medulloblastoma subgroups with high confidence, including samples derived from formalin-fixed paraffin embedded (FFPE) material [104, 105]. RNA-based methods such as the nanoString assay have also shown utility at subgrouping of samples preserved in FFPE [106] (Fig. 9.9). Further validation of these methods and potentially others in the setting of medulloblastoma clinical trials is expected in the near future as the interest to subgroup patients in a prospective manner increases.

In light of the excellent prognosis associated with WNT medulloblastoma patients, plans to de-escalate craniospinal radiation or even eliminate it in these patients will be implemented in forthcoming clinical trials. Similarly, prospective stratification of all Group 3 patients into a high-risk treatment category is likewise being considered.

Another subset of patients that appears to be of significant clinical relevance are those with *TP53*-mutated SHH medulloblastomas. Using large retrospective patient cohorts, Zhukova et al. demonstrated that the dismal outcome sometimes attributed to medulloblastomas harboring *TP53* mutation [107] can be explained by considering patient subgroup information [37]. *TP53*-mutated cases within the SHH subgroup exhibit a significantly worse outcome compared to either subgroup-matched non-mutated counterparts or WNT cases likewise harboring *TP53* mutation. Furthermore, the prevalence of chromothripsis and oncogene amplification observed in *TP53*-mutated SHHs suggest these patients should be stratified as a unique risk-group and possibly subjected to treatments tailored for their genotype.

Targeting Medulloblastoma with Rational Therapies

One of the major goals motivating the comprehensive genomic characterization of medulloblastoma is the identification of targets that can be specifically exploited for future treatment of the disease. To date, antagonists of the SHH pathway, acting mainly at the level of SMO, have demonstrated the most promise [108–112]. Compounds such as GDC-0449 from Genentech have shown dramatic although transient tumor regression when administered to patients with metastatic medulloblastoma, with patients eventually becoming resistant to the targeted therapy [111, 113]. Similar acquired resistance has been noted when related SMO antagonists (i.e., LDE-225) have been used to treat mouse models of the disease [110]. Ongoing efforts aim to combine SHH antagonists with additional inhibitory agents targeting cooperating pathways in hopes of achieving an improved and more sustained response to treatment. Furthermore, genomic analysis of human SHH medulloblastomas suggests that not all SHH-driven cases are likely to respond to inhibitors acting at the level of SMO, as subsets of cases such as those exhibiting amplification of downstream pathway components (i.e., *GLI2*) are likely to have primary resistance to these agents [34, 67]. As such, screening patients for both SHH subgroup affiliation and their mutation/copy number status in select SHH pathway genes prior to treatment with the current generation of SHH pathway inhibitors could improve their likelihood of response in the future. In addition, novel approaches targeting pathway components downstream of SMO, including the use of agents inhibiting GLI family transcription factors, are currently being evaluated and may increase the likelihood of response when combined with conventional SHH antagonists [114, 115].

The frequent deregulation of chromatin modifiers in medulloblastoma and the potential consequences of these events on the underlying epigenome make the prospect of epigenetic therapy an attractive possibility for medulloblastoma patients [65, 66]. Histone deacetylase (HDAC) inhibitors such as Vorinostat are currently being evaluated in medulloblastoma clinical trials [116, 117]. Similarly, 3-deazaneplanocin A (DZNep), a potent inhibitor of EZH2, is now being prioritized as an agent to be tested in upcoming clinical trials for medulloblastoma [118]. Similar agents targeting histone-modifying enzymes are presently being evaluated in the research setting and undoubtedly will enter the clinical trials arena for medulloblastoma patients in the near future.

Summary

Considerable advances have recently been made with respect to our understanding of the molecular genetics underlying medulloblastoma (Fig. 9.10). Acknowledgement of unique molecular subgroups and an improved knowledge of the

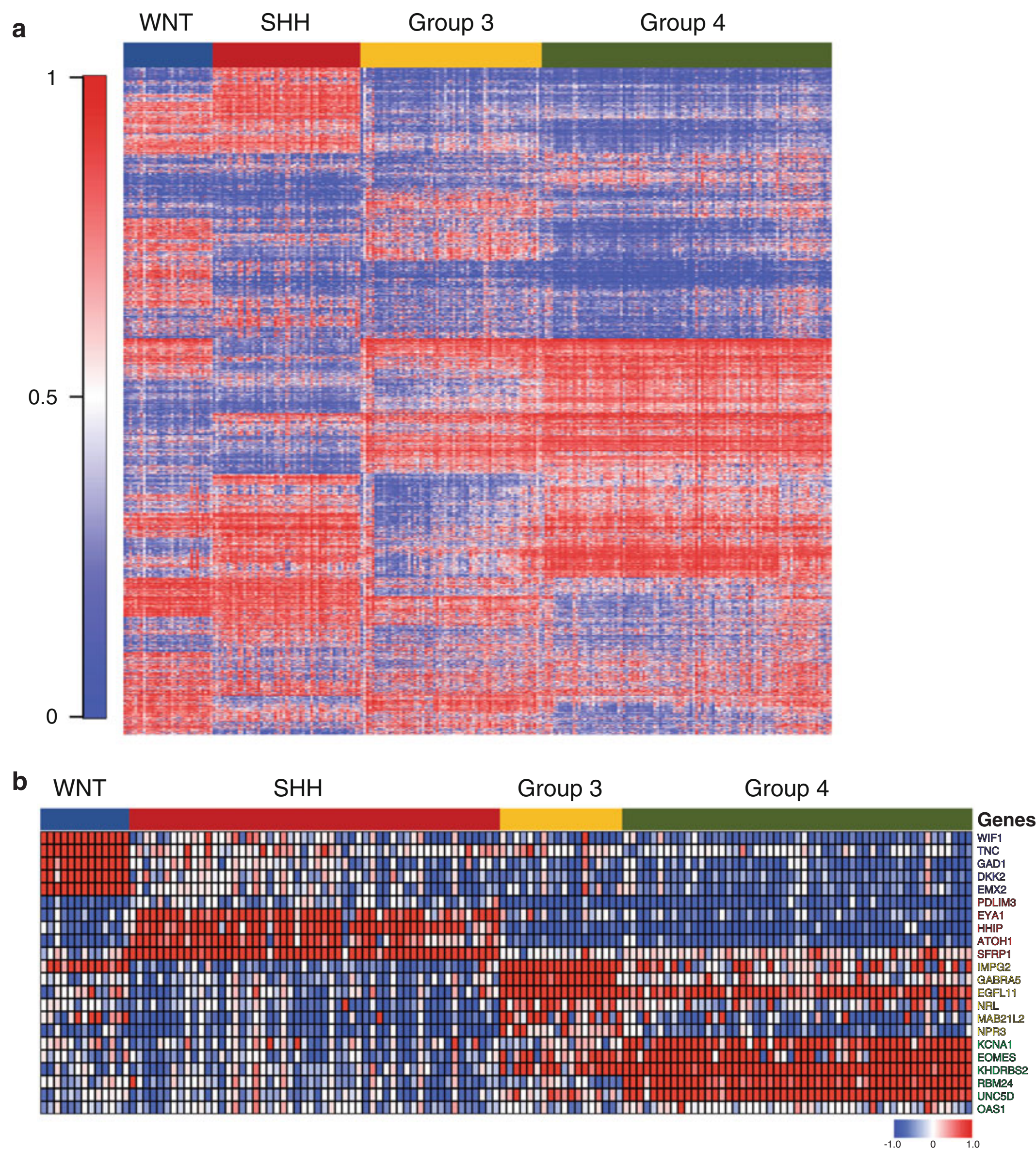

FIG. 9.9. Conventional assays for the molecular classification of medulloblastoma. (**a**) Heatmap of DNA methylation array (Illumina 450K platform) data for >250 primary medulloblastomas classified according to their appropriate molecular subgroup. Data was generated using DNAs extracted from either fresh-frozen tumor tissue or FFPE material. (**b**) Heatmap of gene expression data derived from a custom nanoString assay consisting of 22-signature genes for >100 medulloblastomas classified by molecular subgroup. Data was generated using RNAs extracted from FFPE material.

genes and pathways responsible for their pathogenesis can now at least partially explain the long-recognized biological and clinical heterogeneity encountered in the disease. Application of next-generation genomic platforms to large patient cohorts has identified new driver genes recurrently mutated in specific medulloblastoma subgroups, in addition to recurrent and often complex structural rearrangements, all at base-pair resolution. Additionally, new assays for rapidly confirming subgroup affiliation with pinpoint accuracy have been developed and are now making their way into clinical trials, as the need to progressively subgroup patients in the clinical setting intensifies. As the medulloblastoma research community harnesses the wealth of information gained during the current medulloblastoma "genomics era," new preclinical models faithfully recapitulating the genetics and the biology of the human subgroups are emerging. These new models will serve as valuable tools for the identification, development, and evaluation of rational therapies, bridging the gap between discoveries made in the research laboratory and the future administration of more specific, less-toxic

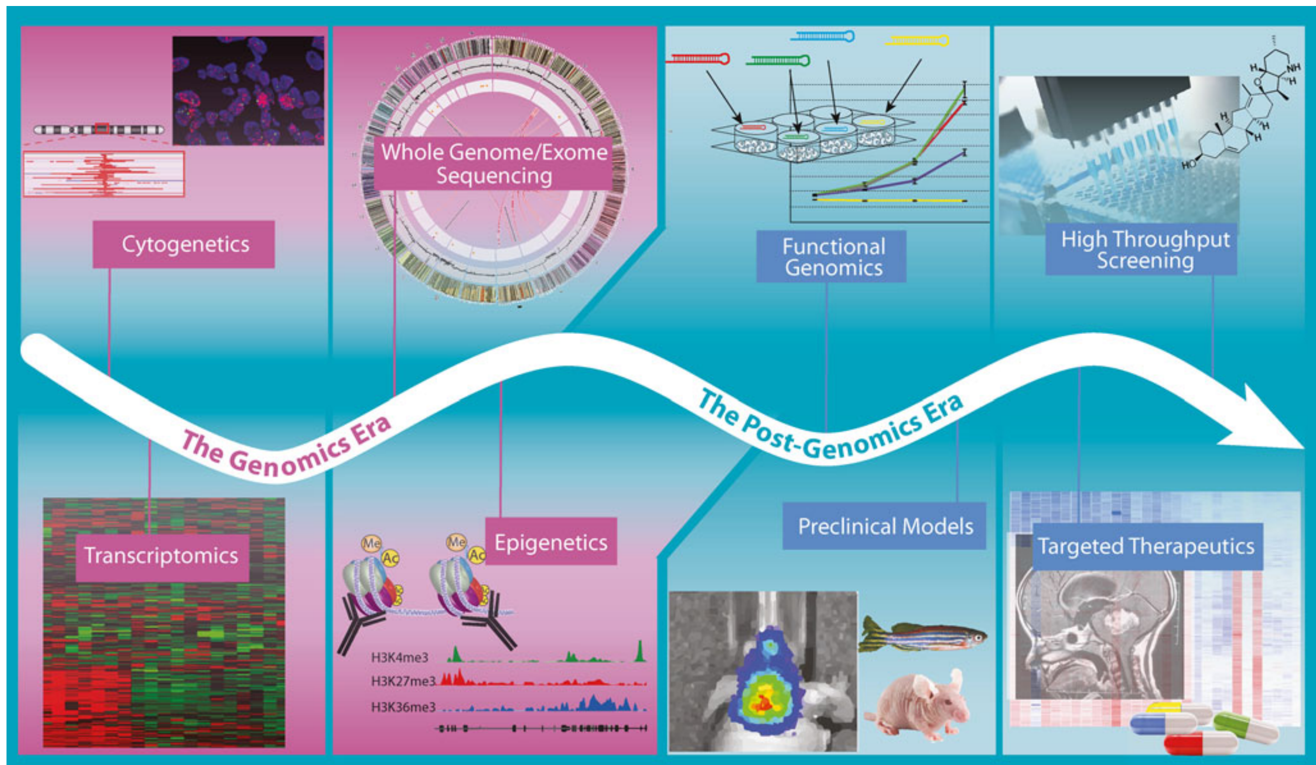

Fig. 9.10. Progression from genomics to the post-genomic era of medulloblastoma. Timeline highlighting the technological approaches being applied in medulloblastoma studies during the current "Genomics Era" and those that will become the focus of the imminent "Post-Genomics Era".

targeted treatment options. It can be anticipated that we are on the verge of an era of personalized medicine for medulloblastoma, whereby patients will be treated with therapies specifically tailored to their underlying genotypes. It is hoped that these novel diagnostic and therapeutic strategies will improve outcomes and quality of life for medulloblastoma patients going forward.

CNS-PNET

Central nervous system primitive neuroectodermal tumor (CNS-PNET, also known as supratentorial PNET to distinguish them from medulloblastoma) is a catch-all term for what is an extremely heterogeneous group of tumors arising in the cerebrum or spinal cord. They are one of the least clearly demarcated entities in terms of their histology, comprising a wide morphological spectrum. The WHO defines them as "An embryonal tumor composed of undifferentiated or poorly differentiated neuroepithelial cells which have the capacity for, or display, divergent differentiation along neuronal, astrocytic, muscular or melanocytic lines" [1]. Four variants of CNS-PNET are described in addition to "not otherwise specified" (NOS), namely: CNS neuroblastoma, CNS ganglioneuroblastoma, medulloepithelioma, and ependymoblastoma (EBL). CNS neuroblastoma is reserved for primitive tumors with solely neuronal differentiation, while ganglioneuroblastoma additionally implies the presence of ganglion cells. A further PNET occurring throughout the brain (including infratentorial locations) and characterized by broad bands of neuropil with true rosettes surrounding lumens, known as embryonal tumor with abundant neuropil and true rosettes (ETANTR), has also been noted but is not yet included in the WHO classification [119]. All of these variants are considered to be of malignancy Grade IV [1].

There is no clear shift in the gender distribution of CNS-PNETs, with a male:female ratio of ~1.2:1. The majority of these tumors occur in children, with a mean age at diagnosis of ~5.5 years [1]. Rarer cases in adults have also been reported, but with an incidence rate approximately one-fifth of that in children [120]. CNS-PNETs diagnosed in older patients are associated with a significantly worse prognosis than those occurring before the age of 40, and also with a worse outcome than for adult medulloblastoma (median survival 16 months for adult CNS-PNET vs. 155 months for medulloblastoma [121]). The latter trend holds true for pediatric cases, with 5-year overall survival typically below 50 % for CNS-PNET compared with 70–80 % for medulloblastoma [122, 123]. Pineal tumors in particular

seem to be associated with poor outcomes. Treatment options are also less well defined than those for medulloblastoma, with some evidence that CNS-PNETs are resistant to standard Packer chemotherapy regimens [124], and no clear rationale for molecularly targeted therapies has been proposed. This is especially true for infants, who do not receive craniospinal radiation due to the risk of severe developmental defects. Slightly better outcomes have been reported, however, in patients receiving risk-adapted radiotherapy followed by high-dose cyclophosphamide-based chemotherapy and stem cell rescue [125]. As with medulloblastoma, survivors often experience significant morbidities and a reduction in quality of life due to tumor- and treatment-related sequelae.

Somatic Copy Number Alterations

Several studies have used methods of varying resolution to investigate SCNAs in CNS-PNET over the last 10–15 years. The general picture that has emerged is that these tumors do not typically display any of the common changes seen in medulloblastoma, such as i[17q], but may harbor other changes. Recently, a region of focal amplification on chromosome 19q has been identified as a highly recurrent alteration in certain subsets of CNS-PNET, as will be discussed in more detail in a later section.

One of the earliest studies comparing the cytogenetics of CNS-PNETs to medulloblastoma, for example, noted a higher frequency of chromosome 14q and 19q loss in supratentorial compared with infratentorial tumors, and no 17q gain in the supratentorial cases [126]. Another early report described an amplification of the *TERT* gene in a recurrence of a medulloepithelioma, which was not seen in the primary tumor, suggesting a role in tumor progression [127]. The authors also described increased expression of telomerase as a common finding in CNS-PNET. A lack of 17q gain but recurrent loss of 13q was noted in an array-based study of CNS-PNETs, which also reported amplifications of *PDGFRA*/*KIT*, *MYB* and 19q, as well as homozygous *CDKN2A*/*B* deletion [128]. One subsequent report did identify chromosome 17 alterations in 2/10 CNS-PNETs, but again noted that this change was significantly less frequent than in medulloblastoma [129]. The same study reported regions of loss on 1p and gain of 19p as being recurrent in CNS-PNET, and described deletions of *CDKN2A*/*B* in 7/21 cases examined [129]. Recurrent gain of 19p was confirmed in a more recent study of 29 CNS-PNETs, along with gains on 2p and 1q, which were seen in more than 20 % of cases [130]. Focal loss of *CADPS* on 3p was observed in 28 % of samples, and tumors showing this loss carried a worse prognosis [130]. In addition to *CDKN2A*/*B* deletions, SCNAs at other key cell-cycle regulatory genes seem to be a relatively common event in CNS-PNET, with 5/20 cases in one study displaying focal amplifications of *CDK4*, *CDK6*, *CCND1*, or *CCND2* [131].

There also appears to be a role for *MYC*/*MYCN* amplification in CNS-PNET, although there are conflicting reports as to the frequency of these changes. Some studies have reported only single cases with this change [129, 130], while in others it was reported in up to 50 % of cases, and was found to be associated with more aggressive tumor behavior [132].

Gene Mutations

As with SCNAs, reports on specific gene mutations in CNS-PNETs are relatively rare. Some alterations described in other pediatric or adult brain tumors have been identified, but typically at a lower frequency. For example, mutations in *SMARCB1*, encoding the INI1 tumor suppressor, have been reported in a small number of patients originally diagnosed with either medulloblastoma or CNS-PNET [133]. In some cases, a pathology review resulted in a change in diagnosis to atypical teratoid/rhabdoid tumor (AT/RT), for which *SMARCB1* loss is extremely common. The authors of this study noted the difficulties in differential diagnosis between AT/RT and CNS-PNET, particularly in young patients where only limited biopsy material is available.

The issue of potential diagnostic use of mutations which are typically highly specific for one entity, but which are occasionally seen in CNS-PNET, was also raised in a recent report by Gessi et al. [134]. The authors identified mutations at glycine 34 of histone variant H3.3 (encoded by *H3F3A*) in 4/33 CNS-PNETs. This mutation is typically found in hemispheric glioblastomas of older children and young adults, raising the question of whether H3.3 G34-mutant tumors can be defined as a distinct entity.

Mutations in *IDH1*, frequent in adult gliomas, are not common in pediatric patients in general, and also not in pediatric CNS-PNET. It does, however, appear to be a relatively frequent event in adult CNS-PNET (occurring in approximately 15–50 % of cases, although the overall number of tumors investigated remains small) [135–137]. This suggests that adult CNS-PNETs may frequently be related to tumors of a more glial origin, and their relationship to other adult gliomas requires additional investigation. Mutations in *TP53* have also been reported to be relatively frequent in adult CNS-PNET (~40 % in one study, but again with small numbers; [137]), which may further support an astrocytic link. In a similar vein, an entity termed "malignant gliomas with PNET-like component," which shares common molecular features of both malignant glioma and CNS-PNET, has recently been proposed—further highlighting the diagnostic uncertainty in this area [138].

As described in the first part of this chapter, practically all WNT MBs harbor an activating change in *CTNNB1*. Reports of this mutation in CNS-PNET, however, are extremely rare [139, 140]. The frequency of mutations in other genes recently identified as being altered in medulloblastoma (e.g., *DDX3X*, *SMARCA4*, *KDM6A* etc., as outlined above) is not

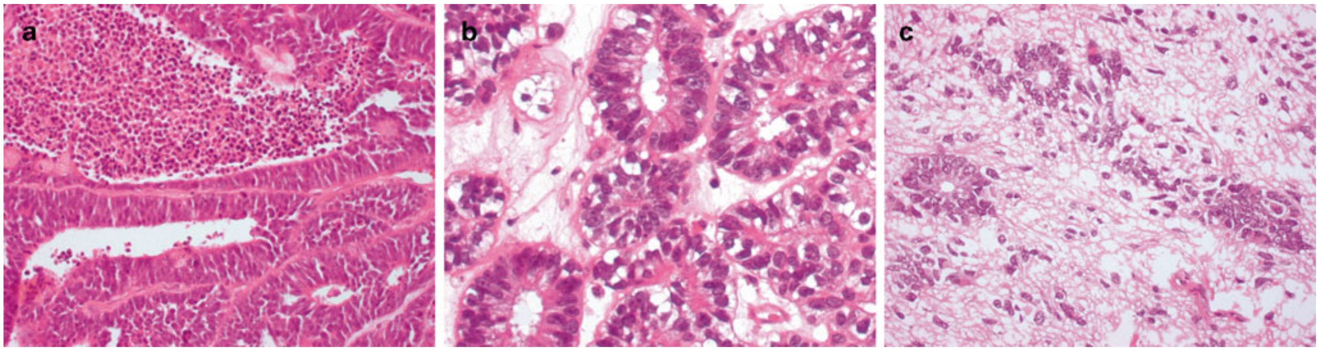

Fig. 9.11. Histological subtypes of CNS-PNET. Representative histology for (**a**) medulloepithelioma, (**b**) ependymoblastoma, and (**c**) ETANTR.

currently known for CNS-PNET. No NGS studies on this entity have been reported at the time of writing, but the results of such studies in the future will be of keen interest.

Signaling Pathways and Molecular Classification

In addition to studies looking at mutations in target genes or focal regions of copy number change, there have also been attempts to investigate deregulated signaling pathways and/or global expression patterns in CNS-PNET. The first seminal study using gene expression arrays looked at a comparison between medulloblastoma, CNS-PNET, AT/RT, and malignant gliomas [23]. This analysis was the first to clearly confirm the hypothesis that medulloblastoma and CNS-PNET do not share a common molecular origin, and display distinct expression signatures.

A possible glial signature in CNS-PNETs, compared with the more neuronal expression pattern in medulloblastoma, was proposed on the basis of a targeted expression analysis looking at various neuroglial developmental genes [141]. The authors identified an up-regulation of SOX2, NOTCH1, ID1, and ASCL1 in CNS-PNET, with higher levels of proneural transcription factors (NEUROD1, NEUROG1) in medulloblastoma.

Some evidence has recently been presented that the WNT/β-catenin pathway may also be playing a role in a proportion of CNS-PNETs [142]. Pathway activation, as assessed by β-catenin IHC, was identified in 11/42 primary CNS-PNETs (26 %). This pathway activation was also associated with a better prognosis among the CNS-PNETs examined (5-years OS 52 % in WNT-activated tumors compared with 13 % otherwise), but not to the extent of the excellent prognosis seen in WNT-activated medulloblastoma (5-years OS >95 %). As noted above, however, the frequency of *CTNNB1* mutation in CNS-PNET is very low, and thus the mechanism of WNT pathway activation is not currently clear.

An important recent study looking at the transcriptional profiles of 51 CNS-PNETs identified subgroups of tumors with differential signaling pathway activation and survival outcomes [143]. Three groups were identified based on their expression signature, showing differences in age and gender distribution, propensity for metastasis, and prognosis. Group 1 tumors showed a “primitive neural” profile, and occurred in young patients with a very poor prognosis. Positive immunohistochemical staining for LIN28A, and frequent presence of focal 19q amplification, suggests that this group may be composed primarily of embryonal tumors with multi-layered rosettes (ETMR), as discussed below. Interestingly, Group 1 was also characterized by a WNT pathway activation signature, and the poor survival of this group is therefore in contrast to the association seen by Rogers et al. [142]. Subgroup 2 tumors were labeled as “oligoneural.” They typically showed OLIG2 immunopositivity as well as recurrent *CDKN2A/B* deletion, and a prognosis only slightly better than Group 1 CNS-PNETs, suggesting some similarity with high-grade glial tumors. Group 3 tumors displayed somewhat better outcomes (particularly in children older than 4 years) despite a much higher incidence of metastasis at diagnosis than the other subgroups. They showed a mesenchymal signature, were typically immunonegative for LIN28 and OLIG2 but positive for IGF2, and harbored recurrent loss of chromosome 14 [143]. Further investigation of these subgroups and their implications in terms of diagnostic/prognostic markers and also their cellular origins is clearly warranted.

Medulloepithelioma, Ependymoblastoma, and ETANTR

Medulloepithelioma (characterized by arrangements of neoplastic neuroepithelium mimicking embryonic neural tube, often with multiple lines of differentiation) and EBL (a densely cellular tumor with multi-layered rosettes) are two recognized CNS-PNET variants, which share the presence of rosette structures as a histological feature (Fig. 9.11). Arguably, however, the most fruitful area of research in terms of our molecular genetic understanding of CNS-PNETs in recent years has been in an additional rosette-forming entity

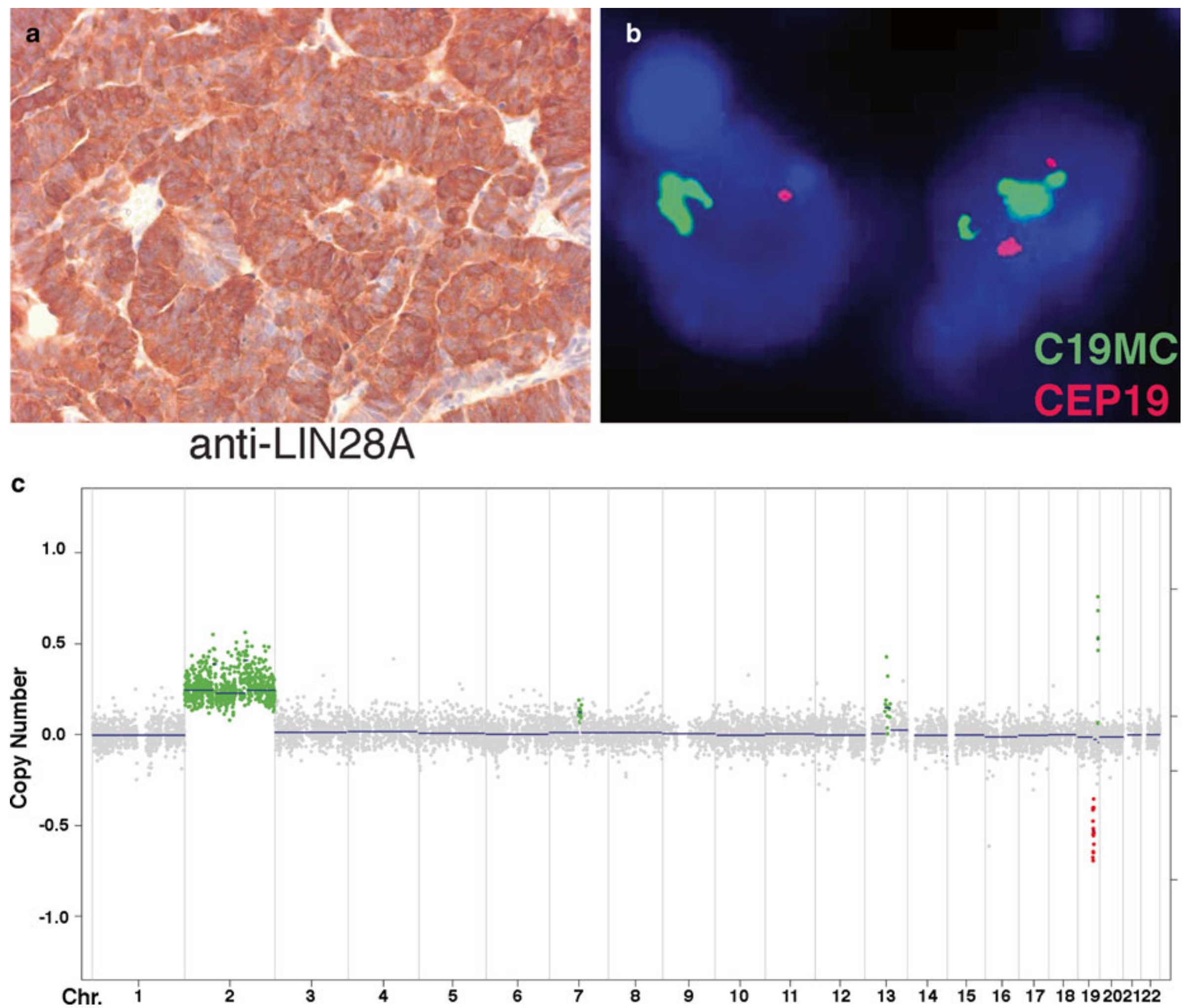

Fig. 9.12. Characteristic molecular features of ETANTR. (**a**) IHC of an ETANTR showing strong immunopositivity for LIN28A. (**b**) An ETANTR possessing signature high-level amplification of the chromosome 19q microRNA cluster (C19MC). (**c**) Genome-wide copy number plot of an ETANTR exhibiting prototypical gain of chromosome 2 and amplification of C19MC.

which is not yet officially recognized by the WHO classification, termed "embryonal tumor with abundant neuropil and true rosettes" (ETANTR) (Fig. 9.11) [119]. This tumor has a distinct histology, typically occurs in infants, can arise throughout the brain (including infratentorially) and is associated with a very poor prognosis [144]. It has recently become apparent that focal amplification of a microRNA cluster on chromosome 19q13 (C19MC), first described in early 2009 [145], is an extremely frequent event in this entity (Fig. 9.12) [143, 146, 147]. Multiple miRNAs within this locus, particularly miR-517c and miR-520g, were found to be strongly up-regulated in 19q13-amplified tumors [146]. Further functional characterization indicated a role for these miRNAs in promoting cell survival and inhibiting differentiation, with oncogenic effects observed both in vitro and in vivo that may partly be mediated by altered WNT pathway signaling [146].

Interestingly, the C19MC amplicon has also been found to be present in a very high proportion of EBLs [146, 147]. This has led to speculation that ETANTR, EBL, and possibly medulloepithelioma may be connected, with similar origins and biology but displaying a spectrum of morphology [148]. Rare reports of recurrent ETANTRs which retained the 19q amplicon but showed altered morphology add a further layer of histological complexity [147, 149]. As such, "embryonal tumor with multi-layered rosettes" (ETMR) has been proposed as an umbrella term to encompass the three entities. Further studies are therefore warranted to determine how closely related these three histological variants are, and whether it may be of diagnostic, prognostic, and potential therapeutic use to consider them as a single entity.

In addition to the C19MC amplification, gain of chromosome 2 is also particularly frequent in these tumors [144–146], but the consequences or targets of this change are

not currently known. Other alterations including rare reports of i[17q] have also been described [150].

As noted above, C19MC amplification and chromosome 2 gain were also prominent features of Group 1 tumors according to Picard et al., together with immunopositivity for LIN28A [143]. Immunostaining for this marker has subsequently been shown to be highly specific for embryonal tumors with ependymoblastic rosettes, with diffuse positivity seen in 100 % of cases and not in any other embryonal, glial, or ependymal CNS tumor investigated, making it a valuable diagnostic tool (Fig. 9.12) [151].

Summary

In conclusion, CNS-PNETs are currently much less well characterized or understood in comparison to medulloblastoma and several other pediatric entities. In some respects, they represent a histological "dustbin" to gather all primitive-looking tumors that do not show a clear differentiation down any one defined lineage. What is apparent is that they do not resemble medulloblastoma in terms of their molecular genetic alterations. While 19q amplification seems to define one subset of CNS-PNET, a lack of other common alterations together with the occasional finding of changes thought to be specific for other entities (i.e., *IDH1* and *H3F3A* mutations) suggests a high degree of diagnostic uncertainty for this class of tumors. It will therefore be of great interest to see whether future studies looking at molecular profiles of larger numbers of CNS-PNETs can shed further light on the origins of this heterogeneous group of entities.

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10
Subependymal Giant Cell Astrocytoma

David H. Harter, Howard L. Weiner, and David Zagzag

Subependymal giant cell astrocytomas (SEGAs) are benign tumors (WHO grade I) that occur almost exclusively in the setting of tuberous sclerosis (TS), a well-defined, multisystem genetic syndrome (Table 10.1). Most commonly originating from the region of the caudate nucleus, these tumors may cause obstruction of cerebrospinal fluid circulation leading to hydrocephalus. Less frequently, they may hemorrhage spontaneously, causing precipitous neurological impairment [1]. Mutations of the *TSC-1* and *TSC-2* genes, both effectors of the mTOR pathway (originally *mammalian* Target of Rapamycin, now formally *mechanistic* Target of Rapamycin), lead to the variably expressed systemic manifestations of TS; cardiac rhabdomyoma, renal angiolipomas, facial adenoma sebaceum, cortical tubers of the brain, and SEGAs. The standard treatment of symptomatic or enlarging SEGAs is surgical excision. Pharmacological effectors of the mTOR pathway, rapamycin (aka sirolimus) and its analogs have recently been shown to induce rapid involution of SEGAs; however, the optimal timing, dosage, safety, and duration of treatment remain areas of active clinical research. SEGAs in the context of TS represent an example of an emerging paradigm: targeted molecular-oncologic therapy.

Incidence and Prevalence

SEGAs (subependymal giant cell tumors) typically occur in the first or second decade of life, predominately, yet not exclusively in patients with TS (tuberous sclerosis). Reports of neo- and prenatal diagnoses illustrate the developmental nature of these tumors [2–5]. Most SEGAs are related to tuberous sclerosis, occurring in approximately 1 per 5,000–10,000 births [6]. Although most SEGAs are associated with TS, the incidence of SEGA is only 5–10 % among patients with TS [7]. A small portion of SEGAs occur without clinical or genetic evidence of TS, or as "forme fruste" of the disorder displaying some characteristics [8]. Tuberous sclerosis occurs across all ethnicities and in both male and female, worldwide estimates are of 1–2 million affected individuals [6].

Genetics and Oncogenesis

Tuberous sclerosis is an autosomal dominant genetic disorder with high penetrance and variable expressivity. The majority of cases are due to de novo mutations, although inherited somatic mutations and gonadal mosaicism may also occur [9]. Somatic mosaicism may result in limited expression of TS. In cases of both spontaneous mutation and gonadal or somatic mosaicism, parental genetic testing may be normal. In cases of gonadal mosaicism, the possibility of transmission to future offspring remains, albeit at an unquantifiable rate. A variety of mutations of within two genes have been identified, TSC1 (chromosome 9) and TSC2 (chromosome 16), both effectors of the mTOR (mechanistic Target of Rapamycin) pathway. Identified aberrations, including mutation and deletion, lead to loss or attenuation of function. Sporadic SEGAs occurring without clinical or genetic evidence of TSC (tuberous sclerosis complex) may be due to dual somatic mutations of TSC1 or TSC2 [10, 11].

The TOR complexes influence many aspects of eukaryote physiology—largely via growth regulation, cell growth, proliferation, and survival (Fig. 10.1) [12]. The mTOR signaling pathway detects and integrates a variety of environmental conditions to regulate growth and homeostasis. Aberrations of the mTOR pathway have been implicated in a wide array of pathological processes including oncogenesis, obesity, type II diabetes, and neurodegenerative conditions. mTOR has been identified as an atypical serinine/threonine protein kinase belonging to the phosphoinositide 3-kinase (PI_3K)-related kinase family. Interacting with other proteins, mTOR forms two complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). These complexes each have

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TABLE 10.1. Diagnostic criteria for tuberous sclerosis complex.

Definite—One primary, two secondary, or one secondary plus two tertiary features
Probable—One secondary, plus one tertiary or three tertiary features
Suspect—One secondary, or two tertiary features

Primary features
Facial angiofibromas[a]
Multiple ungual fibromas[a]
Cortical tuber (histologically confirmed)
Subependymal nodule or giant cell astrocytomas (histologically confirmed)
Multiple calcified subchondral nodules protruding into the ventricle (radiographic evidence)
Multiple retinal astrocytomas[a]

Secondary features
Affected first-degree relative
Cardiac rhabdomyolysis (radiographic or histologic confirmation)
Other retinal hamartoma or achromic patch[a]
Cerebral tubers (radiographic confirmation)
Noncalcified subependymal nodules (radiographically confirmed)
Shagreen patch[a]
Forehead plaque[a]
Pulmonary lymphangiomyomatosis (histologic confirmation)
Renal angiolipoma (radiographic or histologic confirmation)
Renal cysts (histologic confirmation)

Tertiary features
Hypomelanotic macules[a]
"Confetti" skin lesions[a]
Renal cysts (radiographic evidence)
Randomly distributed in a multiparous in the deciduous and/or permanent teeth
Hamartomatous rectal polyps (histologic confirmation)
Bone cysts (radiographic evidence)
Pulmonary lymphangiomyomatosis (radiographic evidence)
Cerebral white matter "migration tracts" or heterotopias (radiographic evidence)
Gingival fibromas[a]
Hamartoma of other organs (histologic confirmation)
Infantile spasms

From Roach ES, Smith M, Huttenlocher P, Bhat M, Alcorn D, Hawley L. Diagnostic criteria: tuberous sclerosis complex. Report of the Diagnostic Criteria Committee of the National Tuberous Sclerosis Association. J Child Neurol 1992;7:221-4, with permission
[a]Histological confirmation not required if the lesion is clinically obvious

independent effectors and effects, as well as differing sensitivities to rapamycin and its analogs [13].

TSC1 (hamartin) and TSC2 (tuberin) form a heterodimer that is a key upstream regulator of mTORC1, functioning as a guanosine triphosphate (GTPase)-activating protein (GAP) for Ras homolog enriched in the brain (Rheb) (Fig. 10.2). The GTP-bound form of Rheb interacts directly with mTORC1, significantly enhancing its kinase activity [13]. TSC1/2 as a Rheb GTPase-activating protein negatively regulates mTORC1 by converting Rheb to its inactive GDP-bound state [14]. TSC1/2 integrates multiple upstream signals that attenuate mTORC1 including growth factors via PI_3k and Ras pathways. The effector kinases of these pathways (Akt/PKB, ERK1/2, RSK1) directly phosphorylate the TSC1/2 dimer to inactivate it, resultantly activating mTORC1 [15]. Cytokines, such as TNFα, may also activate TORC1 by phosphorylation of TSC1/2 via Iκβ kinase β (IKKβ) [16]. The Wnt pathway, a regulator of diverse cellular processes including differentiation, proliferation, and polarity, also modulates mTOR. By inhibiting glycogen synthase kinase 3b, phosphorylation of TSC2 is reduced leading to activation of mTORC1 [17].

Hypoxia, mediated via transcriptional regulation of DNA damage response 1 (REDD1), activates TSC2 function [18]. mTORC1 is activated by DNA damage through a p53-mediated mechanism. The induction of TSC2 and Pten results in downregulation of PI_3K-mTORC1 [19], and also, through induction of Sestrin1/2, activates AMPK [20]. Phosphatidic acid also activates mTORC1 [21].

mTORC1 may also be activated by amino acids (leucine and arginine), which are also required for activation of mTORC1 by some growth factors [22]. The mechanism of mTORC1 activation remains poorly understood, although it has been shown to involve the Rag GTPases and translocation of mTORC1 to the lysosomal surface [23].

Cellular processes regulated by mTORC1 include protein synthesis, lipid synthesis, energy metabolism, cell fate determination, autophagy, and cytoskeletal organization [13]. The role of the mTOR pathway in oncogenesis is evinced by mutations identified in human cancers and cancer syndromes. The loss of p53, a common observation in human cancers, promotes mTORC1 activation. Upstream from mTORC1 and mTORC2, components of the PI_3K pathway are also often mutated in human tumors. Several human cancer syndromes, including TS and neurofibromatosis type I, are defined by mutations in upstream signaling components of mTOR complexes. Dysregulation of translation and protein synthesis downstream of mTORC1 by interaction with initiation factor 4E-binding proteins ($_4$E-BP1/eIF$_4$E) likely plays a significant role in tumorigenesis by promoting cell cycle progression [24]. Another hallmark of proliferating cancer cells, lipid synthesis is regulated by mTOR-PI3K activation of the lipogenic factor SREBP1, which requires mTORC1 signaling [25].

A complex, TSC1-TSC2 (hamartin-tuberin), via GTPase-activating protein acts as a negative regulator of mTORC1, a controller of anabolic processes. Multiple factors and cellular signaling pathways are integrated, leading to phosphorylation events, and resultantly mTORC1 activity [26]. Dysregulated mTOR activity subsequently results in abnormal cellular division and differentiation across tissue types and abnormal cellular enlargement is seen, as is the case in SEGAs.

Clinical Presentation

SEGAs usually present with signs and symptoms of cerebrospinal fluid obstruction due to the encroachment of the foramen of Monro either uni- or bilaterally. The onset of symptoms is usually insidious, with progressive headache,

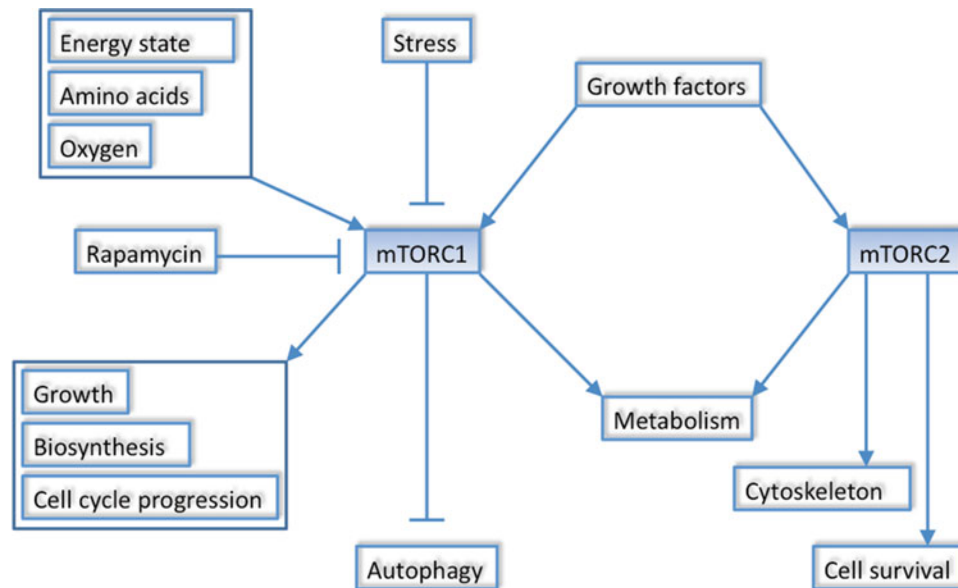
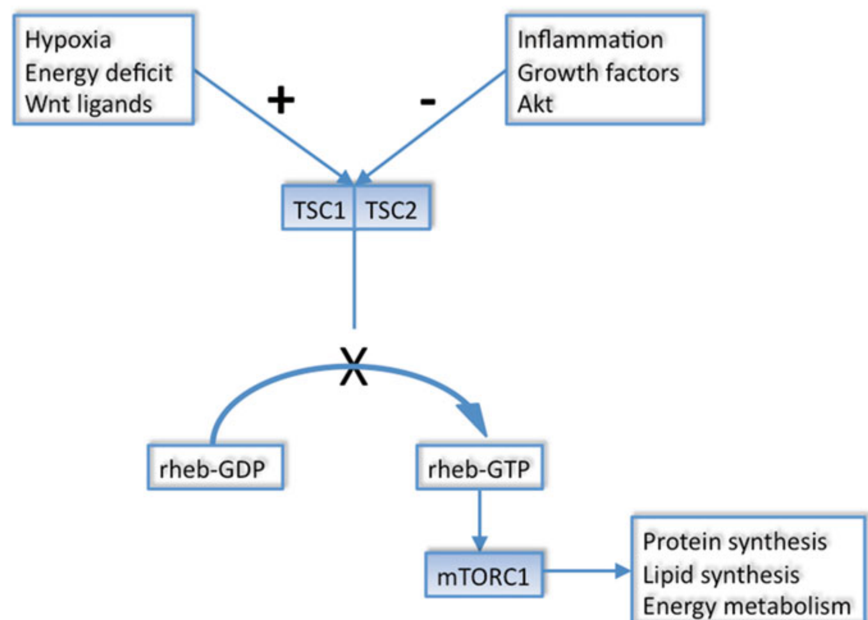


FIG. 10.1. Overview of mTOR1 and mTOR2 interactions and effectors.

FIG. 10.2. TSC1/2 complex interactions via a Rheb GTPase mediator leading to mTORC1 activation.



cognitive impairment, lethargy and finally, if unrecognized, coma and death. Occasionally, precipitous neurological decline or death due to acute hydrocephalus or intratumoral hemorrhage may occur [27–31]. Clinical history and findings suggestive of tuberous sclerosis may be present; epilepsy and other systemic manifestations may also lead to the diagnosis. SEGAs usually become symptomatic within the first two decades of life.

The diagnosis of TSC is based on clinical examination and confirmed with genetic testing. Cutaneous findings include hypomelanotic macules, facial angiofibromas, and shagreen patches. Oral lesions may include ungula or gingival fibromas. The three hallmark pathologies of TSC in the central nervous system (CNS) are cortical tubers, subependymal nodules, and SEGAs. Functional impairment of effected individuals may be due to seizures, intellectual disability, and/or developmental delay. Renal manifestation may include angiomyolipomas (AML), cysts, and renal cell carcinoma. Cardiac conditions, including rhabdomyoma and arrhythmias may be present. Pulmonary involvement is restricted to lymphangioleiomyomatosis (LAM). Consensus clinical diagnostic criteria (Table 10.1) were developed prior

to reliable genetic testing and allow for stratification as *definite*, *probable, or suspect* TSC [32]. Patients with somatic TSC2 mutations, as a group, are most severely affected. Somatic mutations of TSC1 are less affected [33]. Patients with genetic mosaicism may have localized, minimal, or no clinical evidence of TSC.

Radiographic Characteristics

Location is of primary consideration in the radiologic suspicion of SEGA. Given that the vast majority of these tumors arise within the lateral ventricle in the caudothalamic groove, medial to the posterior caudate nucleus, SEGA should be strongly considered in the differential diagnosis of tumors in this region [34]. Growth on serial neuroimaging differentiates SEGAs from subependymal nodules. The radiologic identification of SEGA may be made on ultrasound (in neonates, and rarely prenates), computed tomography (CT), and magnetic resonance imaging (MRI) [4, 35] (Figs. 10.3 and 10.4).

CT may show uni- or bilateral hyper-dense foci of calcification medial to the genu of the internal capsule (Fig. 10.3). In cases associated with TS, multiple calcifications and subependymal nodules (candle guttering) may be seen along the caudothalamic groove [36]. Ventriculomegaly may be identified unilaterally or bilaterally [37].

MRI characteristics mirror the heterogeneous pathology of SEGAs, with mixed signal intensities on T1- and T2-weighted imaging. SEGAs are usually hypo- and isointense on T1-weighted imaging, and iso- to hyperintense on T2-weighted imaging. Dense contrast enhancement is usually present, although it may occur in a heterogeneous pattern [38]. Calcified portions of the tumor, usually near the base of the tumor, typically appear hypodense on T2-weighted imaging (Fig. 10.4).

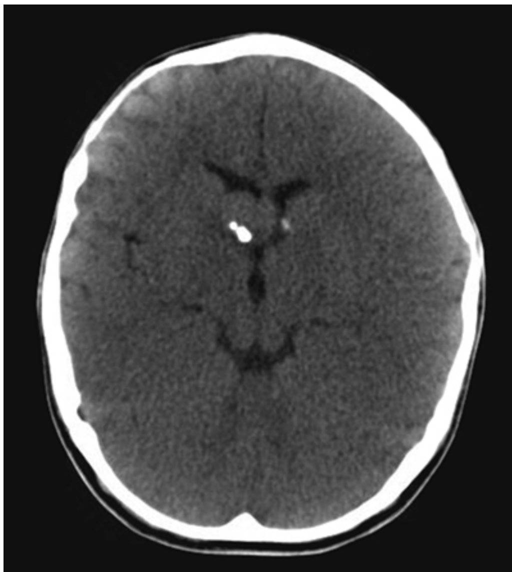


Fig. 10.3. Noncontrast CT scan, 3-year-old with TSC2 mutation. A SEGA is seen on the right, note calcification at the thalamocaudate groove.

Pathology

The origin of almost all SEGAs is the wall of the lateral ventricle, from the region of the posterior caudate/basal ganglia, just medial to the genu of the internal capsule with projection into the frontal horn or body of the lateral ventricle. A focus of dense calcification is often present at the base of the tumor. They are well circumscribed, lobulated, angiomatous, and slow growing. Tumor-related cysts may be present. Malignant transformation is uncommon [39]. SEGAs in other locations have been reported, including the cerebral cortex [40], pineal region [41], and retina [42–44].

Histologically, SEGAs may display a wide range of astrocytic, glial, and neuronal differentiation. Three cell types predominate: small spindle cells, gemistocytic astrocytes, and giant cell with ganglionic features (Fig. 10.5). Mitotic index is usually low and necrosis is an uncommon finding. Nucleoli are usually distinct in all of the cell types and a finely granular chromatin pattern is common. SEGAs may display features associated with malignant potential, pleomorphism, mitotic figures, necrosis, and vascularity; however, true malignant behavior is exceedingly rare [39, 45]. (Table 10.2).

Immunohistochemical staining is variably reactive for S-100 and GFAP—a reflection of the mixed astrocytic/glial composition and heterogeneity of the tumor. Neuronal markers including cytoskeletal components (neurofilaments, MAP2, class III Beta tubulin) and neurosecretory substances (serotonin, Beta endorphin, somatostatin) may also be positive [46]. The presence of both glial and neuronal markers within tumor cells supports the possibility that the originating cells of SEGAs have the potential to differentiate along glioneuronal in addition to neuroendocrine lineage, and to a greater degree than other mixed glioneuronal neoplasms [46]. Reported occurrence of SEGAs in the retina [42–44], with Mueller cell origin capable of dedifferentiation into pluripotent progenitor cell as their putative source, illustrates the potential mechanism of a common progenitor producing multiple cell types.

Treatment Options

The optimal treatment of SEGAs and other TSC-related conditions is an area of intense basic, translational, and clinical research. Recognition of the benign nature of these tumors, along with the potential for long life-expectancy mandates that treatment strategies not only result in long-term disease-free or progression-free survival, but also consider potential long-term complications and cost [47].

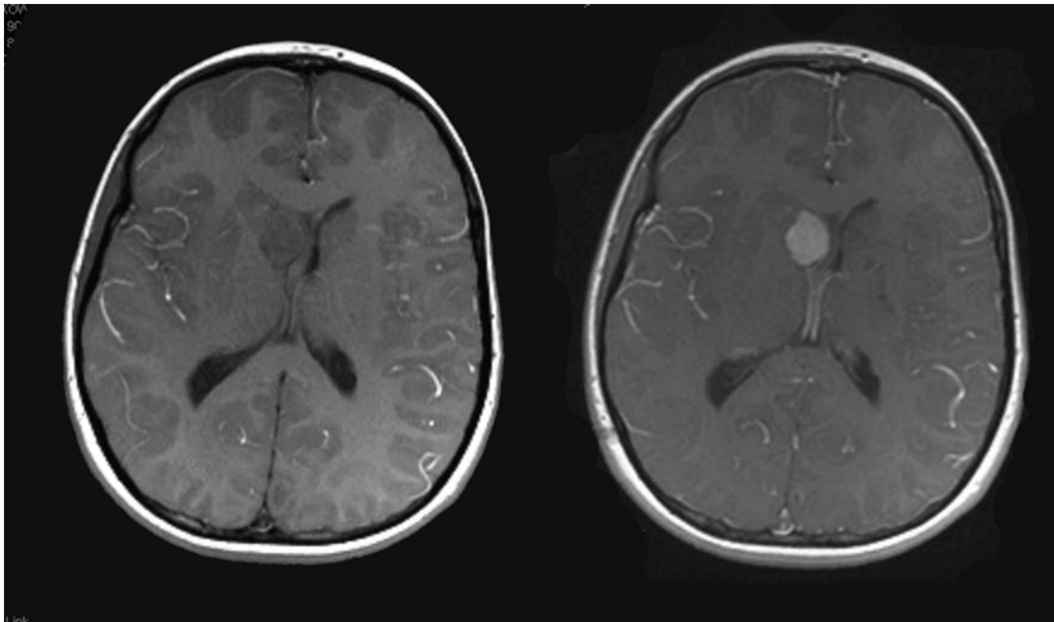


Fig. 10.4. Pre- and postgadolinium T1-weighted axial MRI, 9-year-old with TSC1 mutation. A SEGA is seen on the right, dense contrast enhancement is seen.

Observation

Serial clinic examination and radiologic surveillance are appropriate for incidentally discovered or small, stable, or slowly growing SEGAs. Rapid enlargement is unusual and clinical symptoms that are typically insidious allow for treatment on an elective basis [48–50].

Surgery

Various approaches for resection of SEGAs including craniotomy by transcallosal and transcortical approaches have been the reported. Early operative case series noted significant morbidity and mortality [51–53]. Contemporary series, however, with the aid of microdissection, stereotactic techniques, and modern pediatric neuroanesthetic techniques have significantly improved upon the results of these historical benchmarks [54]. A high rate of gross total resection, with little or no permanent neurological morbidity, can be expected at high-volume surgery centers [55–57]. Tumor recurrence after radiographically confirmed gross or radical subtotal resection is infrequent.

The preferred surgical approach depends upon a number of factors, ventricular size, prior surgery, and surgeon experience. Generally, smaller ventricular size favors a transcallosal approach. Significant ventriculomegally and the presence of an existing frontal resection cavity (i.e., from cortical tuber resection) may favor a transcortical approach. Additionally, success of a purely endoscopic approach via a single frontal burr hole has been reported and may be appropriate for some SEGAs [58].

Medical Therapy

SEGAs are slow-growing tumors with low, if any, potential for malignant transformation [39, 59, 60]. Conventional cytotoxic compounds do not have a role in their treatment. However, targeted medical therapy directed specifically at the implicated signal transduction pathways has emerged as a potentially effective and safe strategy to control SEGAs and other manifestations of TS. Progress in this area was initiated literally with the unearthing of rapamycin.

The discovery of the macrolide compound rapamycin began with a "bioprospecting" expedition to Easter Island ("Rapa Nui" in the native language). A soil sample obtained from the site included the bacterium *Streptomyces hygroscopicus*, from which a secondary metabolite with strong antiproliferative properties was obtained—rapamycin [61]. Eventually, the antifungal properties of rapamycin led to the discovery of its molecular targets—TOR1 and TOR2. Acting to suppress T-function, rapamycin was used in post-transplant patients as an immunosuppressant.

The mechanism of rapamycin and related compounds, known as rapalogs, upon the mTOR pathway is complicated; however, it is known to form a gain-of-function complex with FKBP12, a 12-kDa intracellular protein. This rapamycin-FKBP12 complex inhibits mTOR as component of mTORC1—although the molecular mechanism of this inhibition has not been elucidated. Current theories include impaired structural integrity of mTORC1 [62] and allosteric reduction of the complex's kinase domain activity [63].

The first rapalog approved in the USA was Temsirolimus for advanced renal cell carcinoma. In 2012 Everolimus was

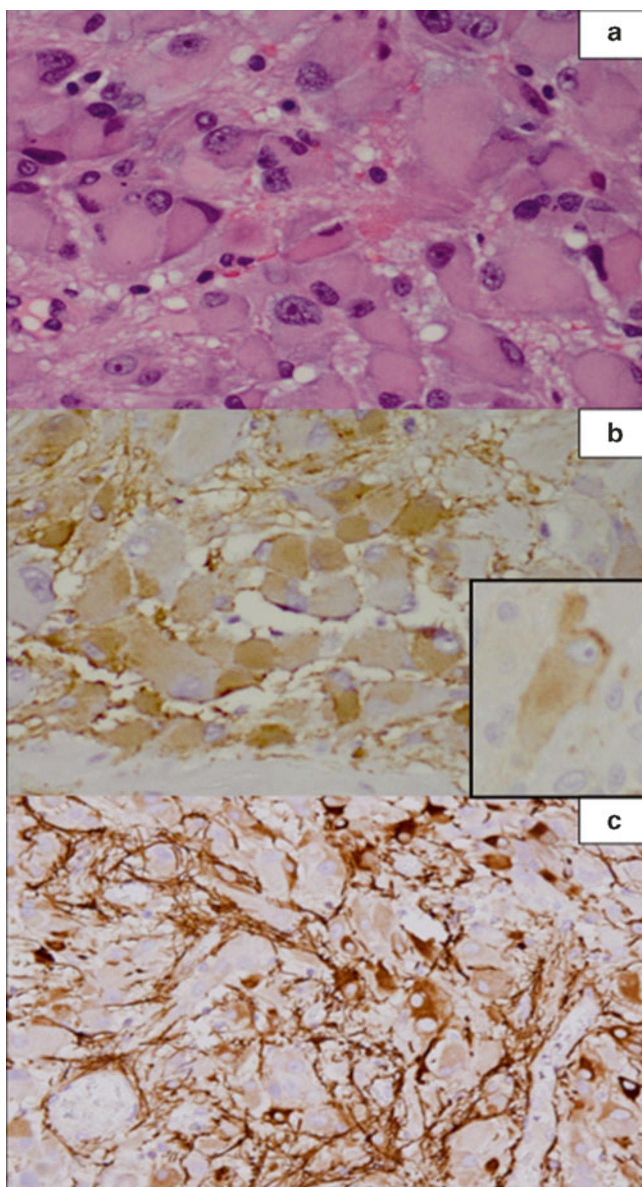


Fig. 10.5. Subependymal giant cell astrocytoma shows large mostly polygonal cells with abundant cytoplasm and often vesicular eccentric nucleus with prominent nucleolus (**a**). The tumor cells share features of glial cells and are immunoreactive for glial fibrillary acidic protein (**b**, **c**) but also have neuronal features and are immunopositive for synaptophysin (*inset*).

Table 10.2. Pathology of subependymal giant cell tumors.

Histology
Low mitotic index
Necrosis uncommon
Calcifications common
Differentiation all multiple lineages—astrocytic, glial, and neuronal
Cell types—gemistocytic astrocytes and giant cell with ganglionic features
Immunohistochemistry
S-100 and GFAP variably reactive
Neuronal markers—neurofilament, MAP2, class III Beta tubulin
Neurosecretory substances (serotonin, Beta endorphin, somatostatin)

approved by the Food and Drug Administration for the treatment of pediatric and adult patients with TSC who have SEGA that requires therapeutic intervention but *cannot be curatively resected* [12]. Case reports, clinical series and clinical trials, including a multicenter, placebo-controlled trial [64–68], have demonstrated ≥50 % volumetric reduction of SEGAs among treated patients. Notably, some trials have also demonstrated a meaningful reduction in seizure frequency during treatment [67, 69]. Common side effects include stomatitis, oral ulceration, and impaired wound healing [66, 70]. Metabolic side effects include hypercholesterolemia, hyperlipidemia, and hyperglycemia [71].

In addition to rapamycin and related compounds, the development of small molecule inhibitors of mTOR kinase activity has been investigated [72, 73]. As adenosine triphosphate (ATP)-competitive inhibitors of mTOR, these molecules block all phosphorylation targets downstream of mTORC1 and mTORC2. As a result, these compounds impair cell growth, proliferation, and tumor formation to a much greater extent than rapamycin, which solely inhibits mTORC1.

Radiosurgery

Stereotactic radiosurgery (SRS) has been used as a primary treatment of SEGAs and for tumor recurrence after initial surgical treatment in a small number of cases. Treatment doses of 13–14 Gee to the 50 % iso dose line have been used [74, 75]. A high rate of local control has been reported; however, instances of tumor progression have been noted, some retreated successfully with SRS, while others required surgical excision [74–76]. The risk of radiation-induced secondary tumors is a concern, especially in young patients, and development of glioblastoma has been reported after radiotherapy for SEGA in TSC [77].

Outcomes

CNS involvement is the leading cause of morbidity and mortality in patients with TSC and is usually related to status epilepticus or SEGAs [78]. Renal disease is the second leading cause of early death [9]. Cardiac or pulmonary involvement is also a potential cause of mortality in TSC [78]. Functionally, poorer cognitive outcomes have been shown for patients with bilateral cortical tubers and early age (<6 months) at the onset of seizures [79]. Tuber count or burden has not been shown to correlate with developmental outcome [80]. Treatment of disorders related to TSC, particularly SEGAs, is likely to undergo a tectonic shift. Rather than palliative and piecemeal strategies, targeted molecular therapies (rapalogs, multi kinase inhibitors, and others) are emerging. These agents may not only control tumor growth, but may also prevent CNS developmental malformations, control or prevent seizures, and ultimately lead to improved quality of life and outcomes.

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11 Germ Cell Tumors

Girish Dhall, Ashley A. Ibrahim, and Eyas M. Hattab

Abbreviations

AFP	Alpha-fetoprotein
βhCG	β human chorionic gonadotropin
CBTRUS	Central Brain Tumor Registry of the United States
CCKBR	Cholecystokinin B receptor
CGH	Comparative genomic hybridization
CNS	Central nervous system
CSF	Cerebrospinal fluid
FISH	Fluorescent in situ hybridization
GCTs	Germ cell tumors
hPL	Human placental lactogen
JMJD	Jumonji domain-containing
miRNA	microRNA
NGGCTs	Nongerminomatous germ cell tumors
PLAP	Placental alkaline phosphatase
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
SNRPN	Small nuclear ribonucleoprotein polypeptide N
WES	Whole exome sequencing
WHO	World Health Organization
YST	Yolk sac tumors

Central nervous system (CNS) germ cell tumors (GCTs) are a rare and heterogeneous group of malignant tumors that occur in children and young adults. According to the Central Brain Tumor Registry of the United States (CBTRUS) 2012 Statistical Report, CNS GCTs accounted for 0.5 % of all CNS tumors in adults, 1.3 % in young adults (ages, 20–34 years), 5.1 % in patients ages 15–19 years, and 3.6 % in patients 0–14 years of age [1]. The incidence of CNS GCTs is much higher in Asian countries where it has been reported to be as high as 9–15 % [2, 3]. CNS GCTs are twice as common in males than in females and 1.5 times more common in whites than in blacks [1]. GCTs typically occur in the gonads but extragonadal sites are more common in children with brain being the most common site in older children. Within the brain, GCTs occur predominantly in the pineal and suprasellar regions with basal ganglia being the third most common location. Approximately 5–10 % of patients have bifocal tumors involving both pineal and suprasellar areas [4].

Pineal tumors tend to be more common in boys while girls have a preponderance of suprasellar tumors. Primary tumors in the suprasellar region and basal ganglia as well as bifocal tumors are more likely to be germinomas whereas nongerminomatous germ cell tumors (NGGCTs) predominate at other sites.

The World Health Organization (WHO) classification of CNS GCTs divides these tumors into germinomas and NGGCTs. NGGCTs include teratoma (mature and immature), teratoma with malignant transformation, yolk sac tumor (YST), embryonal carcinoma, choriocarcinoma, and mixed tumors. While germinomas occur as pure tumors in 60–65 % of cases, nongerminomatous tumors more frequently occur as mixed tumors, which most commonly include germinoma and teratoma along with more malignant elements [5]. Hence, the term NGGCT is a misnomer in this sense and some investigators prefer to use the term mixed malignant germ cell tumors (MMGCT).

Histopathology

Germinoma

The classic germinoma is histologically identical to ovarian dysgerminomas and testicular seminomas comprising large monomorphous tumor cells separated into lobules by thin fibrous septa (Fig. 11.1). The septa contain varying amounts

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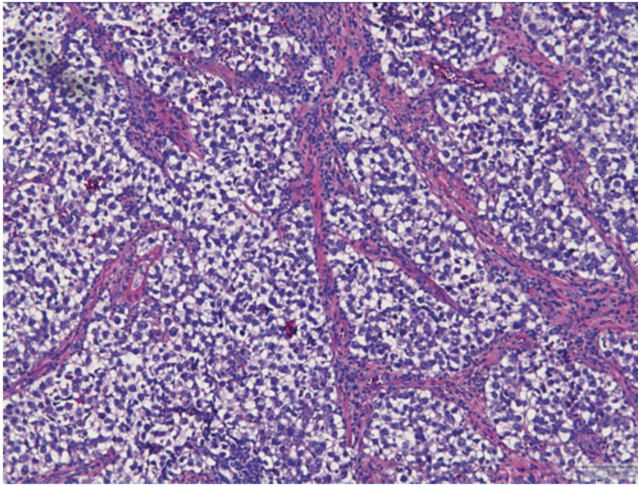

Fig. 11.1. Germinoma. Large monomorphous tumor cells separated into lobules by thin fibrous septa.

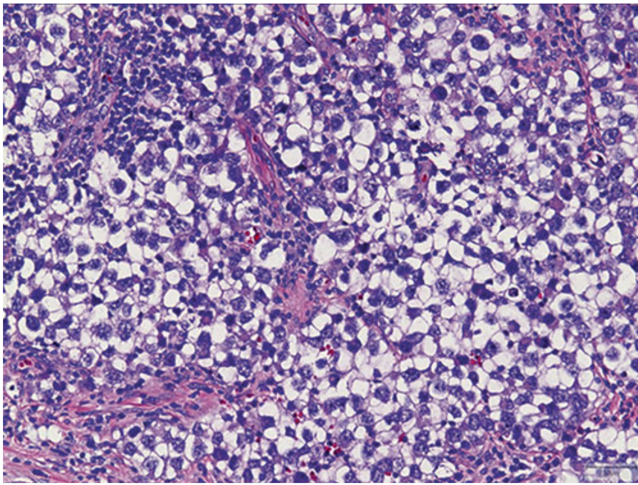

Fig. 11.2. Germinoma. Tumor cells have “squared off” nuclei, abundant clear cytoplasm, and distinct cell borders.

of T lymphocytes and occasionally noncaseating granulomas. In well-preserved, formalin-fixed samples, tumor cells have abundant clear or vacuolated cytoplasm, reflecting their high glycogen content and distinct cell borders. The nuclei are centrally located and have a squared-off appearance (Fig. 11.2). A single conspicuous nucleolus is characteristic. Both individual cell and confluent necrosis can be seen. Calcifications and syncytiotrophoblastic giant cells are additional infrequently encountered phenomena (Fig. 11.3). The latter may be responsible for trace levels of β human chorionic gonadotropin (βhCG) in the cerebrospinal fluid (CSF) but should not be confused with choriocarcinoma where a solid proliferation of syncytiotrophoblasts and cytotrophoblasts is needed for the diagnosis (see below).

Of practical significance is the fact that these tumors are not easily surgically accessible, often yielding very small

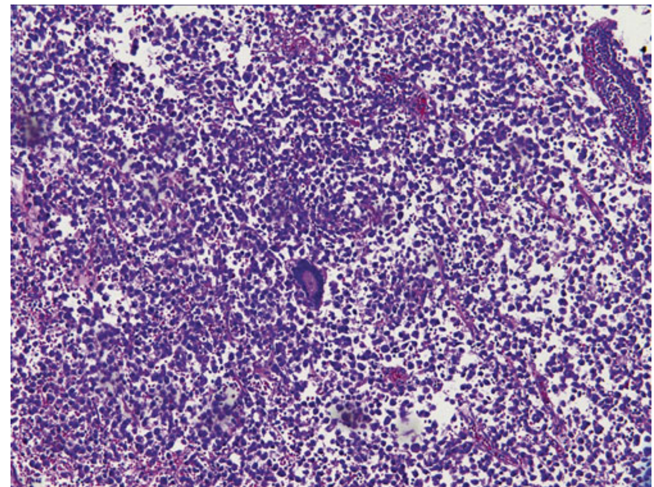

Fig. 11.3. Germinoma. Note the syncytiotrophoblastic giant cell.

samples. As such, in samples where the lymphocytic infiltrate predominates, the diagnostic tumor cells may be obscured and difficult to identify on hematoxylin and eosin-stained sections. Other samples may show an unusual single cell infiltration of the juxtaposed brain parenchyma similar to malignant gliomas or lymphomas. Such scenarios underscore the necessity of routinely employing immunohistochemical studies, including GCT markers, in the work-up of midline CNS lesions.

Yolk Sac Tumor

In contrast to germinomas, yolk sac tumors rarely occur in pure form. More commonly, they are a component of a mixed germ cell tumor. Cytologically, neoplastic cells are large and polygonal in shape with faint eosinophilic or clear cytoplasm and well-defined cytoplasmic borders. Nuclei are moderately atypical, but generally lack marked pleomorphism (Fig. 11.4). Tufts of malignant cuboidal-to-columnar tumor cells surrounding central blood vessels, known as Schiller-Duval bodies, are common though not universal, and not necessary for the diagnosis. The characteristic intercellular and extracellular PAS-positive/diastase-resistant eosinophilic hyaline globules and intercellular longitudinal bands of eosinophilic basement membrane material offer additional diagnostic clues.

Perhaps the most consistent finding in yolk sac tumors is the variety of different morphologic patterns often encountered within the same tumor; a mixture of patterns is the rule (Figs. 11.4 and 11.5). The most common among these is the *reticular* or *microcystic* pattern consisting of cysts lined by a loose network of flattened-to-cuboidal cells. A *macrocystic* pattern is seen when the microcysts coalesce. The *polyvesicular vitelline* pattern displays larger vesicles lined by flat-to-columnar epithelial cells. The *endodermal sinus* pattern shows a predominance of Schiller-Duval bodies and the *papillary* pattern shows papillae rimmed with

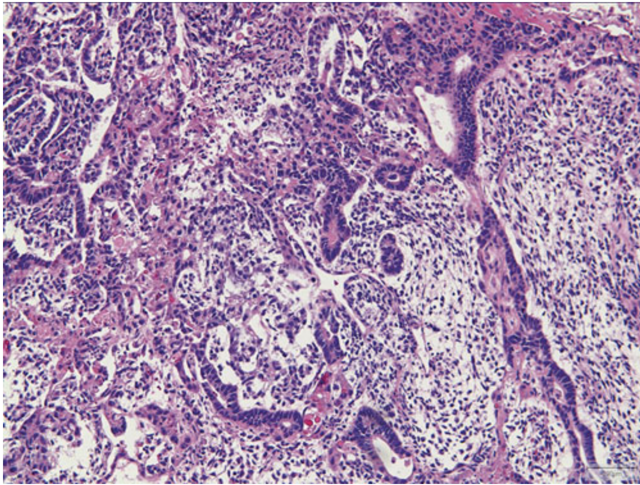

Fig. 11.4. Yolk sac tumor. Microcystic pattern.

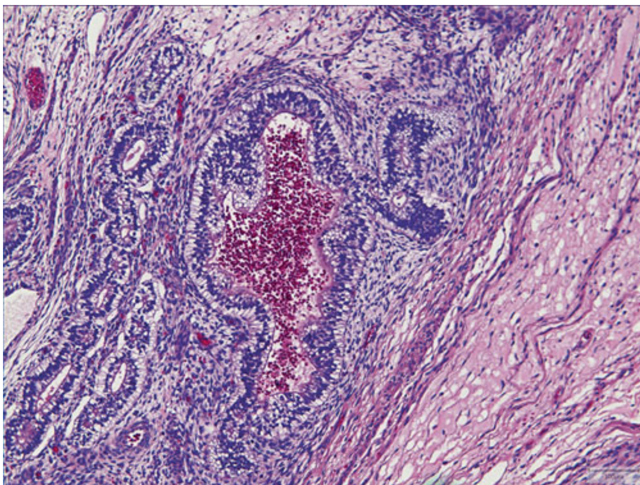

Fig. 11.5. Yolk sac tumor. Glandular pattern.

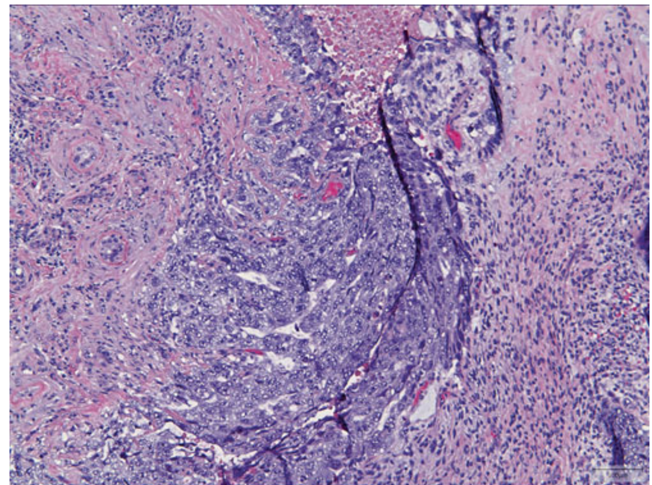

Fig. 11.6. Embryonal carcinoma. Tumor cells are larger than those of germinoma and more carcinoma-like.

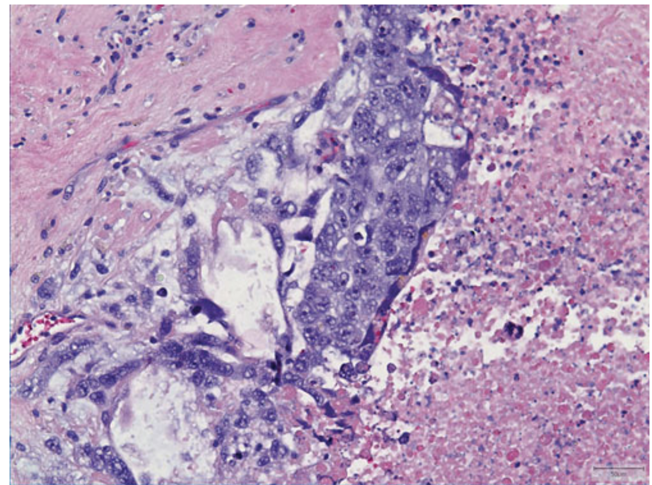

Fig. 11.7. Embryonal carcinoma. Tumor cells show oval-to-round hyperchromatic nuclei with large nucleoli.

tumor cells. Clusters of liver-like tumor cells are seen in the *hepatoid* pattern and neoplastic cells embedded in a matrix of basement membrane-rich material are evidence of *parietal* differentiation. *Glandular*, *myxomatous*, *solid*, and *sarcomatoid* patterns are often found in association with the aforementioned patterns.

Embryonal Carcinoma

Tumor cells are highly atypical and are generally larger, more pleomorphic, and more carcinoma-like than those of germinoma (Fig. 11.6). Nuclei are oval-to-round and hyperchromatic with irregular nuclear contours and large single or multiple nucleoli. Their cytoplasm is fairly abundant, somewhat granular, and variably staining. Cytoplasmic borders are not well defined, accounting for the syncytial appearance of the tumor (Fig. 11.7). The malignant cells can be arranged in solid sheets, cords, papillae, or gland-like patterns. The so-called *appliqué* pattern, characterized by smudged, degenerate-appearing cells seen towards the periphery of tumor nests, imparts a superficial resemblance to choriocarcinoma. Necrosis is common and the mitotic rate is typically high. As with germinomas, syncytiotrophoblastic giant cells are not an infrequent finding. Small foci of neoplastic poorly differentiated stroma, considered by some investigators to be teratomatous in nature, may accompany embryonal carcinoma and account for chemotherapy failure.

Teratoma

Akin to their gonadal counterparts, teratomas of the CNS can comprise both mature and immature elements. Pure mature teratomas tend to behave in an indolent fashion whereas teratomas occurring as part of a mixed GCT are more aggressive

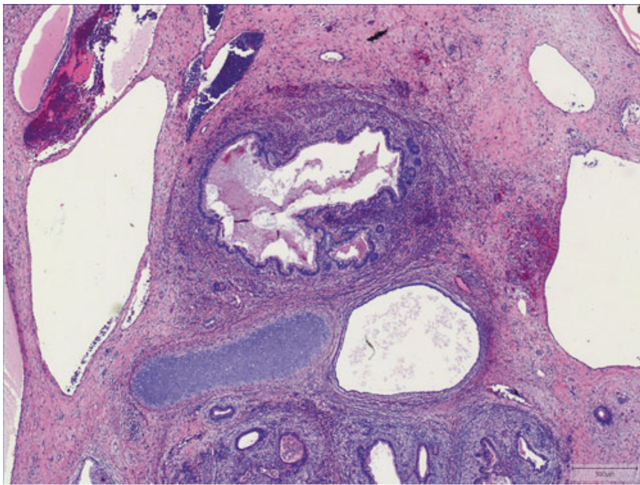

Fig. 11.8 Mature teratoma. Mature teratomas are made up of an admixture of differentiated tissues from more than one germ cell layer.

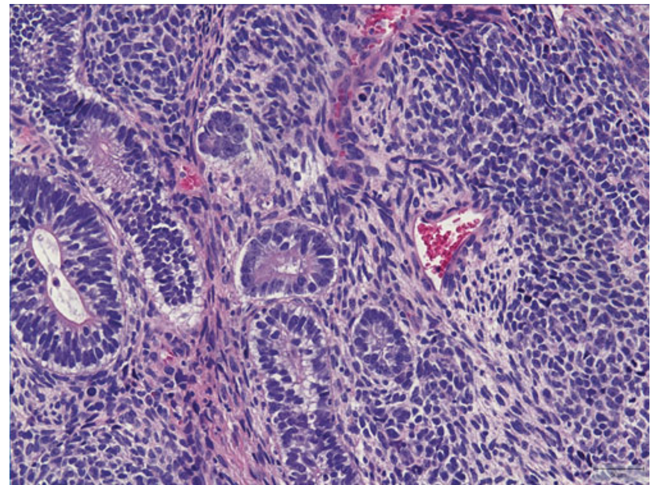

Fig. 11.9. Immature teratoma. Immature teratomas contain tissues that resemble primitive embryonic tissues. Note the immature neuroepithelium.

regardless of their degree of maturity. Their biologic behavior, thus, is likened to that of ovarian teratomas. Congenital teratomas, on the other hand, have a universally poor prognosis regardless of their composition.

Mature Teratoma

Mature teratomas are made up of an admixture of differentiated, adult-type tissues from more than one germ cell layer (Fig. 11.8). Skin and glial tissue are common ectodermal components and enteric, respiratory or transitional type tissues account for endodermal derivation. Focal increased cellularity, mitotic activity, and/or moderate cellular atypia are acceptable and should not prompt a diagnosis of immature teratoma, nor should the presence of "fetal" type tissues (e.g., fetal cartilage). The diagnosis of immaturity, rather, should only be made when tissues closely resemble embryonal (not fetal) type tissues (see below).

Immature Teratoma

Immature teratomas, by definition, contain varying amounts of incompletely differentiated tissues that resemble primitive embryonic tissues (Fig. 11.9). Most commonly, the immature tissue shows neural differentiation with rosette or tubule formation (i.e., primitive or embryonic-type neuroepithelium). Blastomatous-type stroma, consisting of small, round-to-spindled cells with hyperchromatic nuclei, apoptosis, and increased mitoses, is frequently encountered surrounding small, immature glands. Increased mitoses and apoptosis are not features of mature tissue, and can be helpful clues to the identification of immature elements. Any amount of immature component, no matter how small, is sufficient to render the diagnosis of immature teratoma. Following the convention of their ovarian counterparts, some observers choose to quantify the volume of the immature component and assign a grade (grade I–III) based on this volume. While the grading of immature teratomas in the ovary has documented prognostic significance, this has not been proven in the CNS to date. It is worth noting that maturation of a previously treated teratoma is a phenomenon often encountered secondary to effects of chemotherapy and irradiation. Conversely, malignant transformation of mature teratoma after treatment has also been documented [6].

Congenital Teratoma

Congenital teratomas are the most common neonatal brain tumor and, by definition, occur within the first 60 days of life [7, 8]. Although, they are histologically similar to those occurring later in life, key differences exist. First and foremost is their location. In contrast to the infratentorial teratomas of older children, congenital teratomas are predominantly supratentorial. Due to their large size, however, a precise determination of their site of origin is often difficult [9]. Secondly, congenital teratomas are generally pure, either mature or immature. Finally, congenital teratomas carry a dismal prognosis (>90 % mortality rate) while those occurring in older children generally have a much better clinical outcome [8].

Choriocarcinoma

Choriocarcinomas are the most malignant GCTs but are, fortuitously, the least common among the primary tumors. They are extensively hemorrhagic and highly necrotic tumors comprising of two cell types: syncytiotrophoblasts and cytotrophoblasts (Fig. 11.10). Syncytiotrophoblasts are easily recognized as large multinucleated cells with smudged vesicular nuclei and dark eosinophilic-to-amphophilic cytoplasm. Cytotrophoblasts are more uniform and have single

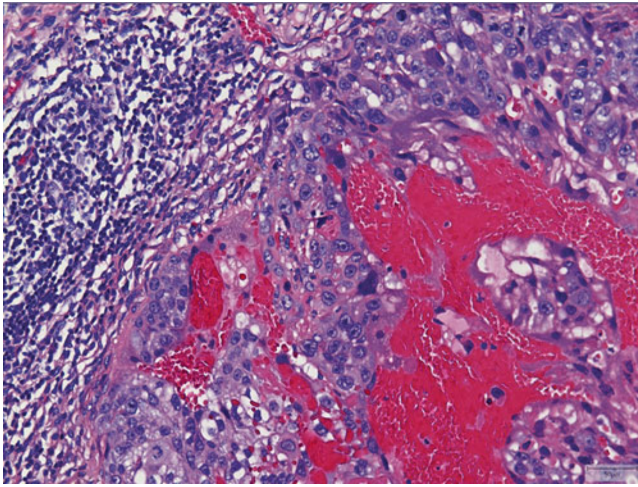

Fig. 11.10 Choriocarcinoma. Tumors show extensive hemorrhage and necrosis with recognizable syncytiotrophoblasts and cytotrophoblasts.

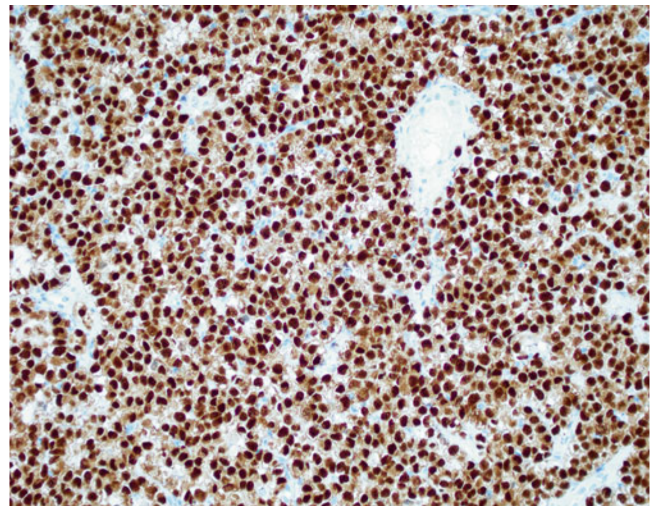

Fig. 11.11. Germinoma. OCT4 immunohistochemistry shows strong nuclear staining.

bland nuclei with vesicular chromatin and pale-to-amphophilic cytoplasm. Cytoplasmic borders are distinct. Syncytiotrophoblasts and cytotrophoblasts are arranged in a biphasic plexiform pattern similar to that seen in chorionic villi where sheets of cytotrophoblasts are surrounded (caped) by syncytiotrophoblasts. Mitoses are easily identifiable in the cytotrophoblastic component, but occur only exceptionally in syncytiotrophoblasts. Rarely, the cytotrophoblastic component dominates. This monomorphic pattern, which can be seen following chemotherapy, may be difficult to recognize and often requires immunohistochemical confirmation. As discussed earlier, it is important to recognize that scattered syncytiotrophoblasts are not uncommonly encountered in other GCTs and their presence alone is not diagnostic of choriocarcinoma.

Mixed Germ Cell Tumors

As previously mentioned, mixed germ cell tumors comprise two or more of the previously described histologic variants. The relative percentage of each component should be reported. It is recommended that the tissue be, at minimum, extensively sampled if not entirely embedded to avoid underreporting of a GCT component.

Ancillary Immunohistochemical Studies

Routine use of ancillary immunohistochemical studies is standard of practice in the work-up of primary CNS GCTs. The most historically utilized immunostain in GCTs, placental alkaline phosphatase (PLAP), is now obsolete and has been replaced by superior, more specific and sensitive transcription markers, such as OCT4 [10]. OCT4 preferentially highlights germinomas and embryonal carcinomas and has the added advantage of being a nuclear marker

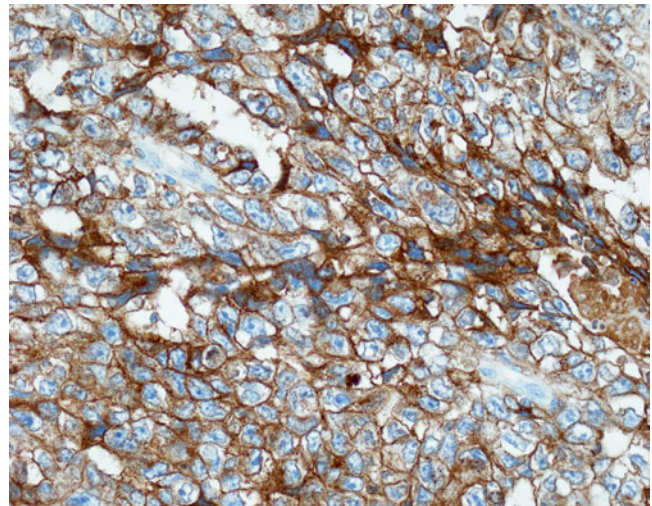

Fig. 11.12. Embryonal carcinoma. CD30 immunostain shows strong membranous staining.

allowing for easier interpretation (Fig. 11.11). CD30 shows strong membranous staining in embryonal carcinomas while other GCTs, including germinomas, are negative (Fig. 11.12). C-kit can also be exploited in the differential diagnosis of embryonal carcinoma versus germinoma as it shows strong and diffuse membranous staining in germinoma but focal or weak cytoplasmic staining in embryonal carcinomas [11–13].

SALL4 appears to be a fairly sensitive and specific pan-germ cell marker and can be used as a screening marker. A recent study by Mei et al. demonstrated 100 % sensitivity for germinomas, embryonal carcinomas, and yolk sac tumors (Fig. 11.13) [14]. Additionally, positive staining was observed in approximately two-thirds of teratomas and

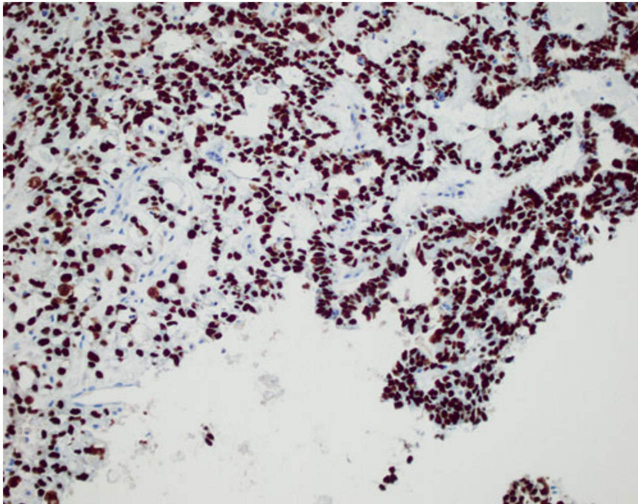

Fig. 11.13. Yolk sac tumor. SALL4, a pan-germ cell marker, shows strong nuclear staining.

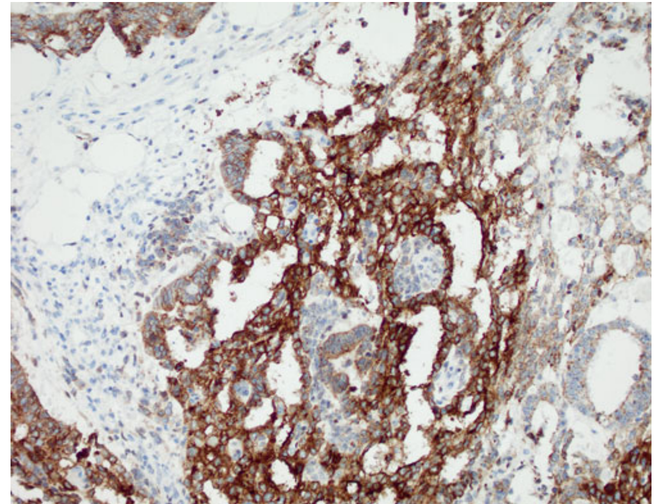

Fig. 11.14. Yolk sac tumor. Glypican 3 immunostain shows cytoplasmic staining.

choriocarcinomas; all non-GCTs were negative for SALL4 staining [14].

Alpha-fetoprotein (AFP) has historically served as the marker of choice for yolk sac tumors. Staining, however, is often focal and patchy and generally varies among the different patterns of tumors. Additionally, abundant background staining is often observed [14]. Glypican-3 is touted as a more superior marker in diagnosing yolk sac tumors of the ovaries and testes. Glypican-3 offers more precise and easy to interpret staining characteristics as well as improved sensitivity (Fig. 11.14) [15]. Studies evaluating glypican-3 staining in CNS yolk sac tumors, however, are limited.

βhCG and human placental lactogen (hPL) strongly stain the syncytiotrophoblastic cells of choriocarcinomas as well as those intermixed with other germ cell tumors [16, 17]. Cytotrophoblastic cells often are weakly positive or negative for these markers. Additionally, cytokeratins can also be used to highlight choriocarcinomas.

Histogenesis

GCTs are thought to arise from progenitor germ cells, mainly due to the facts that the germinoma component resembles progenitor cells very closely, intracranial GCTs resemble their extracranial counterparts morphologically and immunophenotypically, a single tumor can have multiple components (mixed GCTs) suggestive of differentiation of progenitor cells along various lines (embryonic and extraembryonic), and because none of the progenitor cells in the brain share any morphologic features with CNS GCTs. It is hypothesized that aberrant migration of the germ cell progenitors ventrally along the midline is responsible for the predominant midline location of these tumors throughout the body. Both testicular and CNS GCTs have been shown to have overexpression of wild type p53 and MDM2 proteins with a low incidence of TP53 gene mutation and a moderate incidence of MDM2 gene amplification pointing towards a common origin of these tumors [18]. Since $p14^{ARF}$, a protein coded by the *INK4a/ARF* gene locus, functions as a tumor suppressor and regulates the interaction between the MDM2 and p53 proteins by stimulating degradation of MDM2, Iwato et al. further tested for gene mutations in the *INK4a/ARF* gene in 21 CNS GCTs. They found that 71 % of tumors (90 % of germinomas and 55 % of NGGCTs) had either a homozygous deletion (14/15) or a frameshift mutation (1/15) in this gene pointing towards a more central role for this protein in the development of CNS GCTs [19]. More evidence linking germ cell progenitors to CNS GCTs is the lack of methylation seen in gonadal and extragonadal GCTs, since the progenitor cells transiently lose methylation of imprinted genes during migration. Small nuclear ribonucleoprotein polypeptide N (*SNRPN*) is an imprinted gene with complete lack of methylation, which is common to GCTs and progenitor cells. However, Lee et al. showed that lack of methylation of *SNRPN* and other imprinted genes is also seen in neural stem cells in the brain providing an alternate hypothesis about the origin of CNS GCTs [20].

Cytogenetics and Molecular Genetics

Little is known about the molecular biology of CNS GCTs. The overwhelming majority of intracranial GCTs are sporadic; however, a few conditions including Klinefelter syndrome and Down syndrome show higher incidence. Based on the predisposition of GCTs in patients with Klinefelter syndrome, Okada and colleagues studied 25 CNS GCTs with fluorescent in situ hybridization (FISH) for X and Y chromosomes and other chromosomal abnormalities described in

systemic GCTs [21]. They found extra copies of the X chromosome in 23 of 25 cases. They showed that extra X chromosomes were hypomethylated in nearly all tumors irrespective of histology, suggesting that these were active X chromosomes with some potential role in the etiology of these tumors [21]. Schneider et al. performed chromosomal comparative genomic hybridization (CGH) analysis on tumor samples from 19 CNS GCT patients (ages, newborn to 25 years; median age, 11.5 years) and then compared these to the CGH profiles of gonadal and extragonadal GCTs. All 15 malignant CNS GCTs had chromosomal imbalance with average number of imbalances being higher in NGGCTs than germinomas and CGH profiles of CNS GCTs being identical to gonadal/extragonadal GCTs. Gain of 12p was the most commonly detected abnormality (11 of 19 tumors and 10 of 15 malignant CNS GCTs). Other chromosomal imbalances detected included 1q gain (1q 21-24) and 8q11-21 gain [22]. In another study, chromosome 12 abnormalities, including 12p gain and isochromosome 12p formation were found at very high frequencies in CNS germinomas (96 % and 57 %, respectively), but only in 20–40 % of cases in others [21–24].

Gene Expression Profiling

Palmer et al. performed gene expression analysis on 27 pediatric malignant GCTs, including 3 CNS GCTs (2 germinomas and 1 YST), and showed that malignant YSTs had a completely different gene expression signature than testicular seminomas [25]. Self-renewing pluripotency genes (*Nanog*, *OCT3/4*, and *UTF*) were overexpressed in seminomas and genes responsible for tumor growth (cholecystokinin B receptor [*CCKBR*]) and differentiation (*KRT19*, *KRT8*, *GATA3*, and *GATA6*), and genes involved in WNT/β-catenin pathway were upregulated in yolk sac tumors. There were no significant differences in gene expression between CNS GCTs of similar histology arising at different sites and different ages within the pediatric age group. In addition, pediatric and adult testicular YSTs exhibited significantly different gene expression signatures suggesting different biologic behavior [25].

MicroRNAs (miRNAs) are responsible for controlling gene expression and also function as oncogenes as well as tumor suppressor genes within tumor cells. Palmer et al. studied miRNA profiles of 32 pediatric GCTs (gonadal and extragonadal), eight control samples, two adult testicular seminomas, and six GCT cell lines [26]. In unsupervised hierarchical clustering analysis, all pediatric GCT samples showed clear separation with seminomas, cell lines, YSTs, and embryonal carcinomas all having clearly different miRNA expression profiles. There was no overlap between malignant and nonmalignant (mature and immature teratoma) GCTs on a heat map based on differentially expressed miRNAs. Nine of the top ten differentially expressed miRNAs belonged to two clusters (miRNA-371 and miRNA-302) and were overexpressed in malignant GCTs compared to nonmalignant GCTs. Similar to gene expression profile, miRNA expression pattern was comparable in various histologic subtypes irrespective of patient age. Both of these miRNA clusters have been shown to be associated with human embryonic stem cells and their overexpression in turn regulates the expression of various transcription factors involved in oncogenesis and malignant progression. They further showed that YSTs and germinomas had a significantly different miRNA expression profile with members of the miRNA-2302 cluster overexpressed in YSTs compared to germinomas resulting in overexpression of transcription factors such as *GATA6*, *GATA3*, *SMARCA1*, and *SOX11*. They further showed that miRNA-451 and miRNA-144 were significantly overexpressed in intracranial compared to extracranial germinomas and miRNA-320, miRNA-487b, and miRNA-491-3p were significantly underexpressed [26].

Murray et al. used TaqMan® quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) to measure miRNA levels in the serum of a 4-year-old boy with a sacrococcygeal mixed GCT with a predominant YST component (AFP 82, 420 kU/L) at diagnosis and followed the levels during treatment. miRNA-71~373 and miRNA-302 were significantly overexpressed in the patient's serum when compared to three healthy controls. miRNA-372 and miRNA-373 levels were 703 and 192 times higher, respectively. The level of miRNA-372 dropped to 5.8-fold higher on day 73 (serum AFP, 5.8), 2.2-fold higher on day 91 (serum AFP, 6), and <1-fold higher on all subsequent follow-up time points (serum AFP, <2) [27]. Terashima and colleagues examined 32 CSF samples from 22 intracranial GCT patients for expression of miRNA-371–373 and miRNA-302–367 clusters. Significantly higher expression levels were found in CSF of GCT patients compared to controls, as well as pretreatment samples compared to those collected during or after treatment. In addition, miRNA-373 expression was significantly higher in germinomas when compared to NGGCTs [28]. These two publications highlighted for the first time the potential for using miRNAs as diagnostic and/or therapeutic biomarkers for CNS GCTs.

Prognostic Stratification and Treatment

The histologic subtype of the tumor remains the best predictor of prognosis in CNS GCTs. Germinomas are sensitive to chemotherapy as well as radiotherapy. Patients with germinoma have been shown to have survival rates in excess of 90 % with craniospinal irradiation (CSI) followed by a boost to the primary site to 50 Gy [29, 30]. However, due to the deleterious long-term side-effects of irradiation on the developing brains of young children, treatment strategies involving upfront chemotherapy along with reduced dose and volume of irradiation have been utilized by multiple cooperative groups all across the world with similar survival statistics.

[31–33] βhCG-producing germinomas (i.e., germinomas with syncytiotrophoblasts) have been shown by some to have a higher recurrence rate than for pure germinomas, justifying a more aggressive treatment approach [34, 35]. However, other studies have shown no difference [36]. The current Children's Oncology Group (COG) study, ACNS1123, is utilizing a treatment approach using carboplatin and etoposide for four cycles followed by low-dose ventricular field irradiation (1,800 cGy whole ventricular irradiation with a boost to a total dose of 3,000 cGy to the primary site) for patients with non-disseminated disease in the pineal or the suprasellar region.

Unlike germinomas, CNS NGGCTs are more resistant to therapy and using irradiation-only strategies resulted in survival figures of only 20–40 % at 5 years [4, 37, 38]. Chemotherapy combined with irradiation is currently considered the standard of care for NGGCTs, with the exception of mature teratomas. Recently closed COG study, ACNS0122, treated CNS NGGCT patients with six cycles of chemotherapy (cycles of carboplatin and etoposide alternating with cycles of ifosfamide and etoposide) followed by full-dose CSI to 3,600 cGy and a boost of 5,400 cGy to the primary tumor site, which resulted in 2-year event-free survival (EFS) and overall survival (OS) of 84 % and 93 %, respectively. Patients with localized disease who achieved a complete response (CR) or partial response (PR; >65 % reduction in the size of the primary tumor radiologically and negative tumor markers) had a 2-year EFS and OS of 91.6 % and 98 %, respectively [39]. Based on these results, the currently open COG study, ACNS1123, is attempting to reduce the dose and field of irradiation (from 3,600 cGy CSI to 3,000 cGy ventricular field irradiation) in patients who achieve either a CR or PR to induction chemotherapy (similar to ACNS0122). Mature teratomas are treated by total surgical resection with 5-year survival rates as high as 93 % [35]. Patients with immature teratomas often require additional therapy following gross total resection. Some recommend the use of local or partial brain field radiation while others advocate aggressive surgical resection alone for "low-grade" immature teratomas and adjuvant chemotherapy and radiotherapy for those with "high-grade" histology [40, 41]. Massive intracranial congenital teratomas are almost universally fatal.

Molecular Signaling Pathways and Targeted Therapies

Japanese intracranial GCT consortium performed whole exome sequencing (WES) on 33 CNS GCTs. *KIT* mutations were the most commonly found abnormality, predominantly in germinomas. Mutations in *MTOR*, *NF1*, and *EGFR* genes were found in tumors negative for *KIT* mutations [42]. Wang et al. performed WES on 28 CNS GCTs (12 germinomas, 12 NGGCTs, and 4 mixed GCTs) [43]. *KIT* mutations were present in 38 % of cases with mutations in *KIT* and *RAS* being mutually exclusive. Mutations in tumor suppressor genes such as *TP53*, *PTEN*, and *PTCH1* were found in 21 % of cases. The authors reported for the first time a significant enrichment in Jumonji domain-containing (*JMJD*) genes in 39 % of the cases [43]. *JMJD* family proteins are a group of histone demethylases that are involved in fundamental processes such as transcription regulation and DNA repair [44]. Fukushima et al. screened 52 CNS GCTs (30 germinomas, 9 teratomas, 12 mixed GCTs, and 1 YST) for mutations in genes involved in MAPK pathway and detected mutations only in *KIT* and *RAS* genes [45]. These mutations were more frequent in germinomas (60 %) versus NGGCTs and were again mutually exclusive. Interestingly, c-kit expression by immunohistochemistry was present in all germinomas regardless of the mutation status [45]. These studies demonstrating alterations in several signal transduction pathways that might play a role in the pathogenesis of CNS GCTs might provide molecular targets for development of novel therapeutic agents in the future.

Osorio and colleagues reported on six patients with CNS GCTs (five pure germinomas and one mixed CNS GCT with predominant germinoma components), who were treated with dasatinib (KIT inhibitor) in an effort to avoid irradiation and/or to delay recurrence [46]. The study could not directly assess the efficacy of dasatinib in this population, since most patients received dasatinib while they were in a minimal residual disease state, i.e., no evaluable target lesions on imaging. However, only 33 % of patients received irradiation in conventional dosing, suggesting a possible role for targeted therapy with KIT inhibitors in combination with chemotherapy with or without irradiation [46].

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12 Choroid Plexus Tumors

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Definition

Choroid plexus tumors (CPTs) are neoplasms derived from the choroid plexus epithelium of the brain. Three types have been described. (1) Choroid plexus papilloma (CPP), with delicate fibrovascular connective tissue covered by a single layer of epithelial cells with round or oval monomorphic nuclei defined as WHO grade I tumors [1] (Fig. 12.1b). (2) Atypical choroid plexus papillomas (APPs) are characterized by increased mitotic activity and are WHO grade II tumors. [1] (Fig. 12.1c). A mitotic index of two or more mitoses per 10 randomly selected high power fields (HPFs) (one HPF corresponding to 0.23 mm^2) can be used to establish the diagnosis of APP [2]. (3) Choroid plexus carcinomas (CPCs) are characterized by frank histological signs of malignancy, including at least four of the following five features: (a) frequent mitoses (usually greater than 5 per 10 HPFs), (b) increased cellular density, (c) nuclear pleomorphism, (d) blurring of the papillary pattern, and (e) necrosis (Figs. 12.1d and 12.2). These tumors correspond to WHO grade III [1].

Clinical Features

Epidemiology

CPTs are rare, representing between 4 and 9 % of brain tumors in population-based studies [3, 4]. The typical age of presentation is the first year of life. However, CPTs can occur as rare congenital brain tumors [5, 6] and in adults, including the elderly [7–9]. These tumors also occur in other species including canines [10–14]. While most tumors are sporadic, CPTs are reported in: Li–Fraumeni syndrome (discussed below), pediatric patients with large melanocytic skin lesions [15], Pierpont syndrome [16], hypermelanosis Ito [17], and Aicardi syndrome [18]. CPPs can also be seen in von Hippel–Lindau disease [19], but it is not known if loss of the VHL allele contributes to the pathogenesis of these tumors. It is possible that these abnormalities indicate rare pathways of tumorigenesis that are yet to be elucidated.

Clinical Presenting Features

Raised intracranial pressure by hydrocephalus is the main presenting feature of CPTs [20, 21]. Other presenting symptoms may include blindness, non-focal symptoms such as convulsions [20] or focal signs such as hemiparesis [22, 23]. Large amounts of cerebral spinal fluid (CSF) production may also be encountered even when a drain or shunt is placed [24]. Sometimes highly vascularized tumors present as acute intracranial hemorrhage without previous symptoms [25]. Tumor locations in the cerebello-pontine angle may result in more specific signs such as unilateral rhinorrhea and otorrhea [26], or trigeminal neuralgia [27].

Imaging and Tumor Localization

The typical imaging characteristics of CPTs include intraventricular location and contrast enhancement indicating a high degree of vascularization. Invasion of brain tissue is a characteristic finding for CPCs as opposed to CPPs. CPTs are more common in the lateral ventricles [28] than in the third or fourth ventricles [29]. The cerebello-pontine angle is a typical location when the tumor originates in the choroid plexus of the fourth ventricle [30]. Ectopic locations unrelated to the ventricles/choroid plexus have also been described [23, 31–34]. Lateral ventricle tumors are more frequent in infants, while cerebello-pontine angle tumors occur more frequently in adults [30]. An unusual feature of CPTs is that even WHO grade I and II tumors have the potential to metastasize [35–39]. The typical metastatic route is through the CSF [39, 40], often to the spine but also with surprising frequency to the internal ear canals, pituitary stalk, and interpeduncular fossa [41]. Metastases can occur sometimes many years after primary tumor resection. Imaging of the entire craniospinal axis is recommended [40, 42, 43]. Rarely, hematogeneous metastases can occur to other

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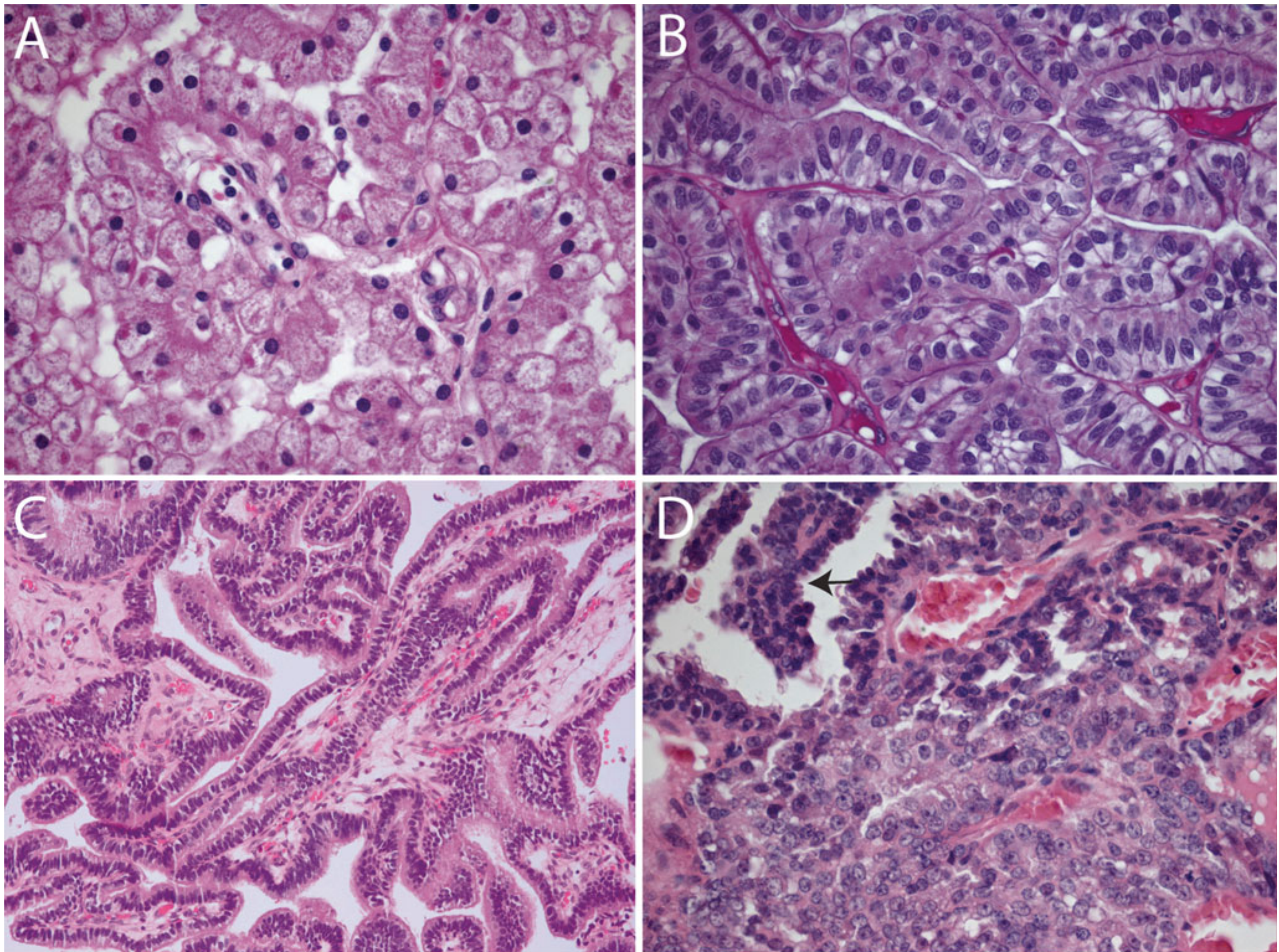

Fig. 12.1. *Histologic features of normal choroid plexus and choroid plexus tumors*. (**a**) Normal choroid plexus demonstrating typical "cobblestone" appearance (40×). (**b**) Choroid plexus papilloma, in contrast to normal choroid plexus in 2A, displays more elongated/columnar cells crowded in a pseudostratified manner and a smooth rather than cobblestone appearance (40×). (**c**) Atypical choroid plexus papilloma, with increased cellularity and focal blurring of the papillary architecture (20×). (**d**) Choroid plexus carcinoma showing focal papillary architecture (*arrow*) adjacent to an area where the papillary architecture is more blurred (40×).

parts of the brain [44], abdomen [45], bone [46, 47], and lung [48]. Abdominal seeding has been described in patients with ventricular-peritoneal shunts [49].

Imaging techniques for CPTs differ in several aspects from that for other brain tumors. As CPTs are the most frequent brain tumors detected on prenatal ultrasound [50–53], ultrasound remains an important diagnostic tool until the fontanels close [6, 51, 54]. On magnetic resonance imaging (MRI), CPTs show inhomogeneity on T2-weighted images, and moderate to marked contrast enhancement. Diagnostic specificity increases when age and intraventricular location are considered. Contrast enhancement, a common finding in these tumors, might be related to the rich vascular stroma. However, extraventricular tumors might not demonstrate contrast enhancement [34]. Differentiating the degree of malignancy on MRI can be difficult. Nevertheless, some general patterns may be observed. CPPs are usually irregular, lobulated, and solid-cystic masses, whereas CPC may present as a poorly defined, mixed-intensity mass [55]. Extensive peritumoral edema and necrosis is more frequent in CPC than in CPP [56, 57]. A thin capsule may be seen in CPP [55].

More recently, nuclear medicine methods and molecular imaging may improve diagnostic ability and also address challenging clinical issues including how to distinguish between tumor and postsurgical scars/postradiation pseudoprogression. For example, sestamibi, an agent that accumulates in mitochondria, has been used to distinguish CPTs from other brain tumors or postsurgical scars [58–62].

Pathology and Diagnostics

Macroscopy

CPTs appear macroscopically as space-occupying lesions located in the ventricles and, less commonly, in extraventricular locations. Grossly, CPPs are well demarcated with a cauliflower-like appearance. They may be attached to the

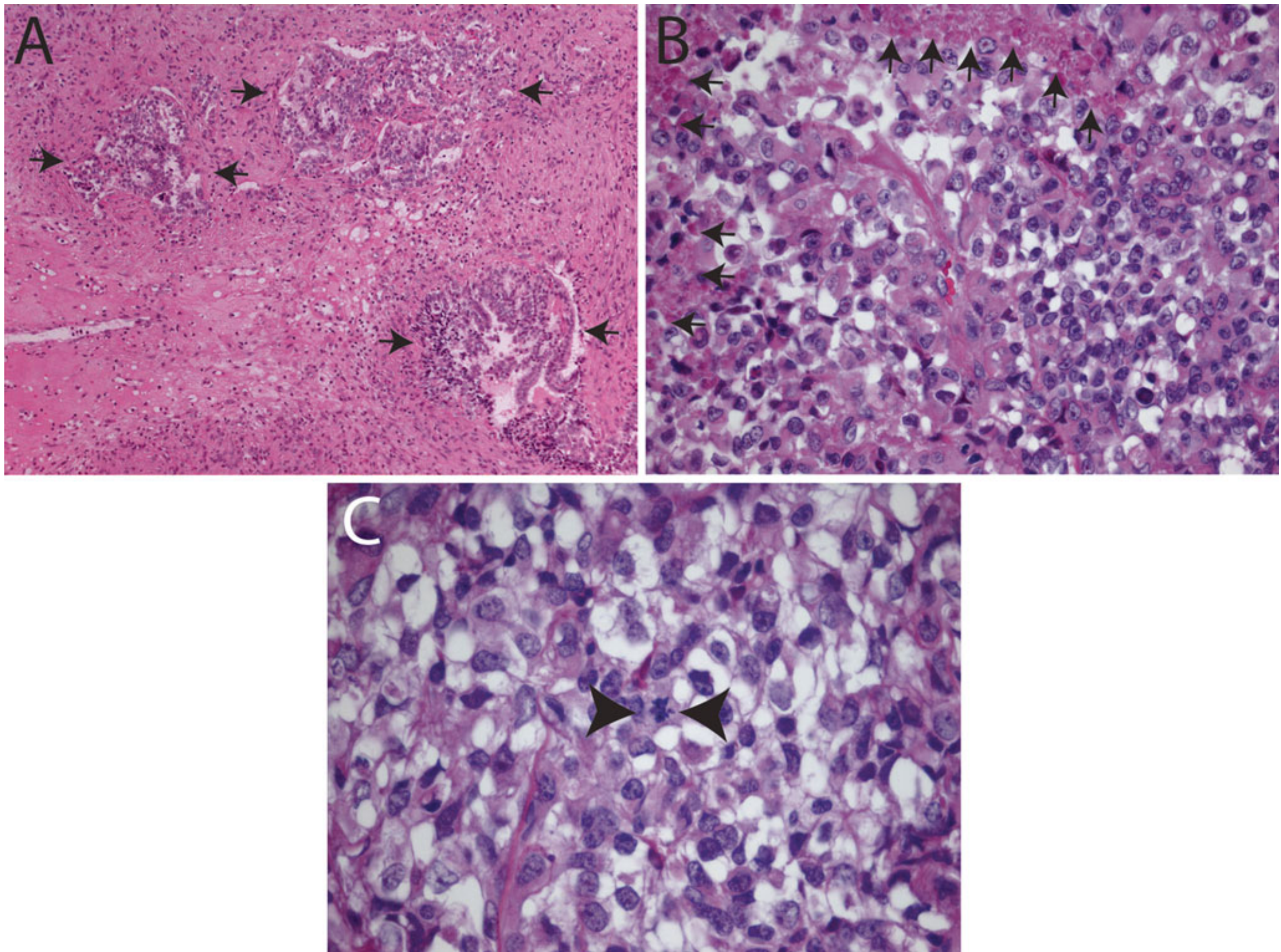

Fig. 12.2. *Histologic features of choroid plexus carcinomas*. (**a**) Choroid plexus carcinoma invading surrounding brain tissue (*arrows*, 10×). (**b**) Choroid plexus carcinoma exhibiting increased cellularity, nuclear pleomorphism, blurring of papillary architecture, and focal areas of necrosis (*arrows*, 40×). (**c**) Choroid plexus carcinoma showing loss of papillary architecture, increased cellularity, nuclear pleomorphism, and a mitotic figure between *arrowheads* (63×).

ventricular wall. CPCs usually show varying degrees of invasion into the surrounding brain. High vascularity and hemorrhages are frequent.

Histopathology

CPTs are typically comprised of epithelial cells with a round or oval nucleus and small amount of surrounding cytoplasm. CPTs are fragile, and drop metastases due to CSF spread are not uncommon [63]. Key cytologic features of CPTs in the CSF include variably sized clusters to frank papillary fragments, and cells that retain epithelial features such as sharply defined cell borders [43, 64].

Specific features of the different CPTs are described below, but from a practical point of view, these tumors can be histologically classified by where they fall along the spectrum of three key histologic features: (1) growth pattern—papillary to solid, (2) mitotic activity/cellular atypia—few or none/absent to moderate/moderate to severe, (3) necrosis—little to none or prominent.

CPPs, the least malignant of CPTs, are composed of delicate fibrovascular connective tissue fronds covered by a single layer of epithelial cells with round or oval monomorphic nuclei (Fig. 12.1b). CPPs can be distinguished from normal choroid plexus (Fig. 12.1a) by an overall increased amount of choroid plexus epithelium with flatter papillae (compared to the typical cobbled stone appearance of normal choroid plexus) comprising cells with increased nuclear to cytoplasmic ratios (Fig. 12.1a, b). These cells rest upon a basement membrane that can be elucidated with special stains for collagen. Mitotic activity is extremely low. Brain invasion, high cellularity, necrosis, nuclear pleomorphism, and focal blurring of the papillary pattern are unusual, but may occur and should prompt the consideration of APP. Rarely, CPPs acquire unusual histological features, including oncocytic change, mucinous degeneration, melanization as well as formation of bone, cartilage, adipose tissue, or neuropil islands. CSF-mediated metastases can occur despite the histologic classification of WHO grade I [38].

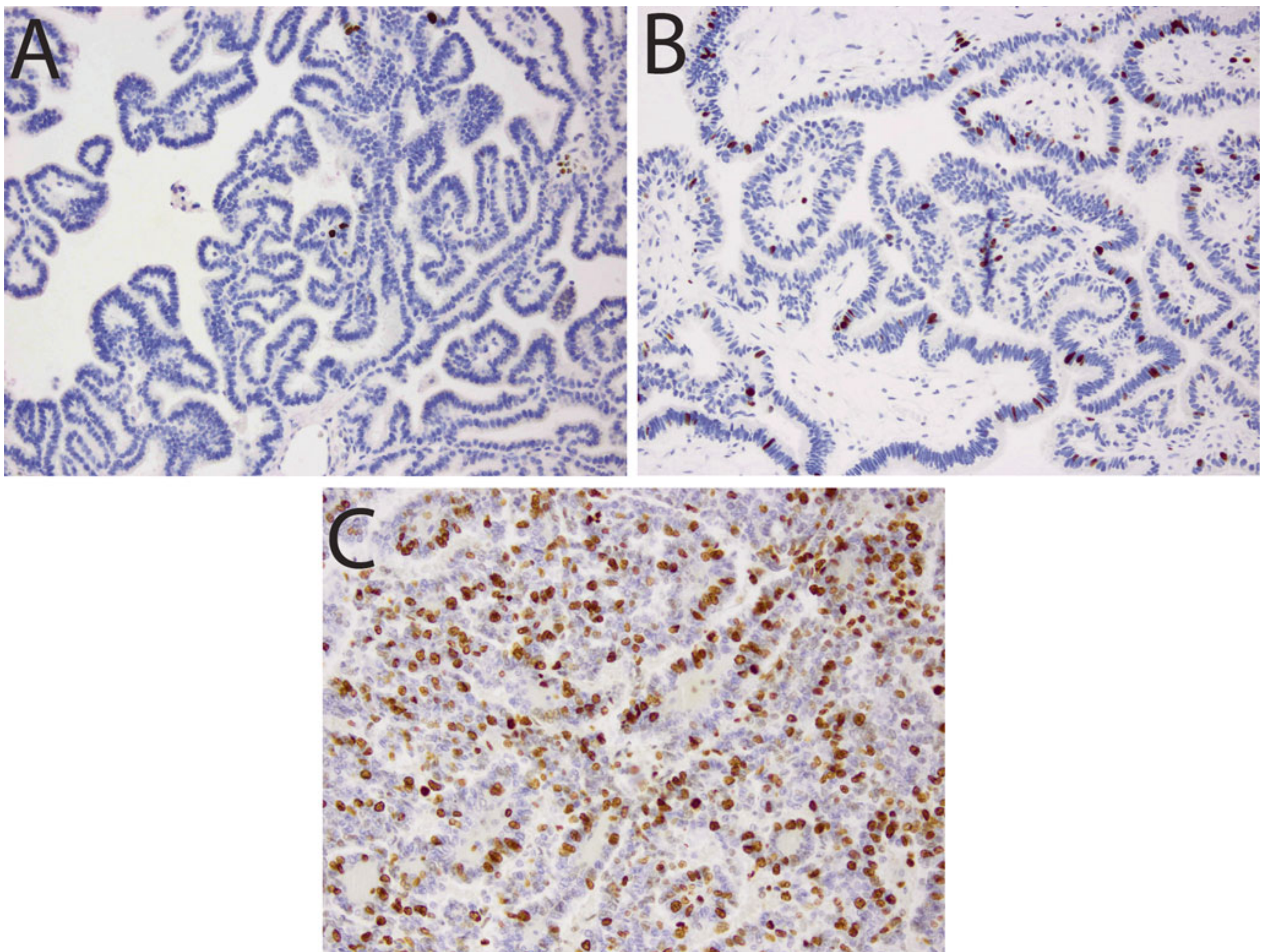

Fig. 12.3. *Proliferative activity in choroid plexus tumors.* Immunostains for Ki67/MIB1 illustrating: (**a**) Choroid plexus papilloma with low mitotic index (20×). (**b**) Atypical choroid plexus papilloma, with intermediate proliferative activity (20×). (**c**) Choroid plexus carcinoma, showing high Ki67/MIB1 labeling (20×).

An intermediate grade CPT, the atypical choroid plexus papilloma (APP) is recognized by the WHO (Grade II) (Figs. 12.1c and 12.3b). These tumors are defined by the presence of two or more mitoses per ten HPFs in what otherwise appears to be a CPP [1, 2]. Additional histologic features that have been reported in APP by some authors include increased cellularity, nuclear pleomorphism, solid growth, and necrosis. However, none of these features are required for the diagnosis of APP and the isolated occurrence of atypical histological features does not automatically imply malignancy.

CPC is a high-grade (WHO III) tumor of the choroid plexus that demonstrates frank signs of malignancy. In contrast to lower grade CPTs, CPCs demonstrate at least four of the following five features (1) frequent mitoses (Fig. 12.2C, typically more than 5 per 10 HPF), (2) increased cellular density (Figs. 12.1d and 12.2 b, c), (3) nuclear pleomorphism (Figs. 12.1d and 12.2b, c), (4) blurring of the papillary pattern (Figs. 12.1d and 12.2b, c), and (5) necrosis (Fig. 12.2b). Invasion of adjacent brain tissue by CPC is common (Fig. 12.2a). In CPCs that are truly anaplastic, identification of epithelial features may become quite challenging.

Immunohistochemistry

CPTs demonstrate expression of a wide range of immunohistochemical markers, a reminder that despite their epithelial appearance, these cells have a neuroepithelial developmental origin. The typical, though variably expressed, pattern includes S-100 protein, synaptophysin, vimentin, cytokeratins (Fig. 12.4d), glial fibrillary acidic protein (GFAP) (Fig. 12.4c), and transthyretin (TTR) (Fig. 12.4a) [65–67]. While TTR is expressed in normal choroid plexus and in many CPTs, it is unfortunately nonspecific and therefore unreliable as a precise marker of choroid plexus origins in any given tumor. However, immunohistochemical detection for membranous expression of the inward rectifier potassium channel Kir7.1 is considered specific for CPT [68] (Fig. 12.5). The Ki67/MIB index can be helpful in refining tumor grade [69] (Fig. 12.3).

Fig. 12.4. *Immunohistochemical analyses in choroid plexus tumors*. (**a**) Immunostain for transthyretin in a choroid plexus papilloma showing diffuse positivity (40×). (**b**) SMARCB1 immunostain in a choroid plexus carcinoma showing strong nuclear positivity (40×). Retained SMARCB1 staining helps differentiate these tumors from AT/RT, which show loss of SMARCB1 expression. (**c**) Focal GFAP expression in a choroid plexus carcinoma (20×). (**d**) Cytokeratin stain (AE1-3) in a choroid plexus carcinoma showing focal immunoreactivity (20×).

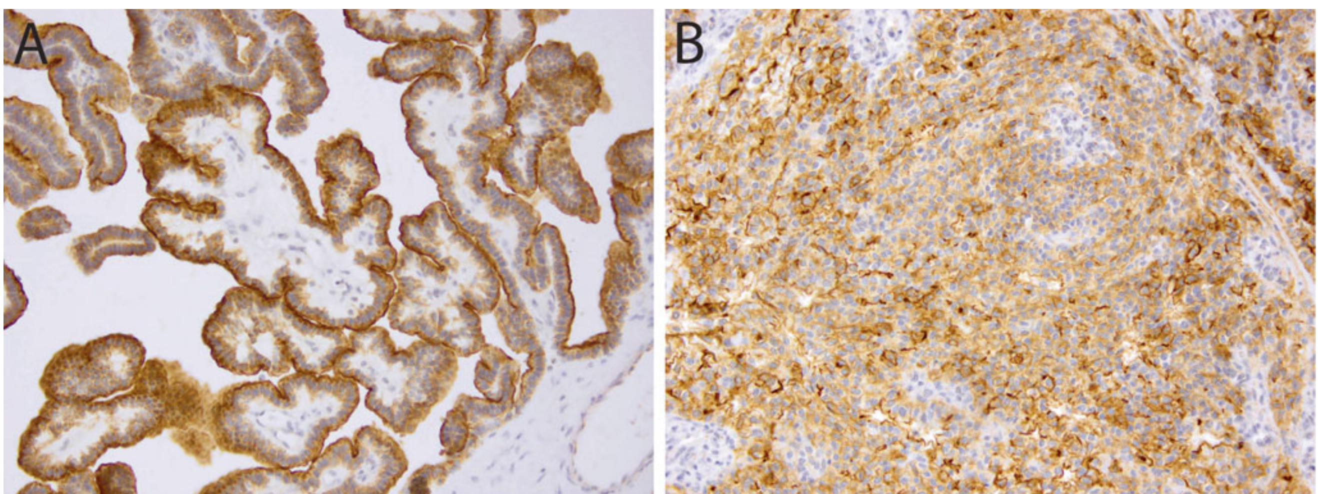

Fig. 12.5. *Immunohistochemistry for Kir7.1 in choroid plexus tumors*. Membranous staining for Kir7.1 in a choroid plexus papilloma (**a**, 20×) and a choroid plexus carcinoma (**b**, 20×).

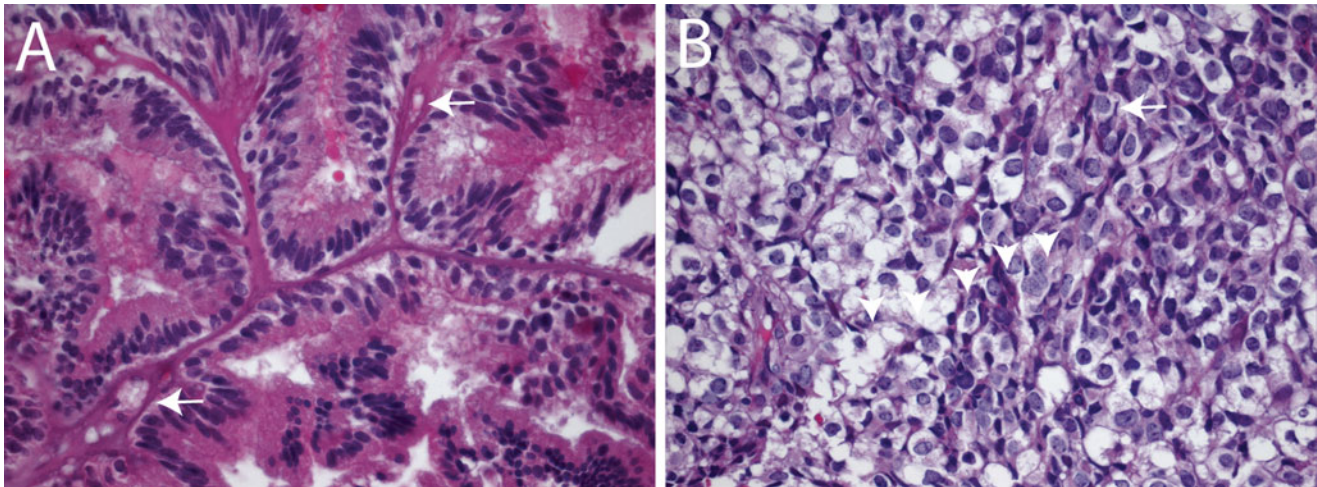

Fig. 12.6. *Histologic mimics encountered in choroid plexus tumors*. (**a**) Choroid plexus papilloma showing elongated tumor cells arranged focally around blood vessels reminiscent of an ependymoma (40×). (**b**) Choroid plexus carcinoma with perinuclear halos surrounding tumor cells mimicking an oligodendroglial neoplasm (40×).

Histological Differential Diagnosis

Depending on the age group, the differential diagnosis of CPTs includes atypical teratoid/rhabdoid tumor (AT/RT), central nervous system (CNS), primitive neuroectodermal tumors (PNET), papillary ependymoma, oligodendroglioma, neurocytoma, papillary tumor of the pineal region (PTPR), and metastases [70–75]. However, the first differential diagnosis to consider is normal choroid plexus. Typically, normal choroid plexus can be distinguished readily enough when the lesion is entirely papillary, with papillae that are not overly cellular and which exhibit a cobblestone surface, and the absence of mitoses (Figs. 12.1a, b). Further, normal choroid plexus epithelial cells express SERCA3, but SERCA3 expression is decreased in CPTs [76].

AT/RT frequently arises in the differential diagnosis, especially in young children [77]. While AT/RT may occasionally demonstrate poorly differentiated epithelial structures, this histologic pattern is generally quite rare. When such cases do arise, the diagnosis may be resolved by immunohistochemical staining for expression of SMARCB1, which is retained in all CPTs (Fig. 12.4b) [73], as well as choroid plexus marker Kir7.1, which stains the majority of CPC but not AT/RT (Fig. 12.5) [78].

Occasionally in pediatric cases, a supratentorial PNET, particularly the medulloepithelioma, may enter into the differential diagnosis. These tumors can usually be distinguished on histologic grounds; they have tubular structures rather than papillary architecture and comprise cells with embryonal rather than epithelial features. Furthermore, medulloepithelioma is characterized by 19q13.42 amplification and LIN28 expression, linking these rare tumors to embryonal tumor with abundant neuropil and true rosettes (ETANTR) [79, 80]. Cribriform neuroepithelial tumor (CRINET) is a rare tumor characterized by cribriform strands and well-defined surfaces, which may be misinterpreted as CPC. Unlike the majority of CPC, however, CRINET is characterized by SMARCB1 loss as well as EMA staining of surfaces [79]. In contrast to AT/RT, prognosis of CRINET seems to be relatively favorable [81].

Papillary ependymomas share the intraventricular location and confusion may arise in CPPs with elongated tumor cells that may give the appearance of overlapping histopathological features (Fig. 12.6a). One important clue to the differential diagnosis is the presence of a delicate basement membrane in CPTs, a feature consistently lacking in ependymomas. While GFAP may be present in both, it is generally stronger and more diffuse in ependymomas. Rarely, synchronous appearance of CPT and ependymoma has been described [82, 83]. PTPR has to be considered in children and young adults with tumors of third ventricular location. The majority of PTPRs can be distinguished from CPTs by absent staining for epithelial membrane antigen and Kir7.1, as well as the presence of distinct MAP-2 immunoreactivity [68].

The endolymphatic sac tumor (ELST) is a low-grade carcinoma originating in the ear. These extremely rare tumors are capable of invading the cerebello-pontine angle and might be mistaken for CPTs in this region. Kir7.1 and EAAT-1 (glutamate transporter) are typically positive in CPTs but absent in ELSTs [84]. The choroid plexus is a common site for metastases and this should be considered in any adult with CPTs. Renal cell carcinoma [85], thyroid carcinoma [86, 87], and cholangiocellular carcinoma [88] primaries have all been reported.

Pathogenesis and Molecular Genetics

Pathogenesis

CPTs were the first models for virally induced brain tumors. Simian Virus 40 (SV40), which naturally infects Asian macaques, has been shown to induce CPTs. The virus is capable of transforming human choroid cells in vitro [89–91] and creates CPTs in hamsters and mice in vivo [92–97]. Transgenic mice harboring the SV40 large T-antigen gene develop CPPs by 80–90 days [98, 99]. SV40 is frequently found in human CPTs [100–103]. The T-antigen of the SV40 virus binds to tumor suppressor genes such as p53 [104] and pRB [99]. This suggests virus-induced tumorigenesis. However, an unintended natural experiment that occurred when the vaccine for poliomyelitis was contaminated with the SV40 virus in India did not produce clear evidence of increased incidence of CPTs. It still remains to be conclusively established if SV40 induces CPTs in humans.

Only limited data are available regarding molecular genetic alterations in CPT. Using comparative genomic hybridization, gains of chromosomes 5, 7, and 9 as well as losses of chromosomes 10 and 22q could be demonstrated in CPP. In contrast, CPC mainly showed gains of chromosomes 1, 4, 12, and 20 as well as losses of 5, 18, and 22q [105]. These findings could be extended using high-resolution methods, showing recurrent copy number gains of chromosomes 1, 2, 4, 12, and 20 as well as losses of chromosomes 5, 6, 16, 18, 19, and 22 in CPC. Clustering analysis separated choroid plexus carcinomas into two groups: one characterized by marked losses and the other characterized by gains across the chromosomes. Chromosomal losses of 9, 19p, and 22q were significantly more frequent in younger children (<36 months), whereas gains on chromosomes 7 and 19, and chromosome arms 8q, 14q, and 21q prevailed in older patients [106].

The involvement of the *TP53* tumor suppressor gene in CPT patients was first suggested by the occurrence of CPC in families with Li–Fraumeni syndrome [107–109], and by the observation of p53 inactivation in tumor tissues [69]. A Canadian group reported a large CPT population with p53 alterations [110]. A Brazilian study confirmed these finding on a larger scale [111–113] for a specific mutation *TP53* mutation: R337H. This *TP53* mutation is also linked to adreno-cortical carcinoma. Interestingly, high-resolution single nucleotide polymorphism (SNP) array analysis did reveal extremely high total structural variation in *TP53-mutated* CPC tumor genomes compared with *TP53* wild-type tumors and CPPs [110]. However, in the absence of *TP53* germline mutations CPTs may still arise through the same pathway driven by somatic mutations [114]. Even though a close association between *TP53* mutation status and nuclear accumulation of p53 protein is often claimed [110], the majority of CPTs show only weak and focal nuclear staining, suggesting that p53 immunohistochemistry might not be a reliable indicator of *TP53* mutations in these tumors.

Other molecular events in the pathogenesis of CPT are not yet as well characterized. *TP53* mutations are unlikely to be the only event in the pathogenesis of CPTs. Patients with multiple resections show progression of CPTs with a tendency to increasing degrees of malignancy [40], and CPTs may arise from teratomas [115], indicating an accumulation of events leading to the final phenotype. Several other pathways have been suggested to be operative in the biology of CPTs. In mice, over-expression of notch3 initiated the formation of CPTs [116]. Some evidence suggests alterations of notch signaling also occur in human CPT [116, 117].

By comparing gene expression profiles obtained from human CPP cells with that of nonneoplastic choroid plexus epithelial cells, the transcription factor TWIST1 was identified to be highly expressed in CPP and also promoted proliferation and invasion in vitro [118]. Amplification and activating mutations of tyrosine receptor signaling pathways play an important role in the biology of human cancer. In CPC, amplification and over-expression of PDGF receptors has been described [119]. Furthermore, in immortalized choroid plexus epithelial cells, PDGF-BB exhibited a time- and dose-dependent proliferative response, which was significantly attenuated by the tyrosine-kinase inhibitor imatinib [120], providing a rationale for the development of treatments targeting PDGF receptor signaling in CPT.

The role of epigenetic alterations in CPT is also poorly understood. In pediatric brain tumors, human telomerase reverse transcriptase (hTERT) promoter methylation has been shown to be associated with tumor progression and poor prognosis. Methylation of the hTERT promoter has also been reported in the majority of CPCs [121]. The clinical utility of these findings for CPCs remain to be elucidated. Similarly, the prognostic and predictive role of MGMT promoter methylation, which occurs frequently in CPTs [122], remains to be determined.

Clinical Aspects and Treatments

Prognosis and Current Treatment

Due to the low incidence of CPTs, few randomized trials have been conducted [123, 124]. Most data come from individual case reports [50, 125], case series [23, 58, 126–129], or systematic literature reviews [130–133]. These data suggest that histological classification appears to be the most reliable prognostic parameter [134, 135]. Patients with CPPs have long-term survival rates exceeding 95 % when completely resected. In contrast, CPCs in patients treated with surgical resection and radiation therapy have 5-year survival rates of approximately 60 %. Primary and metastatic CPC in infants from Li–Fraumeni families fare even worse, with 5-year survival rates of less than 5 % [130].

Tumor resection is of very high therapeutic value in CPTs [28, 135–141]. In particular, gross total resection was found to be of significant prognostic value in meta-analyses, [130, 134, 141] thereby confirming the institutional experiences of many groups [23, 124, 142]. Meta analyses also confirmed the value of a second resection [143]. However, attempts at radical resection should be made with caution, since the high vascularity of these tumors also translates into a high rate of intratumoral bleeding [136], and other surgical complications such as tension pneumoventricle [144] and hyperacute disseminated intravascular coagulation [145]. Newer surgical techniques might reduce morbidity and mortality. These include endoscopic [137] and combined endoscopic and microsurgical approaches [146]. For tumors of the foramen of Luschka, a telovelar approach has been proposed [147]. Preoperative embolization may reduce the operative risk [6, 50, 148, 149]. In one case the tumor regressed after embolization without the need for resection [150]. Similarly, preoperative intensive chemotherapy may reduce the risk for intraoperative hemorrhage even when the size of the tumor does not shrink significantly [151].

Radiation therapy can increase survival of CPTs in patients old enough to receive therapeutic doses [129, 130, 134, 152, 153]. For example, CPPs may be sensitive to radiation therapy [39] and CPCs may show a survival benefit with craniospinal irradiation [152]. However, long-term sequelae of radiation are particular devastating for the developing brains of young children and limit the use of this modality.

Chemotherapy improved survival rates at least in the subgroup of incompletely resected CPC [42, 132]. The five most frequently used drugs are cisplatin, vincristine, cyclophosphamide, carboplatin, and etoposide. Of those, etoposide (VP16) was most frequently used in protocols and had the most convincing survival benefit in various multivariate analyses [133]. More recently reports suggest temozolomide is less promising [125]. In a prospective clinical trial, CPT-SIOP-2000, cyclophosphamide was found equally effective to carboplatin. As a result of these experiences, the benefit of chemotherapy (including high-dose chemotherapy recently reported for an adult patient [154] is becoming more widely accepted, at least for young children [135, 153, 155, 156]. However, intensive chemotherapy is associated with its own risks, and fatal complications have been described [136]. These studies highlight the need for better targeted and less toxic agents.

The influence of germline *TP53* mutations on prognosis and efficacy of treatment remains controversial. A large series of patients treated mainly with intensive chemotherapy including ifosfamide etoposide carboplatin (ICE) show a significantly worse prognosis in patients with Li–Fraumeni syndrome [110]. A second report of patients treated with various chemotherapeutic protocols, among them head start III, described long-term survivors among the Li–Fraumeni population [157]. However, data from the Brazilian family with *TP53*-R337H mutations failed to show statistically significant differences in survival on treatment [111]. Finally, in the international CPT study, there was no significant difference between Li–Fraumeni and non-Li–Fraumeni families. These differences in results prompt further prospective evaluations.

Unique biological features of CPTs may provide leads to novel therapeutic approaches. The blood–brain barrier is located typically in the vascular wall and is characterized by tight junctions between endothelial cells. In contrast, choroid plexus capillaries are leaky. As this feature of leaky blood vessels is maintained in CPTs, systemic medication may reach tumor cells even among the most differentiated CPTs without hindrance from the blood–brain barrier. The normal choroid plexus also functions as an immunological gate to the CNS, including interferon-γ signal mediated entry of circulating leucocytes for immune surveillance [158], and IL-6 production [159]. It is possible that these immune pathways could be leveraged to develop novel therapeutic approaches against CPTs in the future.

Summary

CPTs are tumors arising from the choroid plexus and based on histologic criteria are classified as CPP, APP, and CPC, which correspond to WHO grades I, II, and III, respectively. CPTs occur in all ages but are more common in childhood, peaking in incidence during the first decade of life. Histological grading remains a key prognostic factor and several ancillary immunohistochemical tests can aid in establishing their diagnoses. CPPs are usually treated with surgical resection, whereas a combination of surgical resection and/or chemo/radiation therapy may be used for higher-grade tumors. The biology of CPTs is poorly understood. Factors implicated in the pathogenesis of CPTs include alterations in p53- and SV40-induced viral transformation. However, the molecular genetics of CPT initiation and progression have not been otherwise elucidated and should provide fruitful avenues for future research.

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13 Atypical Teratoid Rhabdoid Tumors

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Definition

Atypical teratoid/rhabdoid tumors (AT/RTs) correspond to World Health Organization grade IV tumors occurring predominantly in infants and young children. These tumors are classically associated with the presence of primitive neuroectodermal cells, variably prominent rhabdoid cells, and have evidence of differentiation along several different lines including neuronal, glial, epithelial, and mesenchymal lineages. They are characterized by biallelic genetic alteration of the *SMARCB1* gene (also referred to as *SNF5/BAF47/INI1*), resulting in loss of SMARCB1 protein expression.

Clinical Features

AT/RTs are highly malignant tumors encountered predominantly, though not exclusively, in young children. These tumors were first described in the mid-1980s [1–3]. It has been challenging to determine the exact incidence of these tumors, since until recently they were often misclassified as other central nervous system (CNS) embryonal tumors. However, since the discovery of presence of *SMARCB1* alterations in these tumors, routine immunohistochemistry analysis has replaced fluorescence in situ hybridization (FISH) to demonstrate the absence of the SMARCB1 protein in these tumors, enabling more accurate diagnostics and a clear-cut molecular genetic distinction from other CNS tumors. The incidence of AT/RT is approximately 1–2 % of CNS tumors in children <21 years of age but rises to 10–20 % in those <3 years of age [4, 5]. Recent retrospective studies from national tumor registries have estimated the median age of children initially diagnosed with AT/RT to be between 1 and 2 years of age [6, 7].

AT/RTs have been described to occur throughout the central nervous system. However, these tumors are most commonly located in the infratentorial region in infants and more often supratentorially in older children [5, 8]. In addition, the incidence of metastatic disease at diagnosis is higher in patients <3 years of age. Seeding along cerebrospinal fluid pathways has been reported in up to 20 % of patients at presentation [9]. Because of the predominant tumor location within the posterior fossa, affected children typically present with symptoms including vomiting, lethargy, and failure to thrive, while headache and hemiplegia are more commonly seen in older patients with cerebral hemispheric tumors.

Rhabdoid Tumor Predisposition syndrome (RTPS) is a complex familial disorder seen predominantly in infants. RTPS results in increased susceptibility to development of AT/RT, malignant rhabdoid tumors (MRT) outside the CNS, schwannomas, choroid plexus carcinoma, central primitive neuroectodermal tumor, and medulloblastoma (MB) [10, 11]. Approximately 30–35 % of infants and children diagnosed with MRT may show RTPS [10, 12–14]. The genetics of RTPS are discussed below.

Histopathology

Macroscopy

Many AT/RTs share macroscopic characteristics with other CNS embryonal tumors such as MB and primitive neuroectodermal tumor (PNET). They are bulky tumors, often well demarcated from the surrounding brain. While typically soft in consistency and pink to tan in color (Fig. 13.1), the presence of firm whitish areas may indicate the presence of mesenchymal differentiation. AT/RT may be heterogeneous with cystic, necrotic, and hemorrhagic regions (Fig. 13.1). Neuroimaging studies are similar to those seen in patients with PNET and MB. These tumors are typically iso- to slightly hyperintense by fluid-attenuated inversion recovery (FLAIR) with restricted diffusion. Most tumors are contrast enhancing and up to a quarter of them demonstrate radiographic evidence of leptomeningeal dissemination.

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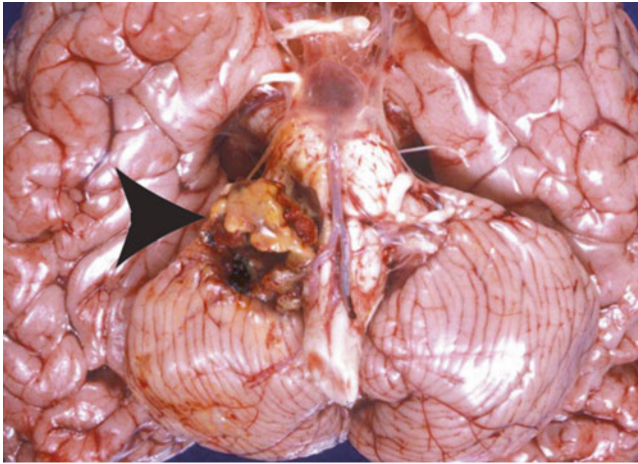

Fig. 13.1 Gross image of AT/RT. Tumor (pink to tan mass) with areas of hemorrhage and necrosis located at the cerebellopontine angle

Histopathology

AT/RTs can be quite heterogeneous and are sometimes difficult to recognize solely on the basis of histopathology. The most striking feature in many cases is the presence of neoplastic cells with rhabdoid features: large cytologically atypical cells with irregular and well-defined cell borders eccentrically placed large nuclei with vesicular chromatin and prominent eosinophilic nucleoli, and abundant pink cytoplasm sometimes containing an eosinophilic cytoplasmic inclusion (Fig. 13.2). In practice, the appearance of these cells often falls along a spectrum, ranging from cells with classic rhabdoid features, to cells with epithelioid features (less striking nuclear atypia and large amounts of pale eosinophilic cytoplasm). Frequently, these cells can exhibit prominent cytoplasmic vacuolar degeneration (Fig. 13.2b). In whatever form they appear, these large cells are rarely the sole or even predominant histopathological feature in AT/RT. Typically, these cells are encountered in small collections or are interspersed among the more numerous primitive neuroectodermal tumor cells (Fig. 13.3). However, it is important to recognize that a relatively diverse range of histopathological patterns may be encountered in AT/RT. Mesenchymal differentiation in these tumors most commonly appears as areas with prominent spindle cell features (Fig. 13.4a) and accumulation of extracellular mucopolysaccharide (Fig. 13.4b). Variable degrees of epithelial differentiation may also be encountered in AT/RT (Fig. 13.5). This is the least common histopathological pattern seen in AT/RT and can manifest as poorly differentiated glandular structures, papillary structures, or poorly differentiated ribbons and cords of cells with epithelial features. Occasionally in the latter case, abundant mucopolysaccharide-rich material separates the nests and cords of tumors in a pattern reminiscent of chordoma. Irrespective of the histopathological pattern, mitotic figures are generally abundant in AT/RT. Both karyorrhexis and areas of geographic necrosis are commonly encountered [8, 9, 15, 16].

Differential Diagnosis

The differential diagnosis of AT/RT includes MB, CNS PNET, pineoblastoma, anaplastic ependymoma, choroid plexus carcinoma, and germ cell tumors [9, 15, 17–19]. Particular care must be taken to avoid confusing anaplastic/large cell MB for AT/RT and vice versa. Misdiagnosis can generally be avoided with attention to a few critical details.

First, at the cytologic level, anaplastic/large cell MB show a range of nuclear features that while overlapping with AT/RT in some regards, generally fail to recapitulate the vesicular chromatin-staining pattern typical of these tumors. While they are variably prominent, at least some proportion of rhabdoid tumor cells demonstrates prominent eosinophilic nucleoli. These are not encountered in anaplastic/large cell MB except in the rare case with predominant or exclusively large cell features; these tumors lack the vesicular chromatin staining and variability seen in AT/RT.

Second, evaluation of the cytoplasmic features is extremely helpful. The presence of tumor cells with rhabdoid cytoplasmic inclusions is reassuring. However, the prominence of such cells within any given case can be extremely variable. This may be a function of biology, sampling, or both. In cases where no classic rhabdoid cells are encountered, it is critical to recognize the presence of poorly preserved rhabdoid tumor cells. The presence of scattered cells with large poorly preserved nuclei, vacuolar cytoplasmic degeneration, and prominent well-defined cell borders may be the only histologic evidence of an AT/RT. In other cases, it is the presence of cells with epithelioid or vaguely epithelioid features that alerts the pathologist to the presence of an AT/RT.

Finally, it is important to evaluate the overall histologic pattern for clues to the presence of an AT/RT. In many cases the overall growth pattern may appear essentially indistinguishable from a classic MB or CNS PNET. However, in some cases it is possible to recognize features suggestive of epithelial differentiation in what is otherwise an unremarkable MB or CNS PNET. These features include evidence of epithelial (the presence of poorly differentiated glandular and epithelial structures or the growth of tumor cells in small nests and cords) and/or mesenchymal structures [most often a spindle cell growth pattern (Fig. 13.4a), but occasionally tumors show evidence of more advanced mesenchymal differentiation including the presence of bone and cartilage]. Finally, the accumulation of extracellular myxohyaline material (Fig. 13.4b) in a CNS embryonal tumor is a histologic feature that should raise the differential diagnosis of AT/RT.

Among the non-CNS embryonal neoplasms, there are several features that deserve consideration in the differential

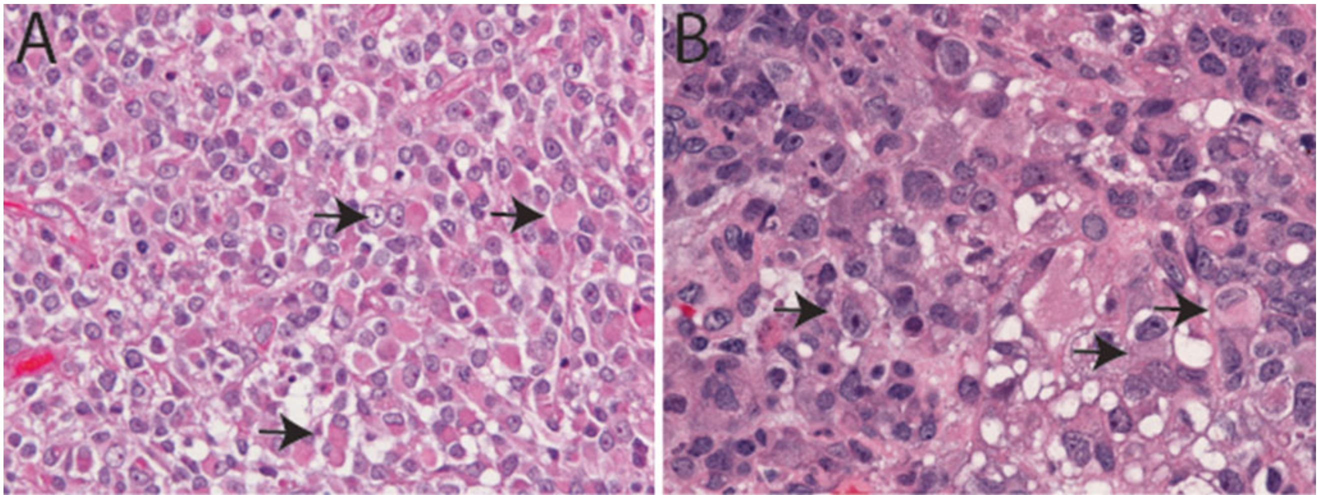

Fig. 13.2 Classic rhabdoid morphology in AT/RT. H&E sections (20×, **a**, and 40×, **b**) illustrating classic rhabdoid tumor cells in AT/RT characterized by large cells with eccentrically placed nuclei with vesicular chromatin and abundant pink cytoplasm sometimes containing an eosinophilic cytoplasmic inclusion (*arrows*). Vacuolar cytoplasmic degeneration typical of ATR/RT in (**b**)

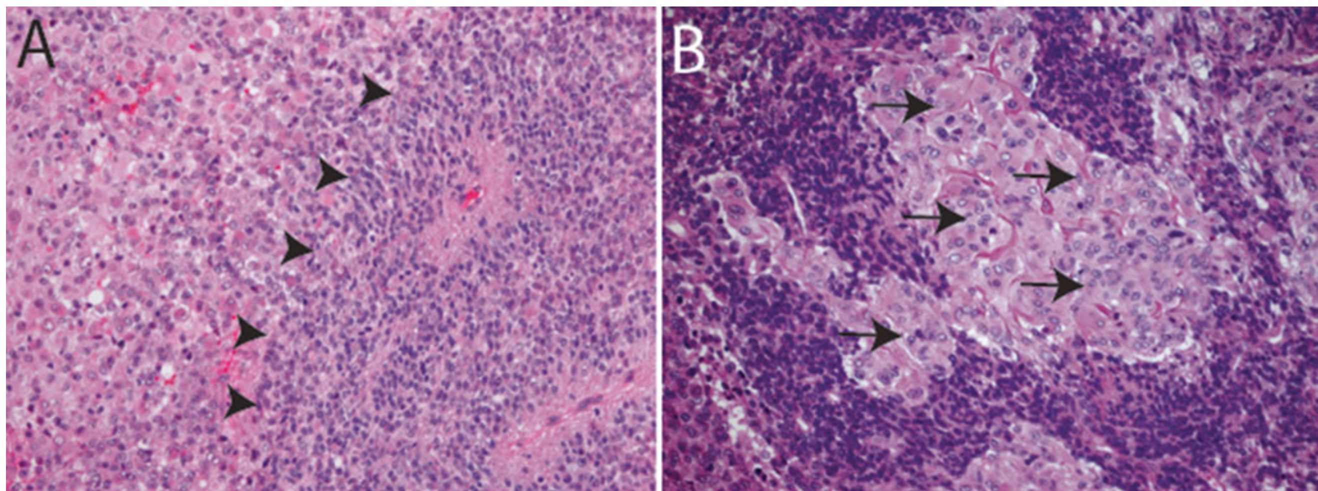

Fig. 13.3 Primitive neuroectodermal features in AT/RT. H&E sections showing primitive neuroectodermal component (*arrowheads* in **a**, 20×) with adjacent rhabdoid areas (*arrows* in **b**, 40×)

diagnosis of AT/RT. Anaplastic ependymomas in the posterior fossa can resemble AT/RT due to their high cellularity and necrosis. It is not uncommon to encounter areas where AT/RTs undermine and appear to grow into the choroid plexus. Combined with occasional papillary features or cells arranged in cord-like structures resembling poorly differentiated epithelial structures, choroid plexus carcinomas should be considered in the differential diagnosis. Prominent spindle cell pattern may resemble a sarcoma and glandular or epithelial differentiations may suggest a teratoma or metastatic carcinoma. In the pineal and other midline locations, germ cell tumors should also enter into the differential diagnosis. Immunohistochemical studies and molecular testing can play a pivotal role in helping the pathologist refine the diagnosis in such cases.

Immunohistochemistry

Immunohistochemical staining for the SMARCB1 protein has been shown to be a highly sensitive and specific tool for detecting the presence of alterations in the *SMARCB1* gene on chromosome 22q11.2 (Judkins et al., 2004). In addition to SMARCB1, immunohistochemical expression of several other markers may play an important role in the diagnosis of

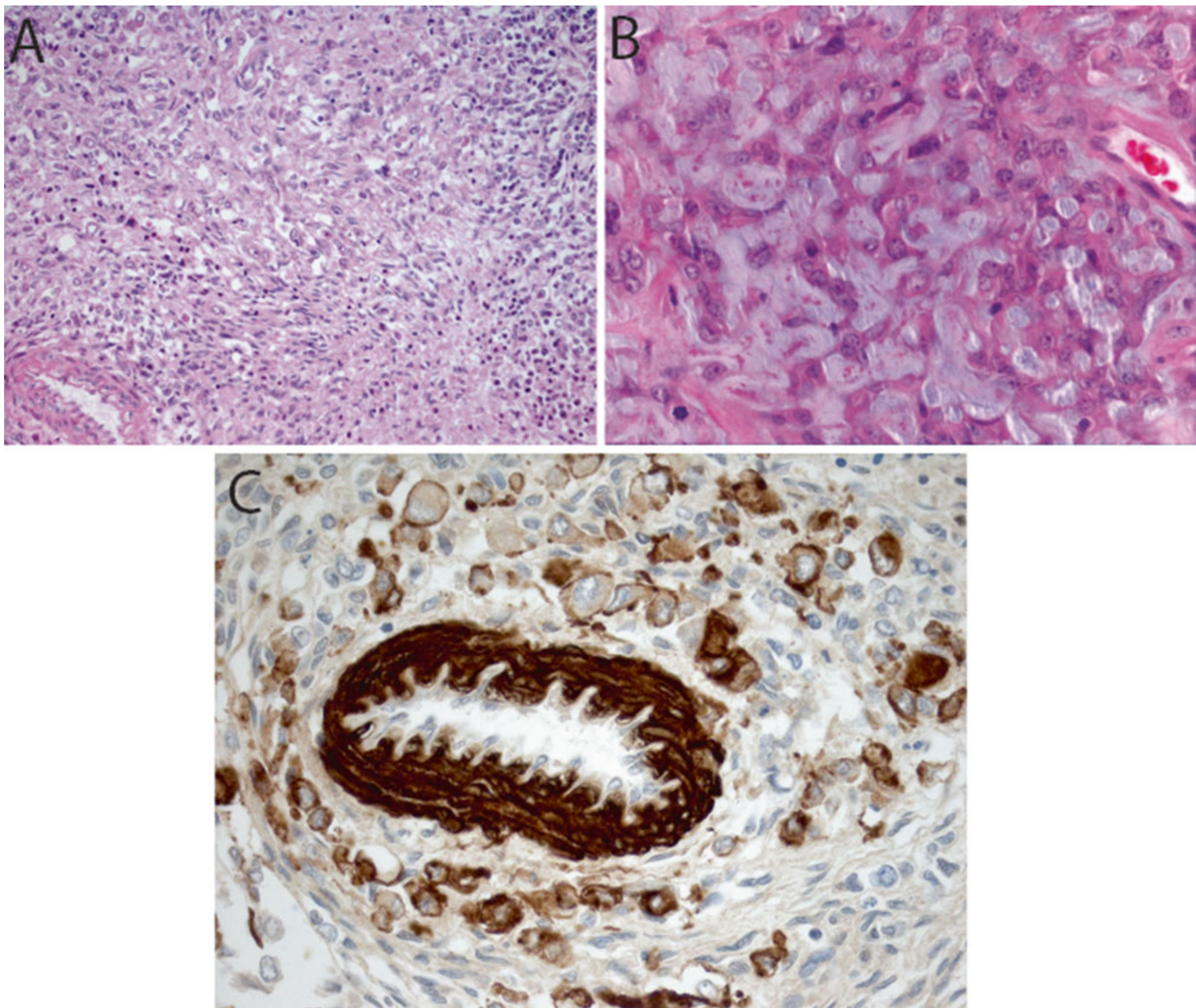

Fig. 13.4 Mesenchymal differentiation in AT/RT. H&E sections showing spindle cell component (20×, **a**) and extracellular mucopolysaccharide accumulation (40×, **b**) in AT/RT. Immunostain for smooth muscle antigen (SMA, 40×) showing staining in vessel wall and tumor cells

AT/RT. The heterogeneity in appearance of AT/RT and suggestion of differentiation along multiple cell lineages is reflected in the polyphenotypic immunostaining profile characteristic of these tumors. AT/RTs demonstrate almost universal expression of smooth muscle antigen (SMA, Fig. 13.4c), epithelial membrane antigen (EMA, Fig. 13.5b) and vimentin (Figs. 13.5a). While the latter stain is of limited specificity, it can occasionally prove a useful tool for demonstrating the presence of hard to detect rhabdoid cells. The combined expression of mesenchymal (SMA, Fig. 13.4c) and epithelial markers (EMA, Fig. 13.5b) is unique for AT/RT among other CNS tumors. Expression of glial fibrillary acid protein (GFAP, Fig. 13.6a) and neuronal markers including neurofilament protein (NFP), synaptophysin (SYN, Fig. 13.6b, and NeuN is typically seen in up to about 75 % of AT/RT. Cytokeratin markers such as AE1.3 are also positive in some AT/RT, though typically they are not as frequently expressed as the other markers discussed above [8, 9]. Classical germ cell markers such as placental alkaline phosphatase (PLAP), β–human chorionic gonadotropin (β–HCG) and octomer-binding transcription factor (OCT-4) may be negative. However, other germ cell markers such as Sal-like protein-4 (SALL4), sex determining region Y-box 2 (SOX2) and Nanog may be positive suggesting the potential for pluripotency in these tumors [20]. The expression of these various markers is highly variable from case to case and may reflect the complex biology of these tumors and their ability to differentiate along multiple lineages.

Loss of SMARCB1 expression in tumor cells enables differentiation of AT/RT from other CNS tumors, which dem-

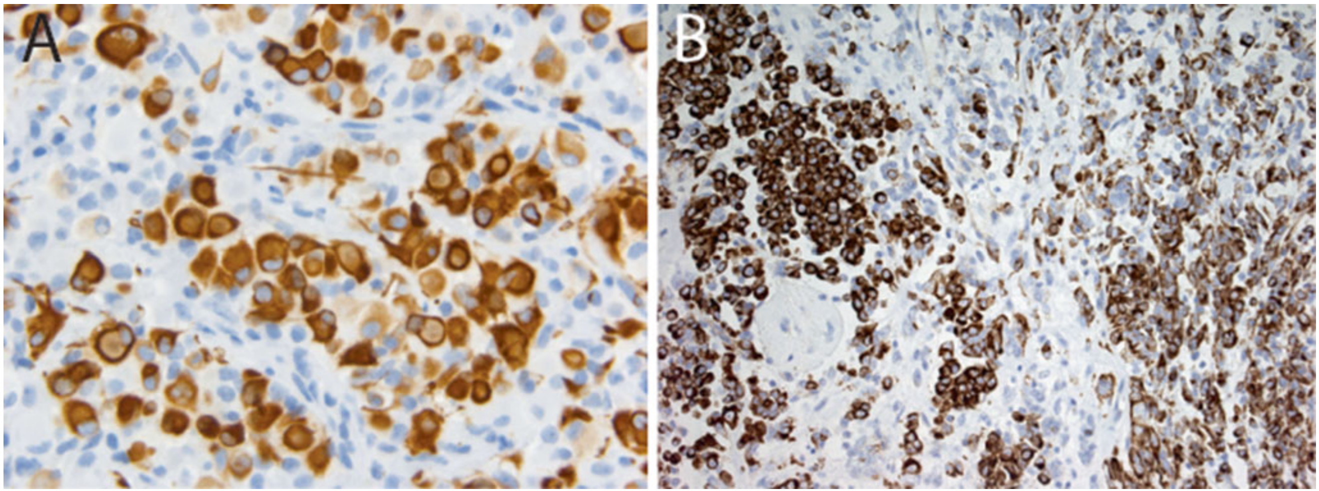

Fig. 13.5 Vimentin and epithelial membrane antigen expression in AT/RT. Immunostains for vimentin (**a**, 20×) and epithelial membrane antigen (EMA, 40×, showing membranous staining, (**b**) in AT/RT

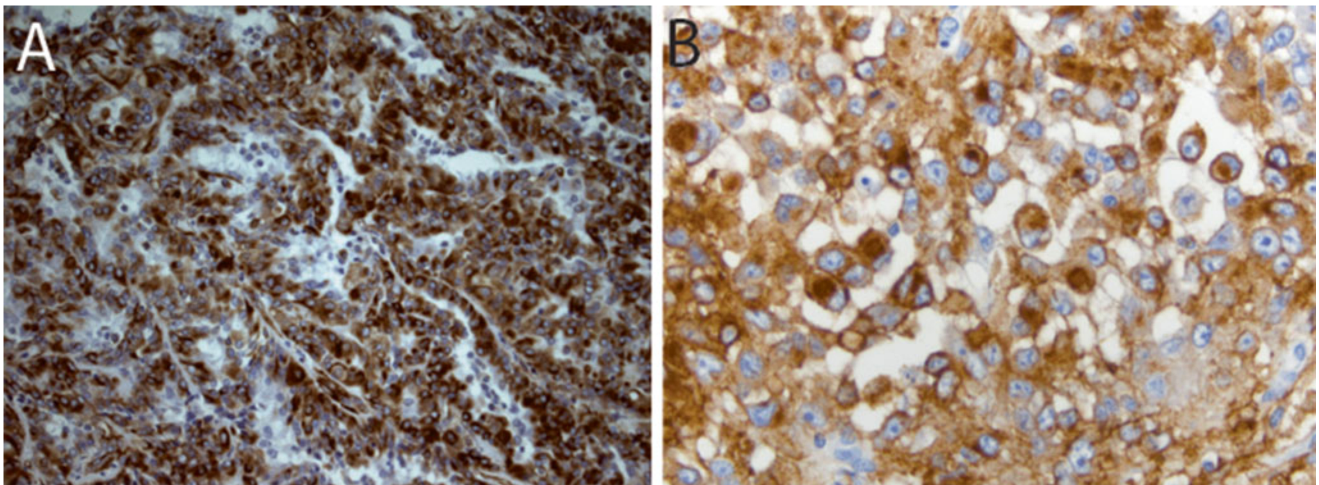

Fig. 13.6 Neuroglial markers in AT/RT. Immunostains for glia fibrillary acidic protein (GFAP, 40×, **a**) and synaptophysin (SYN, 20×, **b**) in AT/RT

onstrate retained expression of SMARCB1 (Fig. 13.7). Correct interpretation of SMARCB1 immunohistochemical staining is aided by the fact that SMARCB1 is a ubiquitously expressed nuclear protein and therefore it demonstrates positive immunostaining in endothelial cells and infiltrating tumor lymphocytes (Fig. 13.7). The loss of expression of SMARCB1 in these cells, as well as the failure of the stain to be expressed in any adjacent normal tissues, should signal to the pathologist the need to repeat the SMARCB1 immunohistochemical staining.

As a result of the utility of SMARCB1, combined with the histopathological diversity of AT/RT, it has become the standard of care to stain all CNS embryonal neoplasms with SMARCB1. By so doing, cases of previously unrecognized AT/RT have been reported in some institutions [21]. Routine application of SMARCB1 immunostaining has led to the recognition of loss of expression in other tumor types including CNS low-grade tumors undergoing malignant transformation (ganglioglioma, pleomorphic xanthoastrocytoma) [22, 23] as well as extra-CNS tumors including epithelioid sarcoma, extraskeletal myxoid chondrosarcoma, renal medullary carcinoma, and epithelioid malignant peripheral nerve sheath tumor [24–26]. The pathogenic role, if any, of loss of SMARCB1 in these non-rhabdoid tumors remains to be determined. It is particularly critical that the neuropathologist who makes the diagnosis of AT/RT recognizes that at least a third of all newly diagnosis AT/RTs are attributable to a germline mutation [12] (see below). It is therefore essential that a referral for genetic counseling and testing be made for the patient and their family.

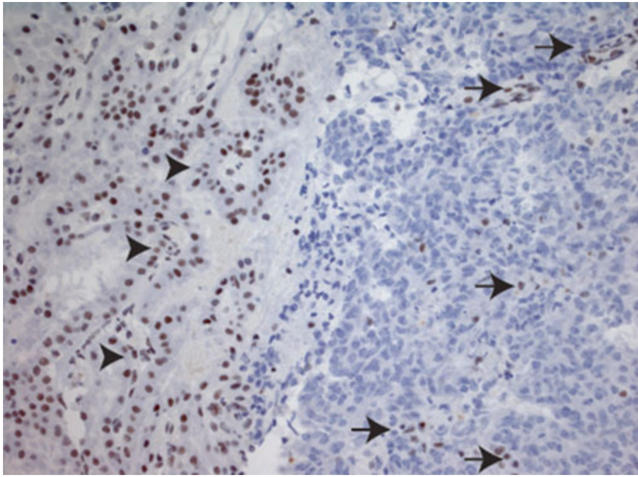

Fig. 13.7 Loss of SMARCB1 staining in AT/RT. Immunostain for SMARCB1 (20×), showing loss of SMARCB1 expression in tumor cells. As an internal control, there is preserved SMARCB1 staining in adjacent choroid plexus (*arrow heads*) and intra-tumoral endothelial cells and infiltrating lymphocytes (*arrows*)

Cytogenetics and Molecular Genetics

Molecular Genetics

SMARCB1 is a tumor suppressor gene biallelically mutated in >95 % of all AT/RTs [27–30]. SMARCB1 is also referred to as: (1) INI1 (Integrase Interactor 1) as the mammalian homologue was first discovered as an HIV-1 integrase-binding protein, (2) BAF47 (BRG1-associated factor 47), and (3) hSNF5 (human sucrose non-fermenting) [31]. SMARCB1 is a component of the mammalian chromatin remodeling SWI/SNF (Switch/Sucrose Non-Fermentable) complex, which reorganizes and/or repositions nucleosomes in an ATP-dependent manner [32]. This complex is believed to be a critical epigenetic regulator for normal development and maintenance of tissue-specific gene expression [33].

The subunits of the SWI/SNF complex are grouped into two major subfamilies: (1) BAF (BRG1 or hBRM-associated factor) complex and (2) PBAF (Polybromo-associated BAF) [34–41]. Among the subunits, there are four core components that are present in all versions of the SWI/SNF complex including: (1) the ATPase enzymatic subunits SMARCA2 (hBRM, hBrahma) or SMARCA4 (BRG1, or Brahma-related gene 1), (2) SMARCB1 (INI1/SNF5/BAF47), (3) SMARCC1 (BAF155), and (4) SMARCC2 (BAF170).

Mutations in almost all subunits of the SWI/SNF complex have been identified in many human cancers [42–44]. SMARCB1 is biallelically inactivated in >95 % of MRT but is variably mutated in other cancer types (discussed below), suggesting that the function of SMARCB1 through SWI/SNF is essential for protecting a specific cell type from becoming cancerous and preventing the genesis of rhabdoid tumors including AT/RT [45].

Mutational Spectrum of SMARCB1 Found in AT/RT

Notwithstanding their histological and immunohistochemical diversity, nearly all AT/RTs involve mutation, deletion, or loss of expression of the *SMARCB1* gene. Approximately 70 % MRT arise due to biallelic loss of the *SMARCB1* tumor suppressor, and an additional 20–25 % exhibit loss of SMARCB1 function due to reduced RNA or protein expression [12].

Several studies have defined the spectrum of *SMARCB1* mutations within AT/RT. These studies have shown that approximately 40 % of *SMARCB1* mutations are homozygous deletions, frequently associated with chromosomal rearrangements of 22q11 [3, 46, 47]. In addition to homozygous deletion of *SMARCB1*, 96 coding-sequence mutations were identified in nine exons of the *SMARCB1* gene among 119 ATRT tumor samples analyzed [12].

These mutations occurred with the highest frequency in exons 5 and 9 in AT/RT, while mutations within exon 8 have yet to be detected, and mutations in exons 1 and 3 are largely underrepresented [12, 48]. The majority of coding-sequence mutations (48/96) were single base-pair point mutations, 47 of which were nonsense mutations predicting premature truncation of the protein, and one of which was a missense mutation in exon 9 [12]. The second-most frequent (31/96) type of mutation was deletion and appeared to be localized to a few spots within *SMARCB1*; 20 were found within exon 9 and 14 involved deletion of one of four cytosines in bases 1,145–1,148, and six involved deletion of one of two guanines at position 1,143 or 1,144 [12]. The remaining mutations identified were duplications of 4–19 bases (7/96), insertions (6/96), and intragenic deletions of one or two exons (4/96) [12]. In addition to coding-sequence mutations, a mutation affecting splicing of the *SMARCB1* transcript has also been identified: an A to G mutation in intron 5, which disrupts the splice acceptor site [49].

Most mutations discussed were likely somatic mutations, however; a study on 49 tumor and matched blood DNA samples showed that 33 % (16/49) mutations were germline [12]. These germline mutations were coding-sequence mutations resulting in introduction of a premature stop codon found within exons 2, 4, 5, 6, and 7 [12].

Some of the mutations identified occur repeatedly in different MRT samples indicating possible hot-spots for mutation [12, 48]. Examples include the cytosine deletion at the 3′ end of the *SMARCB1* coding sequence (detected in ten different AT/RTs) and C601T (p. Arg201x, found in 12 RTs), and C472T (p. Arg158x, found in seven different tumors). Interestingly, the C601T and C472T mutations have also been identified as germline mutations in families with rhabdoid tumor predisposition syndrome (RTPS) [47]. Other germline mutations identified in RTPS included deletions and insertions causing frame shifts, nonsense mutations, missense mutations, and mutations affecting splicing of the *SMARCB1* transcript [11, 47, 50–54]. Notably, many of the

SMARCB1 mutations associated with RTPS are *de novo* germline mutations [11, 47]. LOH is common in RTPS, causing loss of the second allele of *SMARCB1* [11, 47, 50].

Exome analysis has revealed that the genomes of these cancers are remarkably simple, showing extremely low rate of mutations with loss of SMARCB1 being the primary recurrent event [55]. This observation is consistent with the data that mouse models with heterozygous deletions of *SMARCB1* gene develop a high frequency of tumors with early onset resembling AT/RT with loss of heterozygosity (LOH) at the *SMARCB1* locus [56].

Rarely, other components of SWI/SNF may be implicated in the development of AT/RT. There are a few exceptions to the above observations. There are reports indicating that tumors that exhibit typical AT/RT features showed the presence of intact *SMARCB1* gene and protein, but show nonsense mutation and inactivation of *SMARCA4* [57]. Conversely, recently, there has been an explosion of data describing pathological SMARCB1 mutations/loss of expression in several other tumor types including schwannoma, epithelioid MPNST, epithelioid sarcoma, extraskeletal myxoid chondrosarcoma, synovial sarcoma, pediatric undifferentiated sarcoma, renal medullary carcinoma, small cell undifferentiated variant of hepatoblastoma and myoepithelial carcinoma, and schwannomatosis [26, 58–68]. The role of SMARCB1 mutation/loss in these tumors remains to be clearly established.

Molecular Diagnosis

The ubiquitous expression of SMARCB1 and its loss in nearly all AT/RTs makes it a powerful target as a diagnostic tool. Immunohistochemical analysis for loss of SMARCB1 expression was shown to effectively establish the diagnosis of AT/RT and distinguish MRTs from other pediatric soft tissue tumors [18, 19, 69]. Earlier studies demonstrated that AT/RTs could be distinguished from other histologically similar CNS tumors by cytogenetic studies using FISH of chromosome region 22q11.2 to visualize loss of *SMARCB1* locus [70]. Additionally, quantitative real-time PCR (q-RT-PCR) can be performed when DNA of the tumor tissues are available to determine the presence of missense and nonsynonymous mutations in the exons of *SMARCB1* gene.

Prognostic Stratification and Treatments

Prognosis and Treatment

The treatment of children with AT/RT presents several challenges. These tumors are very aggressive and often resistant to even the most intensive therapies. In addition, because these tumors are more commonly found in very young children, treatment modalities such as craniospinal irradiation are not always an option.

Chemotherapy

One of the earliest regimens resulting in long-term survival even in young children with AT/RT incorporated the approach used by the rhabdomyosarcoma group for patients with parameningeal tumors known as Intergroup Rhabdomyosarcoma Study-III (IRS-III). Weinblatt and Kochen treated a patient with a CNS rhabdoid tumor who underwent gross total resection of the tumor followed by 4,140 cGy focal irradiation and intensive chemotherapy using the IRS-III regimen [71]. The patient was alive and without evidence of disease 4.5 years at the time of the report. Olson et al. published their results on three additional patients with AT/RT using a similar approach [72].

Chi et al. published one of the largest prospective studies to date incorporating the IRS III chemotherapy into an intensive multimodality treatment approach [73]. They reported the results of 20 children newly diagnosed with AT/RT treated between 2004 and 2006. The 2-year progression-free survival rate was 53 ± 13 % with an overall survival rate of 70 ± 10 %.

High Dose Chemotherapy

Finlay et al. have used a strategy known as Head Start therapy for very young children newly diagnosed with malignant brain tumors including AT/RT. This approach uses high dose chemotherapy with autologous stem cell reinfusion in order to avoid or at least postpone the use of irradiation in very young children. The initial report included patients treated on Head Start I and Head Start II [74]. Patients enrolled on Head Start I received 5 cycles of induction chemotherapy with cisplatin, vincristine (during the first 3 cycles), cyclophosphamide, and etoposide. This was followed by a single course of high dose chemotherapy including carboplatin, thiotepa, and etoposide with autologous stem cell reinfusion. Head Start II had identical therapy except for the addition of high dose methotrexate with each induction course. One patient received craniospinal irradiation following autologous stem cell reinfusion, but prior to relapse. At the time of the report, there were three patients treated on Head Start II who were event-free survivors 42+, 54+, and 67+ months following diagnosis without radiation therapy.

Lafay-Cousin et al. have recently reported the experience of the Canadian Brain Tumor Consortium. This was a retrospective review of children treated between 1995 and 2007 [75]. The majority of the patients were <36 months of age and over 1/3 had metastatic disease. . Of the 40 patients who were treated, 22 received standard dose chemotherapy and 18 received high dose chemotherapy with autologous stem cell reinfusion. Adjuvant radiation therapy was administered to 15 patients and nine received intrathecal chemotherapy. Children who received high dose chemotherapy had a 2-year overall survival of 60 ± 12.6 % compared to 21.7 ± 8.5 % for those treated with standard dose chemotherapy. Half of the survivors did not receive any irradiation.

Radiation Therapy

The group from St. Jude Children's Research Hospital has investigated the role of radiation therapy in a retrospective review of patients with AT/RT treated at their institution between 1987 and 2007 [76]. The timing and field of irradiation were age- and risk dependent with the youngest patients receiving irradiation at least a month or more following resection and usually limited to the involved-field. Cox regression modeling revealed that overall survival in their cohort was adversely affected by disease progression prior to irradiation, time from diagnosis to start of irradiation and age at diagnosis. They concluded that early postoperative irradiation is important for local control particularly in patients without evidence of metastatic disease at diagnosis.

The Children's Oncology Group has recently closed accrual to a study for children newly diagnosed with AT/RT. This is a single arm study which incorporated high dose methotrexate during induction and consolidation with triple high dose chemotherapy using carboplatin and thiotepa following the previous infant brain tumor study, CCG99703. Radiation therapy was administered between induction and consolidation to children >6 months of age with localized posterior fossa tumors and to those >12 months of age with supratentorial disease. The remaining patients received irradiation following consolidation. Although the study was closed for a year as a result of a toxic pulmonary death, accrual was recently completed and results should be available shortly.

Molecular Signaling Pathways

AT/RTs are relatively free of the genomic instability and widespread accumulation of mutations that are common in many cancers [55]. However, since SMARCB1 is a component of the SWI/SNF complex, its loss may result in modifications of the epigenome that result in large expression changes which may lead to the development of tumors. It has been demonstrated that SWI/SNF regulates transcription of approximately 2–10 % of cellular genes [77].

The SWI/SNF complex mediates both activation and repression of transcription by chromatin remodeling. The exact function of SMARCB1 within SWI/SNF complex is not understood. However, SMARCB1 is one of the subunits that may bridge the interaction of SWI/SNF with specific transcription factors. For example, SMARCB1 interacts with cMYC and the SWI/SNF complex is required for the transactivation functions of cMYC [78]. Another transcription factor that has been shown to bind to SMARCB1 is Hedgehog-Gli1 transcription factor. Inactivation of SMARCB1 may lead to disruption of specific nucleosome patterning and occupancy with accompanying gene expression changes [79].

Loss of SMARCB1 results in a widespread but specific deregulation of genes and pathways important for the cell cycle, differentiation, senescence, or apoptosis, all with tumorigenic potential. Gene expression profile studies carried out to define the changes in transcriptome that occur when SMARCB1 is reintroduced into SMARCB1-null MRT cells identified downstream effectors and several pathways and genes important for normal cell division and homeostasis [80–82]. Genes upregulated by SMARCB1 tend to be antiproliferative or involved in inducing senescence and differentiation, while genes repressed by SMARCB1 tend to be involved in cell cycle progression [82–84]. Several important cell cycle regulatory genes are over-expressed in AT/RT due to loss of SMARCB1 including *Cyclin D1* and *Aurora A* [84–86]. Experimental studies in murine models and human RT cell lines have demonstrated that *Cyclin D1* is a key regulator of AT/RT tumor cell growth and that abrogation of *Cyclin D1* leads to complete loss of tumor formation in genetically engineered *smarcb1*± heterozygous mouse models [84, 85, 87]. Furthermore, reintroduction of SMARCB1 leads to induction of senescence [85, 88–90].

In mammalian cells SMARCB1 loss results in transcriptional activation of EZH2 gene, a polycomb gene (PcG) protein and a component of the mammalian polycomb complex, PRC2 as well as in repression and increased H3K27-trimethylation of polycomb targets [91]. It thus appears that SMARCB1 represses and EZH2 activates stem cell-associated programs [91]. This is consistent with the observation that there is frequent activation/upregulation of PcG proteins and frequent inactivation of SWI/SNF components in human cancers [91, 92]. Thus it appears that SWI/SNF modulates the expression and activities of PcG complex to maintain the epigenome and proper expression of cellular genes required for preventing tumor formation [91, 92].

Molecular Targeted Therapies

Although a few children with AT/RT are long-term survivors following therapy with surgery, intensive chemotherapy, and irradiation, new treatment modalities are desperately needed. Current treatments are very toxic and largely ineffective. Several groups are trying to develop more targeted therapy by focusing on unique aspects of these tumors.

As previously noted, the majority of AT/RTs have biallelic inactivation of the SMARCB1 gene. Many investigators are trying to develop therapy directed towards the effects of the loss of this gene. Kalpana et al. were one of the first teams to study downstream targets of the loss of the SMARCB1 gene [82]. They used cDNA microarray analyses to identify downstream targets and then defined the functional significance of these targets. They found that SMARCB1 activated interferon-stimulated genes and repressed polo-like kinase 1 suggesting that interferon and down modulation of polo-like kinase 1 may be potential therapeutic options.

This same group has suggested that cyclin D1 could be a valuable target since loss of SMARCB1 results in derepression of cyclin D1 and rhabdoid tumors are very dependent on

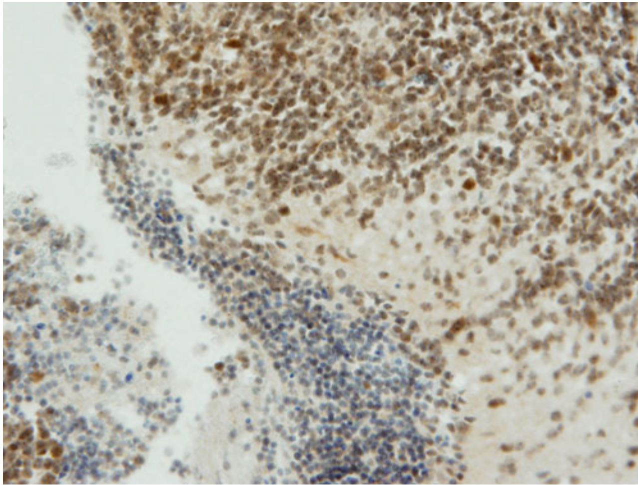

Fig. 13.8 Expression of downstream effector, Aurora Kinase A (*AURKA*) in AT/RT: Aurora Kinase A (*AURKA*) is upregulated in primary human AT/RTs. A primary human AT/RT subjected to immunohistochemical analysis using α-Aurora A antibodies. The tumor cells are strongly positive for AURKA expression (*stained brown*). However, the tumor adjacent normal brain granular cells (blue due to the nuclear stain) are negative for AURKA staining (From Lee S, Cimica V, Ramachandra N, Zagzag D, Kalpana GV (2011) Aurora A Is a Repressed Effector Target of the Chromatin Remodeling Protein INI1/hSNF5 Required for Rhabdoid Tumor Cell Survival. Cancer Res 71:3225–3235, with permission)

cyclin D1 for survival [87]. Furthermore, the same group identified *Aurora Kinase A* (*AURKA*) as a repressed downstream target of SMARCB1 and demonstrated that AT/RT tumors that are deficient in SMARCB1 over-express Aurora A (Fig. 13.8) [86]. Furthermore, this group demonstrated that siRNA-mediated knockdown of *AURKA* leads to mitotic catastrophe and cell death in RT tumor cell lines [86]. Histone deacetylase inhibitors have been shown to decrease cyclin D1 expression [93]. Several different histone deacetylase inhibitors have been shown to alter gene expression and inhibit AT/RT cell growth in vitro and in xenografts [94–96].

Hertwig et al. found that loss of SMARCB1 resulted in an increased sensitivity to phosphorylation of a cytoplasmic unfolded protein response component, eIF2α [97]. They showed that bortezomib, a proteasome inhibitor FDA approved for multiple myeloma, resulted in increased apoptosis of SMARCB1 knockdown cells.

Ogino et al. were one of the first groups to suggest a role of insulin growth factor (IGF) II and insulin growth factor receptor (IGFR) in the pathogenesis of AT/RT [98]. They used immunohistochemistry to demonstrate cytoplasmic positivity for IGFII and cytoplasmic and membranous expression of IGFRI.

D'cunja et al. also used immunohistochemistry to confirm the expression of IGF-1R on 8/8 AT/RT primary tumors [99]. IGF-1R antisense oligonucleotides were used in two AT/RT cell lines resulting in significant downregulation of IGF-1R mRNA and protein expression, apoptosis and increased sensitivity to the chemotherapy drugs doxorubicin and cisplatin.

Arcaro et al. found increased expression of IGF-1R and insulin receptor on AT/RT cell lines compared with normal brain tissue [100]. They found that the AT/RT cells secreted insulin, which potently activated Akt. Inhibitors of the insulin receptor as well as the PI3K/Akt pathway impaired AT/RT growth and proliferation.

Darr et al. also noted persistent Akt activation in Smarcb-1-deficient tumor cells as a result of PI3K-mediated signaling [101]. They, too, were able to prevent proliferation of the Smarcb-1-deficient cells in vitro through inhibition of Akt and inhibited the development of xenografted tumors in their mouse model.

The Pediatric Oncology Experimental Therapeutics Investigators Consortium has used a panel of large drug libraries to identify potential drug therapies for a number of different pediatric tumors [102]. They have recently published their evaluation of three AT/RT cell lines. Their screening studies revealed that agents which altered a number of pathways including Erb2, mTOR, proteasomes, Hsp90, Polo-like kinases, and Aurora kinases were cytotoxic to all three cell lines. Additional studies with the FDA-approved tyrosine kinase inhibitor, lapatinib, revealed cytotoxicity in vitro, in xenografts and in combination with IGF-1R inhibitors.

Although several targeted therapies have been tested in vitro and in animal models, very few have been tested in humans. The Children's Oncology Group recently conducted a phase I/II study using the Aurora kinase A inhibitor MLN8237 in children with a variety of solid tumors. Other investigators have recently begun to use this agent in children with recurrent or progressive AT/RT [103]. Although dosing and safety data are available, efficacy results are pending.

Summary

AT/RTs are characterized by alterations in the SMARCB1 gene and are primarily seen in the pediatric age group. These tumors may demonstrate a wide spectrum of histopathologic features that raises important differential diagnostic considerations. Assessment of molecular alterations in SMARCB1 is vital to the diagnosis of AT/RT and can be performed by immunohistochemistry for the SMARCB1 protein or by molecular tests such as FISH or PCR. We discuss the various molecular alterations seen in SMARCB1 in AT/RT, which include somatic and germline mutations. The pathogenesis of AT/RT is not completely understood but loss of SMARCB1 can contribute to tumor formation by affecting various cellular processes including cell cycle proteins such as Cyclin D1 and the epigenome by altering the transcriptional activity of EZH2 in the regulation of stem cell-associated programs.

While the prognosis for patients with AT/RT remains poor, there are several current regimens available to treat patients that target a variety of different pathways and biological processes.

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14 Hemangioblastoma

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Hemangioblastomas are slow-growing, but highly vascular tumors that arise in specific regions of the central nervous system (CNS) and retina. They constitute about 0.9 % of total brain tumors [1]. Hemangioblastomas may occur sporadically, or as tumors associated with von Hippel–Lindau syndrome (vHL) in about 35–40 % of patients [1–3]. In some series, as much as 80 % of hemangioblastomas are associated with vHL.

Genetics

vHL syndrome is associated with a germline mutation in the *VHL* gene on chromosome 3p25. However, according to the genetic “two-hit hypothesis” proposed by Knudson, tumorigenesis requires a second somatic inactivation of the other *VHL* allele.

Other than mutations in *VHL*, there is a paucity of data regarding other genetic hits in hemangioblastomas that might contribute to tumorigenesis. Sprenger et al. performed comparative genomic hybridization (CGH) of ten sporadic hemangioblastomas and found that the most common genetic aberrations in sporadic tumors are loss of chromosome 3 (70 %), loss of chromosome 6 (50 %), loss of chromosome 9 (30 %), loss of 18q (30 %), and gain of chromosome 19 (30 %). Based on the frequencies and co-occurrence of these genetic changes, they hypothesized that the loss of chromosome 3 is an early event in oncogenesis in sporadic hemangioblastomas, followed by loss of chromosome 6 and subsequently chromosomes 9 and 18q, and lastly by the gain of chromosome 19 [4]. In another study, CGH results of 7 vHL-associated and 16 sporadic hemangioblastomas were compared. Mutations in the *VHL* gene on 3p25-56 were found in 100 % of hereditary hemangioblastomas, but only in 30 % of sporadic tumors. Conversely, complete loss of chromosome 3 occurred more commonly in sporadic hemangioblastomas (69 %) than in vHL-associated hemangioblastomas (14 %). Thus, it can be concluded that sporadic mutation in the *VHL* gene is not the primary oncogenic event in sporadic hemangioblastomas [5].

Epigenetic and other means of somatic inactivation of *VHL* are also being investigated. It has been proposed that inactivation of promoter CpG islands, due to hypermethylation, leads to transcriptional silencing of *VHL* [6].

Prowse et al. examined 53 vHL-related tumors, including 30 renal cell carcinomas (RCCs), 15 hemangioblastomas, 5 pheochromocytomas and 3 pancreatic tumors, for genetic changes such as LOH (loss of heterozygosity), intragenic somatic mutations as well as DNA hypermethylation. In this series, hypermethylation of the vHL gene was detected in 33 % of tumors (6 out of 18 tumors; 2 RCCs and 4 hemangioblastomas). Two tumors, both hemangioblastomas, showed intragenic somatic mutations in a wild-type gene [6].

In a subsequent study, Rickert et al. performed CGH of 20 hemangioblastomas (one vHL and the remainder sporadic), which revealed that the most common cytogenetic changes associated with hemangioblastomas include the loss of chromosomes 19, 6, and 22q, which are seen in 35 %, 30 %, and 15 % of patients, respectively, and the loss of chromosome 6 being significantly associated with the cellular variant. Loss of chromosome 3 was uncommon in this series of sporadic hemangioblastomas, in contrast to the earlier studies by Sprenger et al. [7].

Lemeta et al. suggested that LOH at 6q is common and concurrent with 3p loss in sporadic hemangioblastomas [8]. This finding was subsequently confirmed by other studies [4, 7, 9]. The same authors subsequently demonstrated high prevalence of LOH at the ZAC-1 tumor suppressor gene region located on 6q24-25. Moreover, they also demonstrated that promoter methylation of ZAC-1 leads to epigenetic silencing of the gene in 90 % of tumors [10].

CGH has demonstrated that the reticular and cellular variants of hemangioblastoma have different cytogenetic profiles, with the loss of chromosome 6 significantly associated with the cellular variants [7].

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Pathogenesis

vHL tumorigenesis can be mediated by both hypoxia-induced factor (HIF) and non-HIF-mediated mechanisms. HIF-1 is a heterodimeric transcriptional factor that regulates genes which respond to changes in oxygen levels in tissues [11, 12]. It is composed of HIF-1α and HIF-1β subunits [13]. Levels of HIF-1α are upregulated under hypoxic conditions and, by translocation into the nucleus and dimerization with HIF-1β, activate genes that promote angiogenesis (VEGF), erythropoiesis (EPO), nitric oxide synthesis (NOS), and glucose transport (GLUT-1). However, under normoxic conditions, HIF-1α undergoes ubiquitin-mediated degradation in the proteosomes, which are mediated by vHL protein [14–19]. The vHL protein binds to HIF-1α only after it undergoes oxygen-dependent hydroxylation of the proline residues 402 or 564 or both by members of the Elongin family (EG1N) [15, 18–21]. EG1N1 is the primary HIF-1 hydroxylase while EG1N2 and EG1N3 play compensatory roles under certain conditions [22]. However, when vHL is mutated, HIF-1α will not undergo degradation and remains constitutionally active [23]. This promotes tumorigenesis by increased transcriptional activation of genes that promote angiogenesis and other growth factors.

It has also been demonstrated that vHL is critical for cellular [24] differentiation during development and its inactivation causes developmental arrest [25] and protracted cellular differentiation [26]. The cell of origin in hemangioblastoma is an embryologically arrested hemangioblast derived from the mesoderm, which retains its multipotent properties and ability to differentiate into both red blood cells and blood vessel endothelium [27, 28]. Accordingly, foci of extramedullary hematopoeisis have been detected in hemangioblastomas. Vortmeyer et al. have detected the presence of fetal hemoglobin in these areas of extramedullary hematopoeisis, suggesting that the vHL deletion leads to primitive hematopoeisis [25, 26]. Moreover, co-expression of Epo and Epo receptor on these hemangioblasts represents a key event in vHL deficiency and further promotes tumor growth via autocrine and paracrine stimulation [25]. Developmentally arrested structural elements composed of hemangioblast progenitor cells have been demonstrated in the cerebella of *VHL*-mutated patients [29]. Hemangioblastic activity in the nervous system occurs in the embryonic stage [30] and hence its presence in adult brain depicts persistence of developmentally arrested hemangioblastic cells. vHL disease produces developmental aberrations giving rise to angiomesenchymal tumorlets resembling hemangioblastomas in the human CNS [31]. More recently, the pleuripotent vHL deficient cells in hemangioblastomas have been demonstrated to give rise to mast cells via the c-Kit signaling pathway. Accordingly, mast cells from tumor samples of patients exhibited LOH in the VHL alleles when compared with the peripheral blood lymphocytes [32].

Pathology

Macroscopically, hemangioblastoma is a well-circumscribed tumor, with both solid and cystic components. The tumor appears yellow in color due to its high lipid content.

Microscopically, the tumor has two components: a network of capillaries lined by hyperplastic endothelial cells with intervening vacuolated stromal cells, which have pale cytoplasms, pleomorphic nuclei and high lipid content. Mitoses are conspicuously absent [33]. A recent study of 156 tumors reports that tumor architecture relates to the size of the tumor; with smaller tumors (<8 mm^3) composed of mesenchymal architecture comprising of a network of capillaries, while the larger tumors composed of enlarged stromal cells clustered in groups (Fig. 14.1) [26]. The stromal cell, which is the tumor cell in hemangioblastoma, is an embryologically arrested hemangioblast derived from the mesoderm that retains its multipotent properties as well as the ability to differentiate into both red blood cells and blood vessel endothelium. The stromal cells are immunoreactive for cytokeratin, S-100, NSE (neuron specific enolase), actin, GFAP (glial fibrillary acid protein), vimentin, and EMA (epithelial membrane antigen). The stromal and capillary endothelial cells express different surface adhesion molecules suggesting different cells of origin. The capillary endothelial cells express endothelium associated adhesion molecules such as ICAM-1, ICAM-2, PECAM, ELAM, and VCAM-1. The stromal cells express neuronal cell adhesion molecule (NCAM), which further supports its mesenchymal origin. Since NCAM is also expressed by metastatic renal cell cancer to the CNS, its expression by hemangioblastoma can present as a diagnostic challenge [34, 35]. The stromal cells also stain negatively for von Willebrand factor, a marker of endothelial origin [36]. Brachyury, a protein transcription product of the T box gene, which regulates the formation of mesoderm, is expressed in the cytoplasm of stromal cells and is highly specific for hemangioblastoma, distinguishing it from morphologically similar lesions such as metastatic clear cell renal cell cancer and angiomatous meningioma [37, 38].

Histologically, hemangioblastomas are classified into two variants: the more common reticular variant (composed of proliferating vascular elements) and the rare cellular variant (composed of epitheloid clusters of stromal cells), which are associated with greater GFAP positivity, higher proliferation index, and probability of recurrence [39].

Receptors for cellular growth factors including proangiogenic factors, such as epidermal growth factor receptor (EGFR), platelet derived growth factor receptor (PDGFR), placental growth factor receptor (PFG-1), and vascular endothelial growth factor receptor (VEGF), are expressed on tumor cells in hemangioblastomas [40]. However, unlike malignant gliomas, the VEGF expression does not correlate with the vascular density as indicated by the expression of

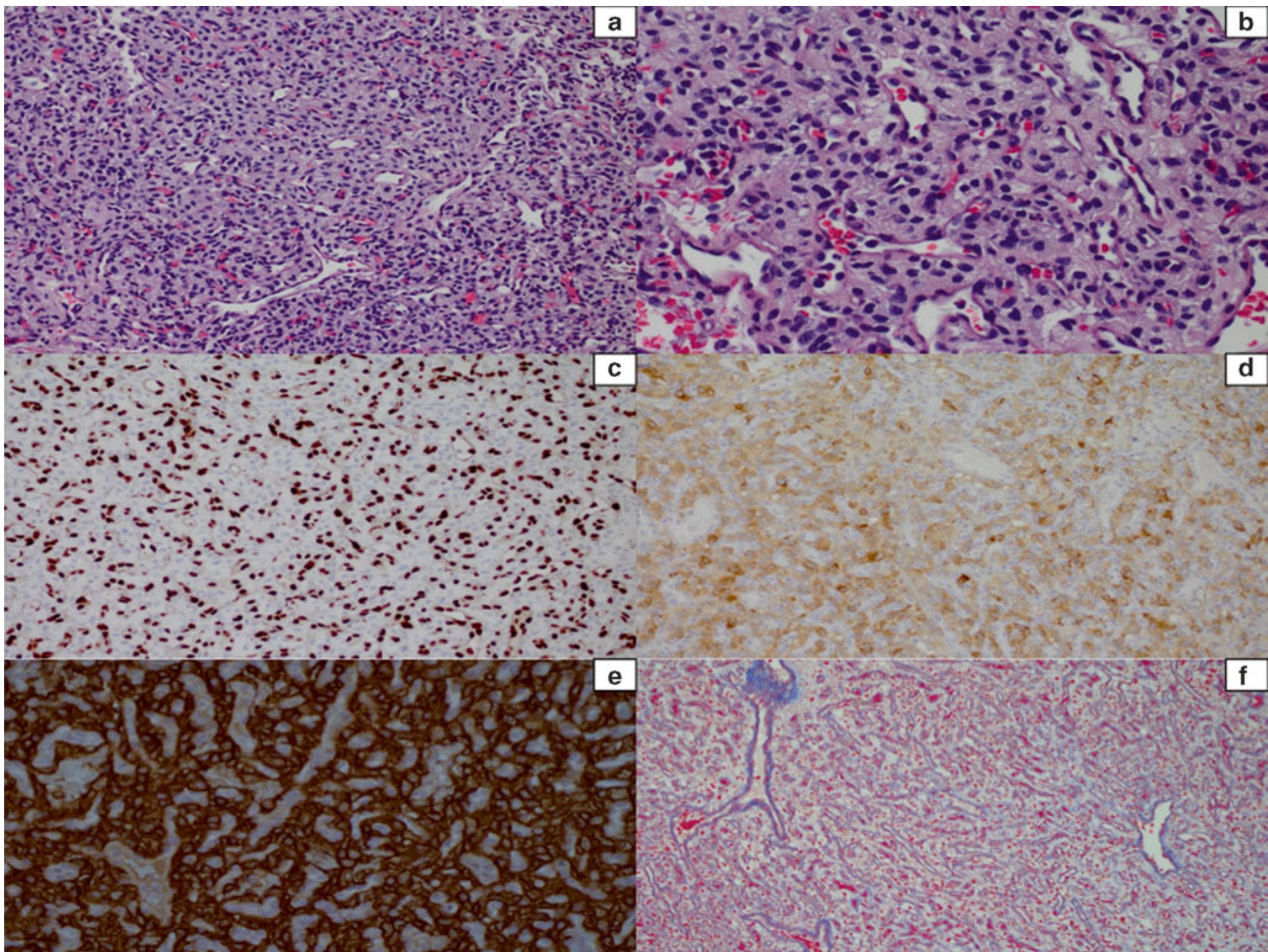

Fig. 14.1. (**a**, **b**) H&E, (**c**) ERG, (**d**) Inhibin, (**e**) Carbomic anhydrase and (**f**) Azocarmine. (**a**) H&E stain shows a highly vascular neoplasm. The tumor is composed of vascular cells and cells with round nuclei designated as "stromal" cells. (**b**) Higher power reveals numerous vascular channels (v) and interspersed stromal cells are seen. Note the nuclear pseudoinclusion in a stromal cell (*arrowhead*). (**c**): ERG immunohistochemistry. Note the intense nuclear staining in vascular cells; (**d**, **e**) Inhibin and carbonic anhydrase immunohistochemistry. Note the intense staining in stromal cells (**f**) Azocarmine stain highlights vascular channels (**a**, **f**, ×50; **b–d**, and **e**, ×100).

CD34-positive endothelial cells. This suggests that pro-angiogenic factors other than VEGF probably contribute to the intense tumor vascularity [41].

Clinical Features

Hemangioblastomas most commonly arise in the CNS especially, but not exclusively, in the posterior fossa. The frequent sites of occurrence in the order of commonality are cerebellum, dorsal part of the spinal cord, brainstem, and retina (Figs. 14.2 and 14.3) [42–44]. The most common site of occurrence of hemangioblastomas in the spinal cord is the thoracic region, followed by cervical and lumbar (48 %, 36 % and 16 %, respectively) [45]. Spinal cord and brainstem hemangioblastomas are frequently associated with tumors at other sites and especially cerebellar hemangioblastomas; in turn, however, cerebellar hemangioblastomas are less frequently associated with tumors at the other sites, suggesting that the spinal cord/brainstem hemangioblastomas are the accompanying manifestation of the latter [46, 47]. Supratentorial (cerebral, sellar/suprasellar, intraventricular) hemangioblastomas are rare [48–50]. It is sometimes difficult to differentiate supratentorial hemangioblastoma from meningioma [38, 51]. Occasionally, hemangioblastomas may arise in extraneural sites such as bone, soft tissue, skin, liver, pancreas, and kidney [52–55].

One-third of hemangioblastomas are associated with the vHL syndrome. The spectrum of tumors [56] associated with vHL is broad and includes hemangioblastomas, renal cell

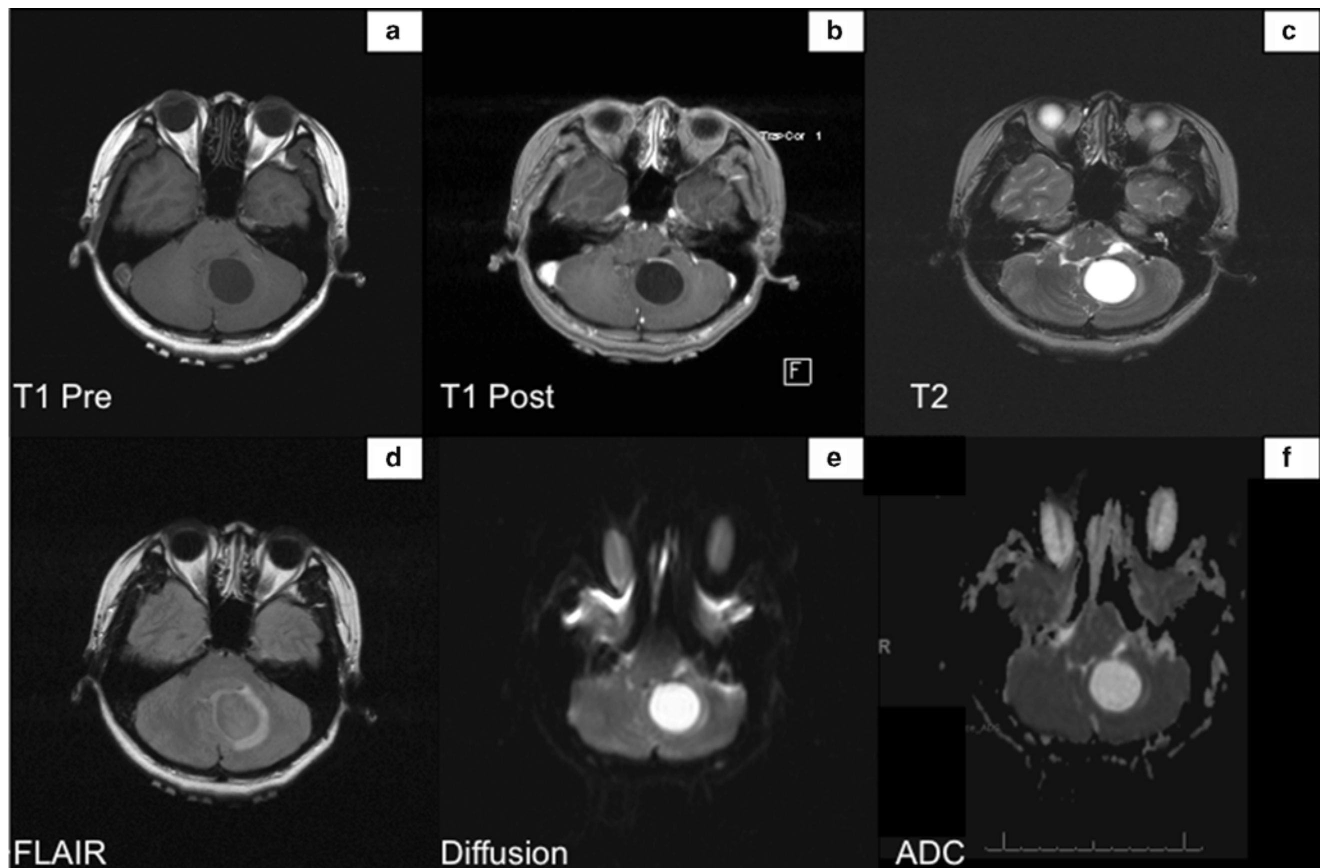

Fig. 14.2 MRI shows the tumor within the inferior medial left cerebellum. Lesion in isointense with the adjacent brain parenchyma on the T1 weighted sequences (Panel **a**), hyperintense on T2 weighted sequence (Panel **c**), and avidly enhances gadolinium (Panel **b**). Diffusion weighted sequence does not demonstrate hyperintense signal within the mass (Panels **e** and **f**)

carcinomas [57, 58], pheochromocytomas [59], extra-adrenal paragangliomas [60, 61], retinal angiomas [62–64], neuroendocrine pancreatic tumors [65–69], papillary cystadenomas of the epididymis [70] and broad uterine ligament [71], as well as endolymphatic sac tumors (ELSTs) of the middle ear [72–74]. vHL-mutated patients with hemangioblastomas are generally younger and present with multiple tumors, while the non-vHL-associated tumors are seen in older patients and are usually solitary.

Based on clinical manifestations, vHL is classified into type 1 and type 2. Type 1 vHL is not associated with pheochromocytoma while type 2 is. Type 2 is further divided into type 2A, 2B, and 2C. vHL-type 2b is associated with high incidence of hemangioblastoma and pheochromocytoma [44, 75–77].

Since patients with vHL syndrome are predisposed to developing multiple hemangioblastomas and require specialized surveillance and treatment, it is imperative to correctly diagnose vHL as early as possible. Genetic testing for vHL in addition to a comprehensive family history should be considered standard practice for all patients with CNS hemangioblastomas, especially those diagnosed under 30 years of age. Clinical screening of vHL-associated tumors consists of complete neuraxis imaging with magnetic resonance imaging (MRI) of the brain and entire spine, MRI of the abdomen, retinoscopy, and measurement of urine catecholamines. Some authors have suggested ophthalmologic screening for family members of vHL disease for early detection of retinal hemangioblastomas [78].

Hemangioblastomas are considered benign tumors, but can cause significant neurological deficits depending on their size and location. Headache, vomiting, cerebellar symptoms, and cranial nerve involvement may be the presenting features. Posterior fossa tumors can also cause cerebrospinal fluid (CSF) flow obstruction leading to hydrocephalus [79, 80]. Patients with spinal cord tumors may present with progressive scoliosis and radicular symptoms until the tumor is large enough to cause weakness. Onset of retinal hemangioblastomas can start prior to 10 years of age until 30 years, after which the risk gradually decreases. It usually presents with unilateral involvement [77]. Hemangioblastomas exhibit a stuttering growth pattern, i.e., there are periods of growth followed by periods of quiescence, which may be as long as 2 years. Indications for treatment relate to the

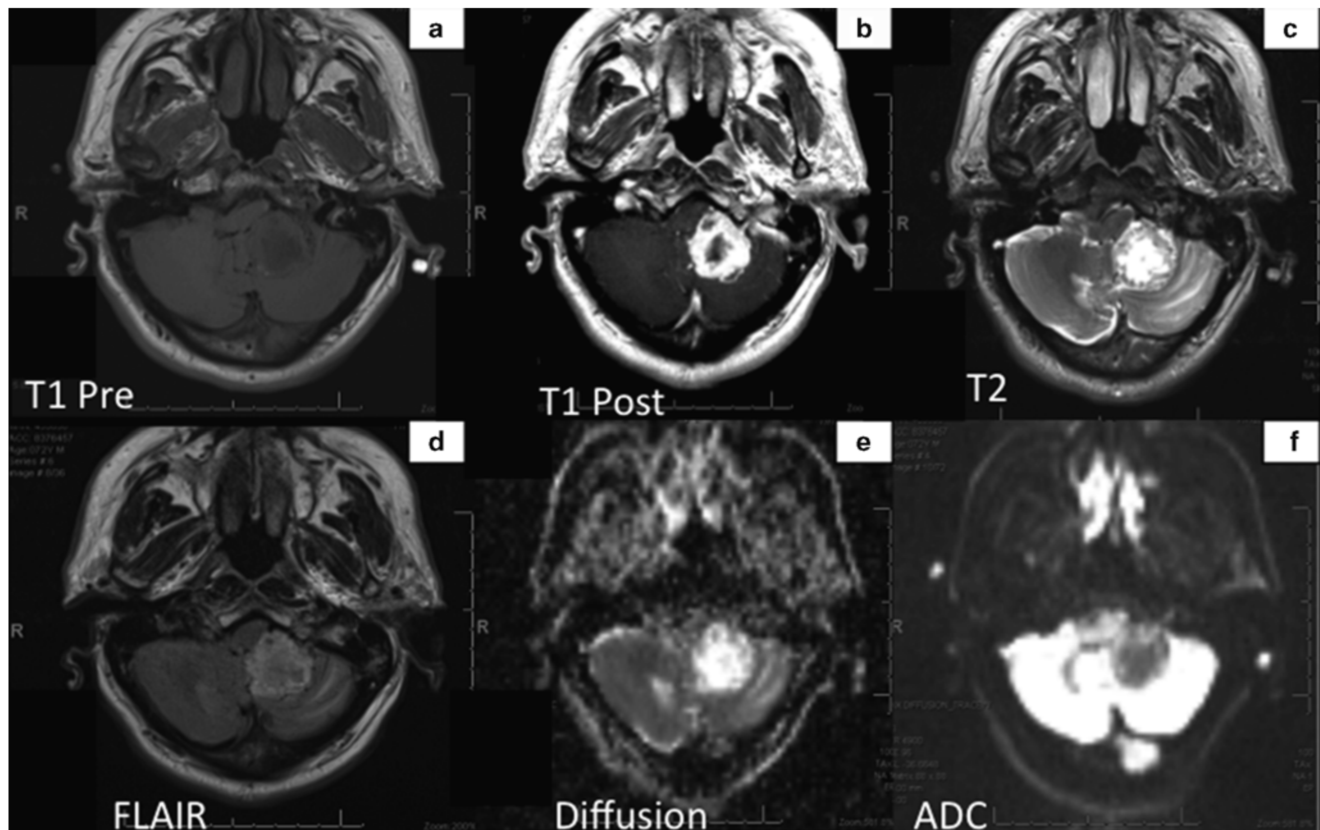

Fig. 14.3. MRI shows the tumor that appears as an irregular thick-walled mass in the region of the left cerebellar tonsil, which enhances intensely with gadolinium (Panel **b**). Mass in predominantly T1 hypointense (panel **a**) but contains several areas of T2 hyperintensity indicating hemorrhagic component (Panel **c**). Diffusion studies show hyperintensity relative to the contralateral white matter (Panels **e** and **f**).

patient's symptoms and tumor size, location, and rate of progression [81]. It is quite common for spinal cord hemangioblastomas to present with syrinx formation [82]. Occurrence of erythrocytosis with male predominance is common in hemangioblastomas due to production of erythropoietin [83, 84]. Due to their arteriovenous malformation-like vascularization, solid hemangioblastomas present a unique neurosurgical challenge [85].

There have been numerous clinical reports of worsening of vHL-associated hemangioblastomas in pregnancy, leading to progressive neurological deficits and obstructive hydrocephalus [86–90]. However, in the first prospective study comparing the rate of tumor growth in pregnant versus the nonpregnant cohorts with vHL-associated hemangioblastomas, Ye et al. observed that there were no differences in tumor growth rate, peritumoral cyst growth and the need for surgery. However, this was a small study with only 27 patients in the pregnancy cohort and it is possible that patients who chose to become pregnant were already in a better state of health leading to selection bias [91].

Imaging

Hemangioblastomas show post-contrast enhancement on computed tomogram (CT) scans and T1-weighed MRI. Imaging studies show the typical appearance of a cyst with mural nodule in approximately 60 % of cases. The nodular portion shows flow voids in the T1 and T2-weighted sequences. Generally, the cysts are slightly hyperintense compared to CSF in T1-weighted images. Both the nodule and the cyst appear bright on T2 and fluid attenuated inversion recovery (FLAIR) sequences [92].

Treatment

While most neurosurgeons agree that surgical intervention of symptomatic hemangioblastomas is required, controversy arises in dealing with asymptomatic hemangioblastomas, which commonly occur in patients with vHL syndrome. Unlike other benign intracranial tumors that exhibit a slow,

progressive growth pattern, hemangioblastomas often have prolonged periods of growth arrest, thus making their natural course difficult to predict [81]. For asymptomatic, radiographically stable tumors, no treatment may be recommended. When asymptomatic tumors show progression on imaging only, the best time for intervention may be difficult to determine [93–96]. Similar to patients with other tumor predisposition syndromes, the optimal clinical management of vHL requires a specialist who oversees and coordinates a multidisciplinary plan of care, including appropriate screening tests.

From a therapeutic perspective, surgical removal remains the treatment of choice for hemangioblastomas and has been successfully employed for cerebellar [97], spinal [98, 99], and brainstem [94, 100] hemangioblastomas. Preoperative cerebral angiography helps surgeons determine the nature of the tumor vascular supply. Following diagnostic imaging, pretreatment with dexamethasone for several days is generally recommended. Intraoperative bleeding increases with tumor size, making en bloc resection of larger tumors difficult. However, modern microsurgical techniques are used to identify feeding vessels and thus help minimize intraoperative bleeding. Dissection should be carried out along the external surface of the tumor in the gliotic brain-tumor interface, to avoid entering the tumor, thus preventing brisk hemorrhage from the hemangioblastoma. The tumor-associated cysts are non-neoplastic and consist of compressed glial tissue, which collapses on its own once the associated tumor is removed. Postoperative complications include temporary worsening of neurological deficits, new neurological deficits, which may or may not resolve during follow-up, cranial postoperative infection, hydrocephalus and aseptic meningitis [101]. A postoperative contrast-enhanced MRI is routinely obtained to verify extent of resection. If no residual is noted, tumor recurrence is rare.

More recently, stereotactic radiosurgery is also being employed with encouraging results especially in spinal hemangioblastomas [102–105]. One advantage of radiosurgery is the ability to treat multiple lesions in a single treatment setting. In a series of 9 patients with 20 spinal hemangioblastomas, 4-year tumor overall and solid tumor control rates with stereotactic radiosurgery were as high as 90 % and 95 %, respectively [106]. In other studies, however, patients with multiple hemangioblastomas associated with vHL syndrome were found to less likely exhibit tumor control after treatment with radiation therapy compared to single sporadic hemangioblastomas [107, 108]. In general, smaller tumor volumes and higher doses of radiation (median 16 Gy) confer a better tumor control [109].

In contrast to surgery and radiation therapy, there is a paucity of data on systemic treatment of hemangioblastomas. Since hemangioblastomas are highly vascular, systemic anti-angiogenic therapies are being investigated as an alternative to surgery, particularly in vHL patients with multiple tumors. Several vHL patients have been treated with semaxanib, a multi-tyrosine kinase inhibitor predominantly active against VEGFR-2. Although disease stabilization outside the CNS was observed in some patients, most of the treatment responses were limited to retinal hemangioblastomas [110]. In a clinical trial for vHL patients with sunitinib, which predominantly targets VEGFR and PDGFR, antitumor activity was seen against renal cell carcinoma, but not hemangioblastomas [111]. EGFR, which is overexpressed and activated in hemangioblastomas, represents an additional attractive target for therapeutic intervention and study in future clinical trials [112]. There have been case reports on the use of anti-angiogenic agents such as bevacizumab [113], pazopanib [114], sunitinib [115], thalidomide [116], and interferon [117] with limited success. However, no prospective clinical trials using these agents have been conducted to date.

Prognosis

Gross total tumor resection was a predictor of prolonged progression-free survival (PFS) in one series [118]. Poor prognostic factors include poor performance status [101], multiple hemangioblastomas, retinal hemangioblastomas, and presence of solid rather than cystic tumors. The risk of recurrence in the future is higher if the age of diagnosis is younger than 40 years with primary sites being the brainstem and spinal cord [119].

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15 Schwannomas

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Schwannomas are benign tumors arising from Schwann cells, named after the German physiologist Theodor Schwann. Schwann cells, or neurolemmocytes, are glial cells that play a number of important roles in peripheral nerve biology, including nerve development, myelination, metabolism, conduction, and repair. The majority of schwannomas arise sporadically, i.e., in patients without a positive family history and no identifiable tumor predisposition syndrome. Schwannomas arise from cranial, spinal, or peripheral nerves and small myelinated nerves in the skin or viscera. The most commonly affected cranial nerve is the eighth cranial nerve, near the vestibular ganglion (vestibular schwannoma, VS), followed by the fifth (trigeminal schwannoma) [1]. VS represents one of the most common intracranial tumor, and the incidence of sporadic VS in the United States is approximately 3,000 per year [1].

Schwannomas arising from spinal nerve roots have a predilection for the sensory (dorsal) roots with the lumbosacral and cauda equina regions most commonly affected. The peripheral nerves most affected are in the flexor surfaces of the upper and lower limbs: the ulnar and peroneal nerves. Rarely, schwannomas occur within the parenchyma of the brain or spinal cord [2] where the tumors are presumed to arise from small myelinated peripheral nerve fibers that accompany vessels into the parenchyma or from aberrant peripheral nerve fibers [3, 4].

Known hereditary tumor predisposition syndromes associated with schwannomas include *neurofibromatosis type 2* (*NF2*), *schwannomatosis*, and *Carney*'*s complex*.

NF2 is caused by inactivation of the *NF2* gene located on chromosome 22q [5, 6]. The *NF2* gene product, Merlin, is a unique tumor suppressor in that it functions both at the cell cortex and in the nucleus [7].

NF2 patients typically develop progressive hearing loss in adolescence or young adulthood due to bilateral vestibular schwannomas (VS), but also develop schwannomas at other locations throughout the nervous system. Patients with *schwannomatosis* suffer from multiple, often painful schwannomas, but with rare exceptions, do not develop VS and meningiomas, which are common in NF2 [8–10]. The disease is familial in approximately 15 % of the patients only, and follows an autosomal dominant mode of inheritance. Germline mutations in *SMARCB1* [11–13], a gene that is also involved in rhabdoid tumor predisposition syndrome (RPDS), can be identified in approximately 50 % of familial and <10 % of sporadic patients [14]. A recent study identified mutations in the *LZTR1* gene, another tumor suppressor, in the majority of patients with schwannomatosis that did not have germline alterations in *SMARCB1* [15].

Carney'*s complex* is an autosomal dominant multiple neoplasia syndrome. The diagnosis is made if two or more major manifestations of the syndrome are present. Major manifestations include skin lesions (lentiginis, blue nevi, and cutaneous myxomas), cardiac myxoma, endocrine manifestations (hyperplasias and adenomas of adrenal and pituitary), and psammomatous melanocytic schwannomas [16–18]. Schwannomas in Carney's complex patients most frequently arise in the paraspinal sympathetic chain or the gastrointestinal tract, although they may occur anywhere in the peripheral nervous system. Inactivating mutations in the regulatory subunit type 1 alpha gene (*PRKAR1A*) located at 17q22-24 can be identified in approximately 30 % of Carney's complex patients [19, 20].

Historically, the mainstay of treatment for schwannomas has included surgery and radiation therapy, both of which have major drawbacks. Although surgical resection is effective at tumor control, serious complications depending on tumor size and location are common, such as disfigurement and further loss of neurologic function. Stereotactic radiosurgery (SRS) or fractionated radiation therapy (RT) have been proposed as alternatives. SRS has the largest clinical experience in VS management with published outcomes, including for NF2 [21, 22]. However, its safety and efficacy in the NF2 population has not been established in patients with large VS and significant hearing impairment and there is concern about long-term efficacy. The risk for a radiation-induced secondary malignancy has also been raised, although

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rare [23, 24]. For a subset of NF2 patients with unilateral hearing only and progressive hearing loss due to a growing ipsilateral VS, surgical resection will most likely result in total deafness. SRS as currently performed at doses of 12–13 Gy, can maintain hearing in some patients, but clinical studies show that hearing commonly declines after 5 years [22]. Over the recent years, our increasing understanding of the biology of NF2 mutant tumors, including schwannomas, has opened avenues for preclinical and clinical development of molecular targeted medical therapies for schwannomas. As a result, some of these therapies have begun to show promise in the clinic, and novel agents are being actively developed in a growing number of preclinical studies and clinical trials.

Pathology and Histopathology

Schwannomas are benign (WHO Grade I) peripheral nerve sheath tumors that are composed of neoplastic Schwann cells. Other terms that may be used as synonyms for schwannomas include neurinoma and neurilemmoma. The term neuroma should not be used as synonym for schwannomas, as it implies a non-neoplastic process, such as a traumatic neuroma, Morton's neuroma [1].

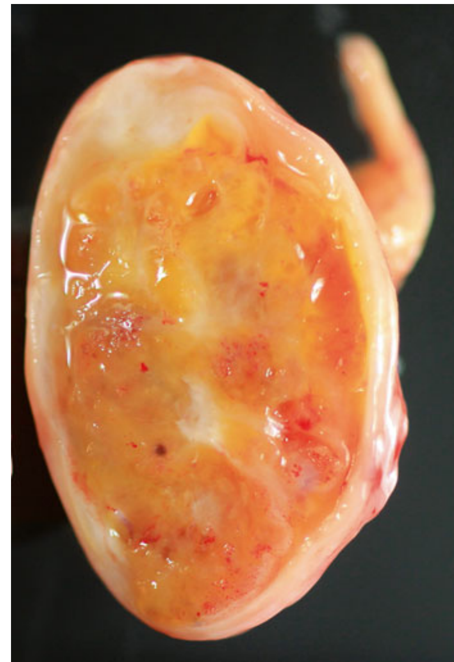

Fig. 15.1. Macroscopic features of a schwannoma: encapsulated mass, associated with a nerve, with homogenous yellow/tan cut surface

Macroscopic Findings

Most schwannomas are globoid, encapsulated, discrete masses. The capsule consists of the epineurium of the nerve in which the tumor arises. The nerve of origin may sometimes be observed entering and/or exiting the tumor mass (Fig. 15.1). The tumor size and shape are dictated by the site of origin: tumors arising in spinal nerve roots often have a "dumbbell" shape with a narrow center, where the tumor is confined within the nerve foramen, and two enlarged extremities where it is unconfined in the extra and intra spinal compartments. Schwannomas that arise in the mediastinum or retroperitoneum where space is not confined, may reach large dimensions and develop degenerative cystic changes.

The cut surface of schwannomas appears tan and homogenous (Fig. 15.1). There may be white areas (fibrosis) or yellow areas which represent fat infiltration or sheets of lipid laden macrophages. In some cases (especially in large tumors) there may be areas of hemorrhage or cystic degeneration.

In some cases, especially in the skin and viscera, schwannomas may grow in a plexiform pattern, expanding and replacing the nerve of origin, in a similar pattern to plexiform neurofibromas.

Histological Features

Most schwannomas have the conventional (classic) histological features. However, other patterns of growth and histological patterns may be seen, especially in association with tumor syndromes (see below) and may be misdiagnosed as other tumor types.

Conventional Schwannomas

The histological appearance of conventional schwannomas is of a benign spindle cell tumor with sharply demarcated margins (encapsulated). Nerve axons and perineurial cells are often present at the periphery of the tumor, and can be highlighted with immunostaining for Neurofilament and Claudin 1, respectively. In contrast with neurofibromas, very few axons (if any) are present within the tumor.

A biphasic pattern with compact (Antoni A) and loose (Antoni B) areas is a characteristic feature (Fig. 15.2). The proportions of these areas vary, and in some tumors only one type will be present. Diagnosis based on the loose (Antoni B) areas can be difficult as the histological features are nonspecific. The hallmark of schwannomas is the formation of nuclear palisades around nuclear free areas (Verocay bodies) which may at times form elongated ribbons (Fig. 15.3) or clusters where tumor cells form groups of "streaming" elongated, narrow nuclei. Longstanding tumors may display degenerative changes such as cysts and sheets of lipid laden macrophages. Some schwannomas may have large clusters of "back to back" large hyalinized vessels mimicking vascular malformations, often associated with thrombosis and hemosiderin-laden macrophages. Another type of degenerative change in schwannomas is the presence of scattered

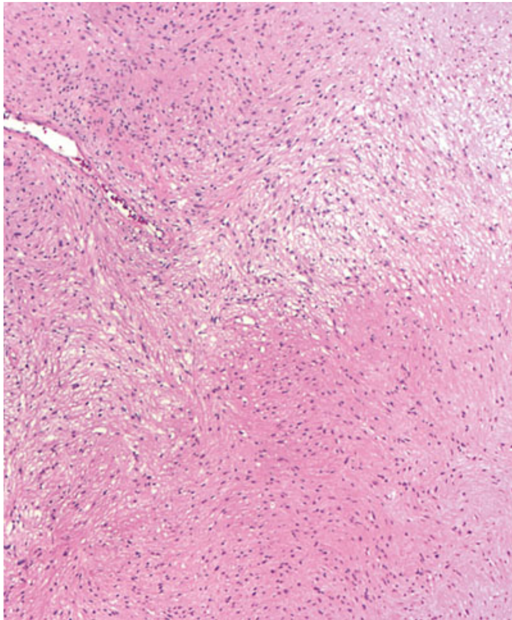

Fig. 15.2. Conventional schwannoma (H&E): Antoni A (compact) and Antoni B (loose, pale) areas are classic features in schwannomas

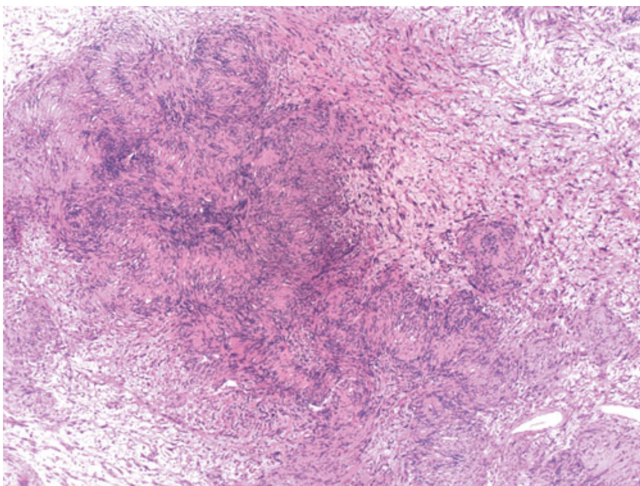

Fig. 15.3. Conventional schwannoma (H&E): Verocay bodies are characteristic of schwannomas and are nuclear palisades around nuclear free areas

large, atypical, hyperchromatic nuclei ("ancient change") which are not indicative of malignant transformation.

Cellular Schwannomas

Cellular schwannomas are characterized by dense cellularity with intersecting fascicles or patternless growth, and lack of the histological hallmarks of conventional schwannomas. The characteristic biphasic (Antoni A/Antoni B) pattern and Verocay bodies are not present and the tumor is often composed of compact (Antoni A) areas only. In addition to

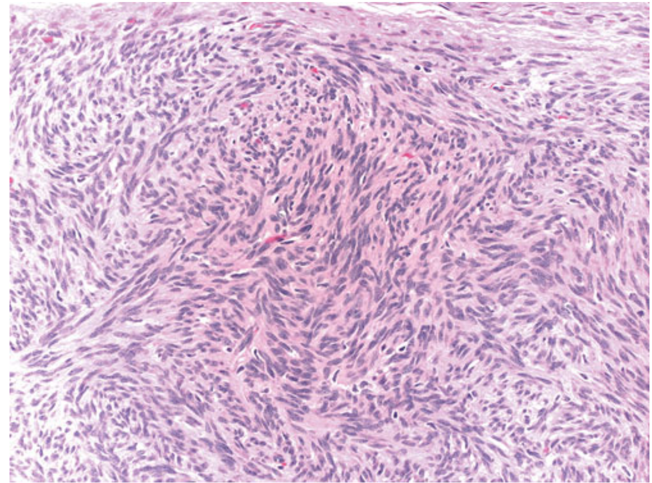

Fig. 15.4. Cellular schwannoma (H&E): Dense cellularity, fascicular growth pattern and scattered mitotic figs. in a cellular schwannoma

hypercellularity, the tumors may display mitotic activity, nuclear atypia, and necrosis (Fig. 15.4). However, despite the presence of these worrisome histological features, cellular schwannomas are benign, and although recurrence rate is variable, they are slow growing and never metastasize [25–27].

The lack of the classical histological features and the presence of dense cellularity and mitoses may prompt a diagnosis of malignancy. The differential diagnosis includes sarcomas such as leiomyosarcoma, or malignant peripheral nerve sheath tumor (MPNST). Helpful distinguishing features that support the diagnosis of cellular schwannoma include the presence of a peripheral capsule, diffuse S100 positivity and collagen IV expression.

Plexiform Schwannomas

Plexiform schwannomas grow within the nerve, expanding and replacing it along its course, so it appears grossly like a rope; a similar pattern as the better known plexiform neurofibromas, with which they may be confused (Fig. 15.5).

Plexiform schwannomas are most common in the skin but may also occur in soft tissue or major peripheral nerves [28–30]. In contrast to other schwannoma subtypes, plexiform schwannomas are often non-encapsulated and may infiltrate adjacent soft tissue, encasing nerves and skin adnexa. Furthermore, entrapped axons are often present within the tumor mass, features that mimic plexiform neurofibromas [31].

Plexiform schwannomas may be associated with NF2 or schwannomatosis; there is no association with NF1 [32, 33]. In contrast to plexiform neurofibromas, there is no risk of malignant transformation. Helpful for the histological diagnosis of plexiform schwannomas are the presence of conventional schwannoma features (Verocay bodies, Antoni A/Antoni B areas) and diffuse S100 positivity.

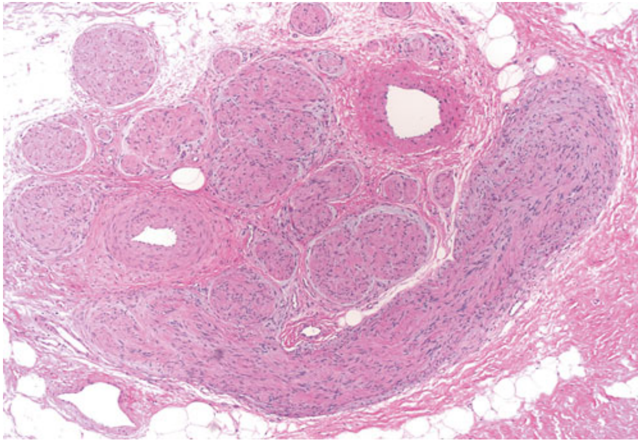

Fig. 15.5. Plexiform schwannomas (H&E): plexiform schwannomas grow along the nerve, expanding and replacing it

Melanotic Schwannomas

Melanotic schwannomas are rare and distinct tumors that are composed of neoplastic Schwann cells that contain melanin. The tumors appear as pigmented, circumscribed masses. Melanotic schwannomas are of two types: non-psammomatous and psammomatous, defined by the presence of psammoma bodies (concentrically laminated bodies that are PAS positive). Non-psammomatous melanotic schwannnomas are benign. In contrast, half of the psammomatous melanotic schwannomas are associated with Carney's complex and may undergo malignant transformation [16, 17, 34]. Therefore, in the case of psammomatous melanotic schwannomas, the possibility of an underlying Carney's syndrome should be investigated.

Melanotic schwannomas lack the classical features of conventional schwannomas (Antoni A/Antoni B areas, Verocay bodies, vascular clusters) and are often composed of large, epitheloid cells, with large round/oval nuclei and prominent nucleoli (Fig. 15.6). The differential diagnosis of melanocytic schwannomas is with melanocytic lesions; melanocytoma and melanoma (primary and metastatic). Electron microscopy may be helpful. Positive collagen IV immunostaining and a rich reticulin network can support the diagnosis of schwannoma.

Sporadic and Syndromic Schwannomas

The great majority (90 %) of schwannomas are single, sporadic tumors [35]. However, schwannomas may also occur as part of the clinical manifestations in patients with an underlying genetic predisposition (syndromic).

In a study in which the histological features of solitary sporadic schwannomas were compared to schwannomas associated with NF2; some histological features were found to be more common in sporadic/solitary schwannomas. In particular, solitary schwannomas were found to have prominent vascular clusters that mimic vascular malformations ("back to back" vessels with thick hyalinized walls or dilated sinusoidal vessels), thrombosis, and inflammation (Fig. 15.7) [36]. In addition, a more recent study found that in contrast to the mosaic pattern seen in NF-associated schwannomas (NF2 and schwannomatosis), most solitary/sporadic schwannomas retain INI1 expression and appear diffusely positive [37] (see below).

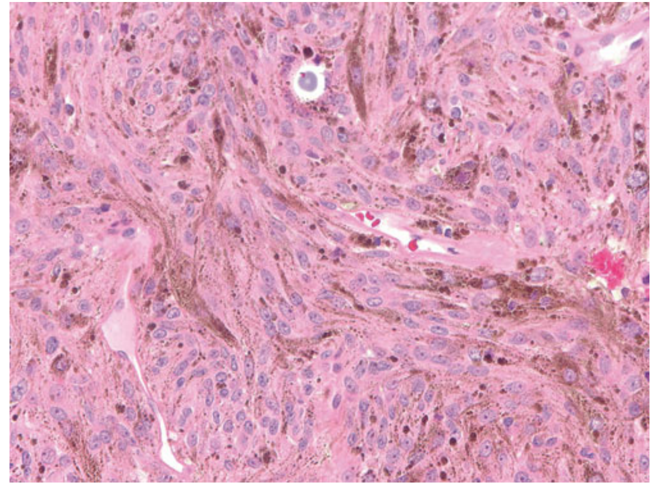

Fig. 15.6. Melanotic Scwhannoma: Melanotic schwannomas are composed of pigmented Schwann cells, and may contain psammoma bodies

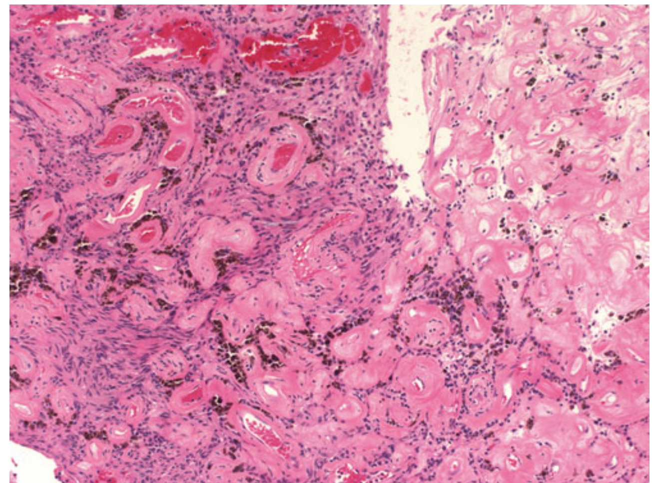

Fig. 15.7. Sporadic/solitary Schwannoma: Clustered thick hyalinized blood vessels mimicking vascular malformations are a common finding in solitary/sporadic schwannomas

Syndromic Schwannomas

The syndromes associated with multiple schwannomas include NF2, schwannomatosis and Carney's Syndrome.

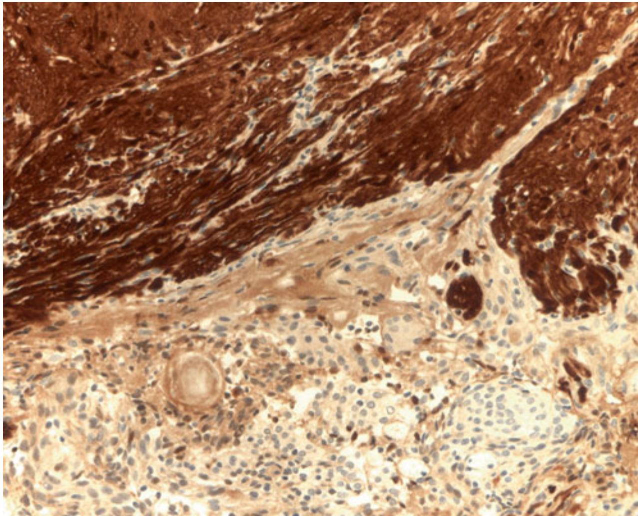

Fig. 15.8. Schwannoma/meningioma collision tumor (S100 immunostain): a collision tumor composed of schwannoma (on the *right*, immunopositive for S100) and meningioma (on the *left*, S100 negative) is pathognomonic of NF2

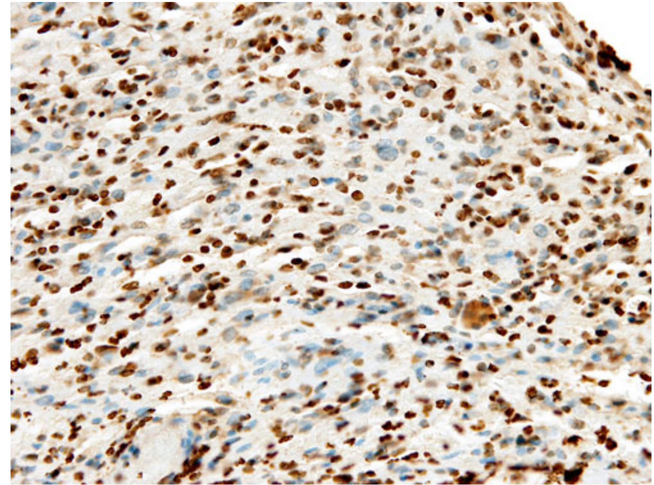

Fig. 15.9. Mosaic INI1 immunostaining (INI1 immunostain): partial loss (mosaic staining) of INI1 expression is common in NF-associated schwannomas

Neurofibromatosis Type 2 (NF2)

NF2 is an autosomal dominant disorder characterized by neoplastic and dysplastic lesions of Schwann cells, meningothelial cells, and glial cells. Patients are predisposed to develop multiple schwannomas, and the hallmark of the disease is bilateral VS. In addition, NF2 patients are predisposed to develop other tumors; multiple meningiomas and spinal ependymomas. Non-neoplastic lesions associated with the syndrome include meningioangiomatosis, glial hamartomas, retinal hamartomas, posterior subcapsular cataracts, epiretinal membranes, and polyneuropathies [1, 38].

The disease is rare, with an estimated incidence of 1 per 40,000 newborns [35]–1:25,000 [39] and is caused by a germline mutation in the NF2 gene on chromosome 22q that encodes the protein Merlin. *De novo* mutations (patients without family history) occur in 30 % of the patients. Particularly difficult to diagnose are patients with mosaic NF2 in which clinical manifestations may overlap with other forms of neurofibromatosis (NF1 or schwannomatosis) or may not fulfill the clinical criteria for the diagnosis of NF [40]. In these scenarios, the pathological diagnosis of nerve sheath tumors may be particularly helpful in supporting a suspected clinical diagnosis. Schwannomas associated with NF2 often present at an earlier age than sporadic, non-syndromic schwannomas [41, 42].

NF2-associated schwannomas frequently have a multilobular appearance ("bunch of grapes") which may be apparent macroscopically and/or microscopically [36]. In some cases, meningioma and schwannoma form a collision tumor, in which the two components are seen on the same slide (Fig. 15.8). Schwannoma/meningioma collision tumors are pathognomonic for NF2. In contrast to sporadic solitary schwannomas, the pattern of INI1 immunostaining of schwannomas associated with NF2 or schwannomatosis is a mosaic pattern, in which there is partial loss, with mixed positive and negative cells (Fig. 15.9); [37]. Therefore, the pattern of growth (multinodular) and the mosaic INI1 expression pattern may support the diagnosis of an NF-associated schwannoma in some cases. Plexiform cutaneous schwannomas may be seen in childhood in NF2 patients and should not be confused with neurofibromas (which would lead to the clinical diagnosis of NF1).

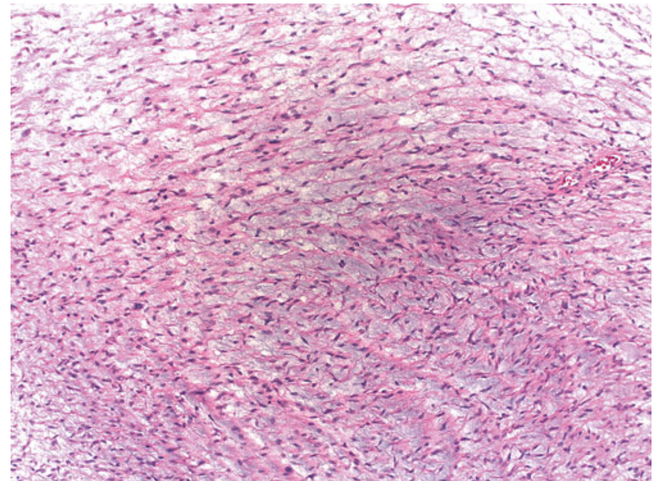

Fig. 15.10. Myxoid schwannoma (hybrid tumor): Schwannomas with abundant myxoid stroma may mimic neurofibroma (hybrid tumor) and are common in schwannomatosis

Schwannomatosis

Histologically, schwannomatosis-associated tumors often have prominent myxoid stroma, which may lead to a misdiagnosis of a neurofibroma [8, 11–13, 43–45]; (Fig. 15.10). Peripheral nerve sheath tumors with mixed features of

schwannoma/neurofibroma are referred to as "hybrid tumors" [46] and may represent a myxoid schwannoma or a "Schwann cell rich" neurofibroma. Hybrid tumors are more common in the context of the neurofibromatoses [47] and misdiagnoses can be avoided by immunohistochemical stains that highlight the different components of the tumor (axons, Schwann cells, perineurial cells). Many of the schwannomatosis-associated tumors (especially familial schwannomatosis) have a mosaic (partial lack) pattern of expression of INI1 protein [37].

Carney's Complex

Schwannomas from patients with Carney's complex are pigmented and often calcified [18]. Histologically, they contain melanin and psammoma bodies and lack the classic features of conventional schwannomas. There is a risk of malignant transformation in 10 % of the cases. Although the histological criteria are not well defined, large nuclei with prominent nucleoli, brisk mitotic activity, and necrosis are worrisome signs of aggressive biological behavior.

Cytogenetics and Molecular Genetics

A number of studies to date have examined the cytogenetics and molecular genetics of schwannomas, including sporadic schwannomas and schwannomas associated with NF2 and schwannomatosis. A number of early studies demonstrated that loss of heterozygosity (LOH) is common in NF2-associated and sporadic schwannomas [48, 49], and subsequent work showed that both sporadic and NF2-related VS harbor mutational inactivation or loss of both alleles of the *NF2* gene [5, 50], consistent with Knudson's two hit model of tumorigenesis [51]. In the case of schwannomatosis, a four hit mechanism has been proposed, involving *NF2* and either *SMARCB1* [12] or *LZTR1* [15].

Studies in schwannomas using comparative genomic hybridization (CGH) [52–55] have identified loss on chromosome 22 (*NF2*) as the most common hit by far, detectable in up to 56 % of sporadic and 62 % of NF2-associated schwannomas, and LOH was caused by mitotic recombination in a subset [53]. Other genetic aberrations observed in subsets of tumors included gains involving 9q, 10q, 17q, 19p, and 19q, as well as losses involving 9p. Of note, and perhaps not surprising, some of the data indicate that genetic aberrations outside of chromosome 22 predominantly occur in tumors that were previously treated with radiotherapy [53]. Other investigators have looked at CpG island hypermethylation of the *NF2* gene as an alternate mechanism of gene silencing, but the results have been largely negative [56, 57].

Gene Expression Profiling

Several studies have been published on gene expression profiling in schwannomas, showing evidence of deregulation in the proto-oncogene *MET*, as well as *ITGA4*, *PLEXNB3*/*SEMA5, and CAV1* [58, 59]. In addition, upregulation of osteopontin (*SPP1*), a gene involved in the protein degradation of the NF2 gene product Merlin, was observed [58]. Gene regulation at the posttranscriptional level has been examined in a recent study, focusing on miRNA profiling of schwannomas [60]. In that study, 12 miRNAs were found to be significantly deregulated in tumors, including miR-7. Targets of miR-7 include several oncogenes relevant to schwannoma biology, including epidermal growth factor receptor (EGFR), p21-activated kinase (Pak1), and associated cdc42 kinase1 (Ack1).

Prognostic Stratification

Extent of resection is the strongest predictor of recurrence free survival. According to published data, recurrence risk for vestibular schwannomas ranges from 0 to 4 % after gross total resection, 9–29 % after near-total resection and 25–65 % after subtotal resection [61–63].

In NF2, a genotype-phenotype correlation exists and is of prognostic value. Compared to other hereditary disorders, NF2 has an unusually high rate of mosaicism of greater than 30 % amongst *de novo* patients [64], and clear associations between type of mutation and disease severity has been recognized. For example, while 5′ truncating mutations are associated with a high tumor burden, severe disease course and early mortality, missense mutations have been linked to a relatively mild phenotype [65, 66]. For individual tumors, it is presently not known whether the type of NF2 mutation present in the tumor, or any other genetic or molecular characteristics are prognostic or predictive of tumor aggressiveness or risk of recurrence after surgical resection.

Molecular Signaling Pathways

The molecular signaling pathways that drive tumor initiation and progression associated with loss of Merlin have been subject to intense research efforts over the past decades. It has become evident that rather than acting through a single pathway or at a single cellular compartment, Merlin regulates a wide variety of cellular processes, including contact inhibition, tumor suppression and growth through signaling at the cellular cortex and nucleus. Loss of Merlin has been linked to loss of contact inhibition and activation of a number of pro-growth signaling cascades, as recently reviewed by Li et al. [7]. These include the Rac-PAK

[67–70], mTORC [71–73], EGFR/PDGFR/c-kit RAS-RAF-ERK [74–82], PI3K-Akt [83], FAK-Src [84], and Hippo pathways [85–87]. In addition, Merlin has been shown to interact with α-catenin and Par3 at adherens junctions [88], and with the scaffold and signaling protein Angiomotin at tight junctions [89]. Recent studies showed that in addition to cortical functions, Merlin also translocates to the nucleus to alter gene expression through inhibition of the E3 ubiquitin ligase $CRL4^{DCAF1}$ [90, 91]. Several of these pathways have been validated in preclinical studies involving in vivo and/or in vitro schwannoma models.

The tumor microenvironment, including angiogenesis, has been recognized as an important aspect of schwannoma biology. VEGF and its receptors are expressed in schwannomas, and expression levels are associated with increased rates of tumor growth [92, 93]. Anti-VEGF(R)-directed therapy with bevacizumab and vandetanib normalized the vasculature of $NF2^{-/-}$ schwannoma xenografts in nude mice and decreased tumor growth [94]. Recent data suggest that Merlin regulates angiogenesis in schwannomas through Rac1-dependent semaphorin 3F expression [95].

Molecular Targeted Therapies

VEGF

The first "molecular targeted" therapy to show clinical success in treating VS in NF2 patients has been bevacizumab, a monoclonal anti-VEGF antibody. Based on retrospective data, bevacizumab may result in radiologic responses and/or hearing improvement in approximately 50 % of patients, although treatment effect is only maintained with continuous administration [96–99]. Recently completed and ongoing prospective clinical trials with bevacizumab (ClinicalTrials.gov identifiers NCT01207687 and NCT01767792) will provide additional data on the efficacy and safety of this therapy, including in children.

ErbB Receptor Family

Preclinical data implying overexpression and activation of ErbB family receptors in promoting schwannoma growth led to a clinical trial using lapatinib, a small-molecule inhibitor targeting EGFR and ErbB2. In this phase 2 study, 24 % of evaluable patients experienced a radiologic response. Median time to overall progression (i.e., volumetric progression or hearing loss) was 14 months, but only one transient hearing response was observed [100].

mTOR

Based on the observation that loss of Merlin leads to activation of mTORC1 signaling [71, 72], several phase 2 clinical trials with everolimus (RAD001) have been conducted. Results of one of these trials have been published recently, suggesting that everolimus is not clinically effective in treating NF2-related VS [101].

PDGFR and c-kit

Schwann cells express PDGFRα and PDGFRβ [76]. Signaling through these receptors activates the RAS-RAF-MEK-ERK and PI3K-AKT signaling pathways, and is important for Schwann cell proliferation in vivo and in vitro [77–79]. Overexpression of PDGFRβ has been observed in VS [74, 80, 81], and PDGFR inhibitors including AG1296, imatinib, and nilotinib are effective in preventing PDGFR-driven proliferation when tested in VS in vitro models [74, 82]. VS cells express activated c-KIT and are growth-inhibited by imatinib [81] and nilotinib [82]. Based on this preclinical data, a phase 2 clinical trial with nilotinib is ongoing (ClinicalTrials.gov identifier NCT01201538).

Other Targets and Future Outlook

Some of the key tumorigenic signaling pathways associated with loss of Merlin, such as the Hippo signaling pathway and activation of the E3 ubiquitin ligase $CRL4^{DCAF1}$, are not directly targetable with currently approved drugs, but of interest for future therapeutic development. Although it appears that loss of NF2 may be sufficient for tumor formation and progression, it is conceivable that other oncogenic drivers may cooperate with loss of Merlin. To identify such molecular genetic drivers in schwannomas, future studies using next-generation sequencing approaches, such as whole-exome/whole-genome sequencing and RNA-seq, could provide valuable information.

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16 Malignant Peripheral Nerve Sheath Tumors

Brian Weiss, Amy Sheil, and Nancy Ratner

Malignant peripheral nerve sheath tumors (MPNSTs) (previously called neurogenic sarcomas, malignant schwannomas, or neurofibrosarcomas) are soft tissue sarcomas, which arise from a peripheral nerve or show nerve sheath differentiation. MPNSTs are associated with a high risk of local recurrence and predominantly hematogenous metastasis [1, 2]. They account for 10 % of all soft tissue sarcomas, and approximately half of these malignancies arise in patients with neurofibromatosis type 1 (NF1) [3]. MPNSTs occur in about 2–5 % of patients with NF1 compared with an incidence of 0.001 % in the general population [1]. In contrast, in a large population-based longitudinal study the lifetime risk of developing an MPNST in NF1 was 8–13 % [4]. In patients with NF1, the majority of MPNSTs arise in a previously clinically detectable plexiform neurofibroma, but MPNST may also develop as a primary tumor [5, 6].

The most frequent sites of metastasis of MPNSTs are lung, liver, brain, soft tissue, bone, regional lymph nodes, and retroperitoneum [1]. Early diagnosis of MPNSTs is crucial, as only complete surgical resection has been shown to be curative. However, the clinical diagnosis of MPNST in patients with NF1 can be difficult to establish, because clinical indicators of malignancy (mass and pain) may also be features of benign plexiform neurofibromas commonly seen in this patient population. For unresectable or metastatic disease, adjuvant or neoadjuvant radiation therapy and/or chemotherapy have been used, but are generally not curative. Therefore, novel molecular targeted agents are being evaluated in this difficulty to treat patient population.

Histopathology

MPNST are malignant tumors of neuroectodermal origin arising from a peripheral nerve with or without a preexisting benign nerve sheath tumor [7]. The diagnosis of MPNST is often challenging, due to a lack of standardized morphological criteria, specific immunohistochemical marker expression, or characteristic karyotypic aberrations. Sarcomas with involvement of a nerve and lacking features indicating an alternative line of differentiation (such as synovial sarcoma or angiosarcoma), or those sarcomas definitively arising from a preexisting benign nerve sheath tumor, are designated MPNST [8]. Malignant spindled tumors in patients with neurofibromatosis (NF1) are also considered to be MPNST unless proven otherwise. Spindled tumors that are unrelated to a major nerve are more difficult to classify. In order to establish a diagnosis, a combined analysis of histological features, immunohistochemical phenotype, and/or ultrastructural features of Schwann cell (basal lamina) or differentiation (such as intracytoplasmic vesicles) in perineurial-like cells is necessary [7, 8].

As noted above, the main recognizable benign precursor to MPNST is the plexiform neurofibroma common in the setting of NF1 (Figs. 16.1 and 16.2). Figures 16.1 and 16.2 are photomicrographs representative of plexiform neurofibroma [9]. Prior irradiation is also a risk factor for NF1 patients to develop MPNST [9]. Figures 16.3 and 16.4 represent the gross pathology of an MPNST arising from a plexiform neurofibroma of the vagus nerve [10].

Notable histological variation may be observed in MPNSTs. Common histological findings include fascicles of alternating cellularity (Fig. 16.5), whorls, palisading or rosette-like patterns, subendothelial condensation of tumor cells, and geographic necrosis [8, 11]. Occasionally, the tumors resemble primitive or undifferentiated sarcoma (Fig. 16.6). Less commonly, rhabdomyosarcomatous elements (malignant Triton tumor), angiosarcoma, melanin, neuroendocrine, or glandular structures are observed. The cell(s) of origin of these divergent features remain uncertain [7, 11].

MPNST grading is separated pathologically into low and high grade categories; the majority of MPNSTs are high grade [8]. Morphological criteria for a low grade MPNST include hypercellularity and nuclear enlargement (approximately 3× the size of a neurofibroma nucleus) and

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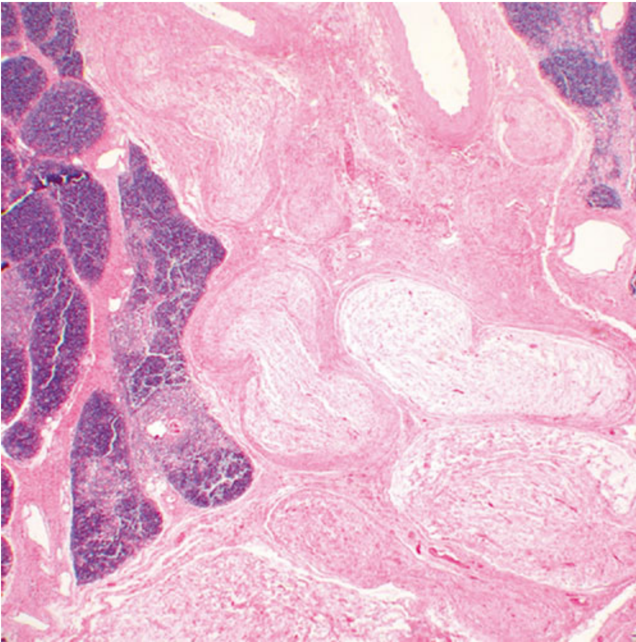

Fig. 16.1 Plexiform neurofibroma arising from the vagus nerve, involving the thymus gland

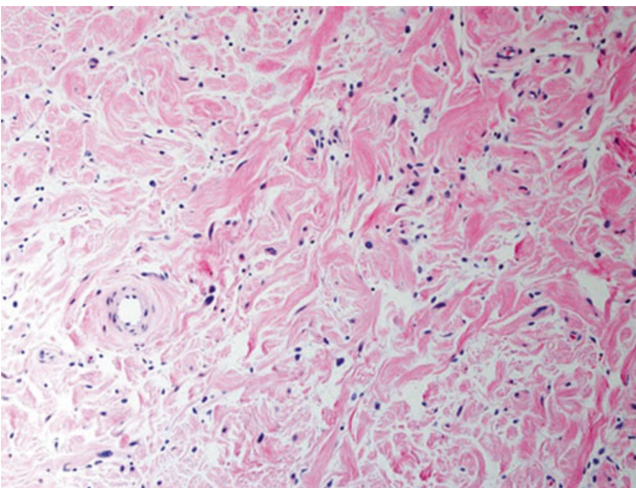

Fig. 16.2 Plexiform neurofibroma with low cellularity and "shredded carrot" collagen

hyperchromasia, features also seen in high grade MPNST; however, low grade MPNSTs exhibit little necrosis and show fewer than five mitoses per 10 high power fields [18, 12]. The isolated presence of one of these features in a neurofibroma is not adequate for a malignant diagnosis. Diagnostic difficulties arise due to the lack of objective criteria for hypercellularity, hyperchromasia, and the extent of changes required for a malignant diagnosis. Features concerning for malignant transformation include increased cellularity and a fascicular pattern of growth not usually seen in conventional neurofibromas (Fig. 16.7). Histological grading systems include the US National Cancer Institute (NCI) system [13], based on the tumor histological type, location, and degree of necrosis, as well as pleomorphism, cellularity, and mitotic activity. The Federation National des Centres de Lutte Contre

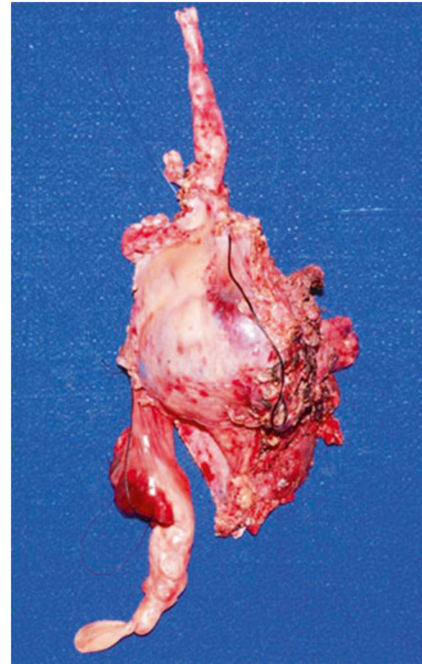

Fig. 16.3 Large mediastinal soft tissue mass (high grade MPNST) arising from vagus nerve (*top*) and encasing and eroding into the superior vena cava, with intravascular thrombosis (*bottom*) (Courtesy of Children's Hospital of Wisconsin, Milwaukee, WI)

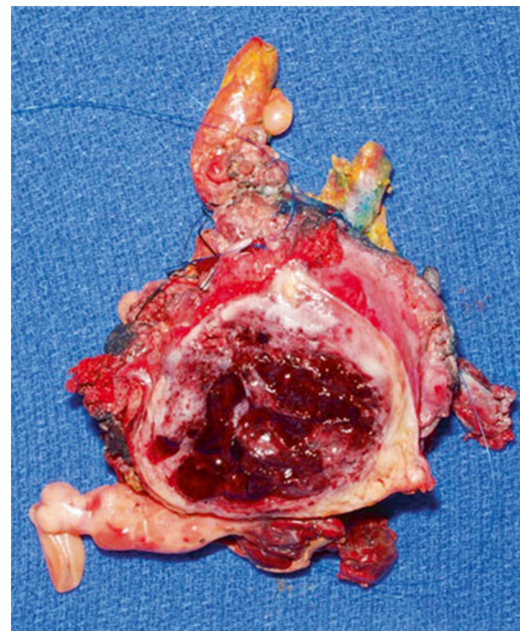

Fig. 16.4 Cut surface of MPNST depicted in Fig. 16.3 with peripheral rim of white-gray tumor and central hemorrhage and necrosis

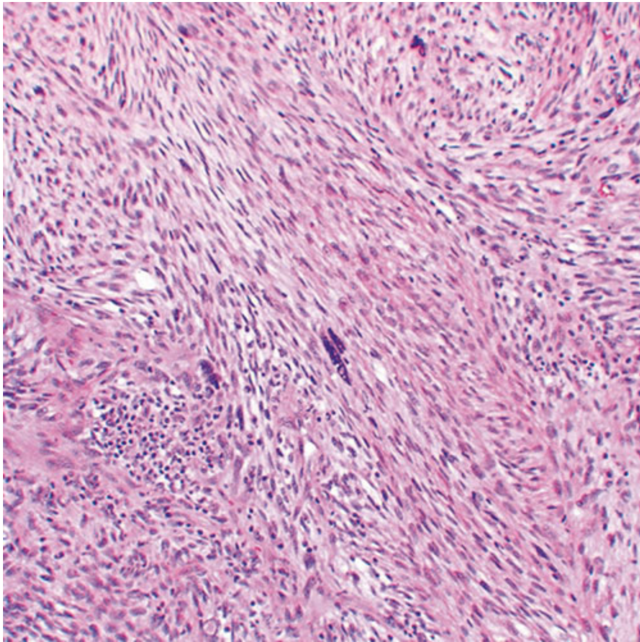

Fig. 16.5 High grade MPNST with spindled cells, fascicular pattern, and focal cytological atypia with nuclear enlargement and hyperchromasia

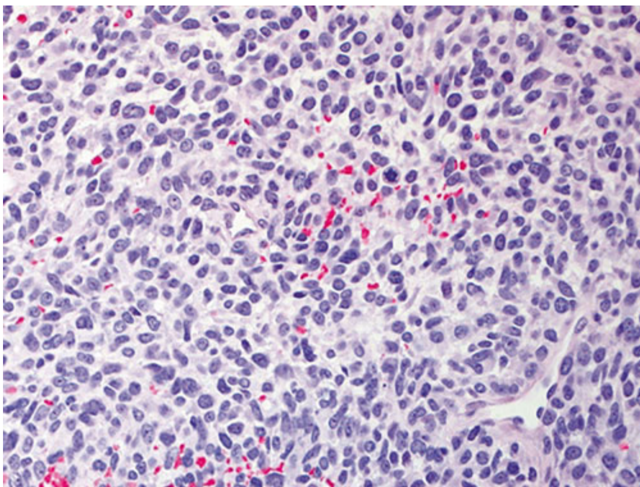

Fig. 16.6 High grade MPNST with appearance of undifferentiated, primitive sarcoma

le Cancer (FNCLCC) is a French grading system which uses a score generated by degree of tumor differentiation, mitotic activity, and extent of necrosis [14]. Neither of these grading systems has proven entirely useful in distinguishing low versus high grade MPNST and predicting clinical behavior. Information regarding the molecular biology of MPNST is anticipated to prove important for not only diagnosis but possible targeted therapy options.

Even more challenging is the separation of atypical neurofibroma (considered benign) from low grade MPNST (malignant), particularly in the setting of NF1. The term "atypical neurofibroma" has been applied to neurofibromas with degenerative nuclear changes [8]. This term, or alternatively, cellular neurofibroma, has also been used for nerve sheath tumors showing worrisome histological features, including high cellularity, few mitotic figures, monotonous cytomorphology, or fascicular growth, which do not fully meet criteria for malignancy. Atypical changes often develop in large, slowly growing neurofibromas [15]. Atypical neurofibromas have generally been regarded as benign. However, a study of NF1 patients suggests that atypical neurofibromas, defined as neurofibromas with increased cellularity and nuclear hyperchromasia and enlargement lacking mitotic figures (Figs. 16.8, 16.9, and 16.10), represent early malignant change in neurofibroma, with *CDKN2A* (p16) deletions (seen in MPNST) in the majority of studied cases [16, 17].

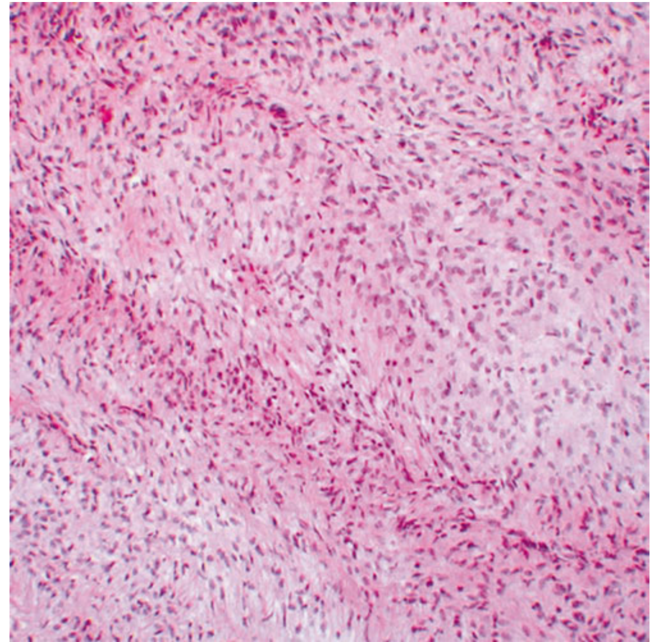

Fig. 16.7 Low grade MPNST with high cellularity and monotonous spindled cells arranged in long fascicles

Histological examination of a soft tissue lesion in which the differential comprises MPNST should include routine-stained H&E sections and possibly reticulin, to clearly outline nerve fibers. In addition, immunohistochemical stains for S100β protein, the skeletal muscle markers desmin and myogenin, and a proliferation marker (MIB-1 or KI-67) may be useful [1]. Increased MIB-1 (Ki-67) and p53 nuclear labeling by immunohistochemistry are seen in high grade MPNST [6, 18]. Genetic loss of the *CDKN2A* locus, and therefore loss of p16 immunoreactivity, are not found in neurofibroma, but are common in MPNST [16, 17]. Both p16 and p27 expression are typically present in neurofibromas and low grade MPNSTs but absent in high grade MPNSTs [6]; loss of expression may highlight foci of malignant

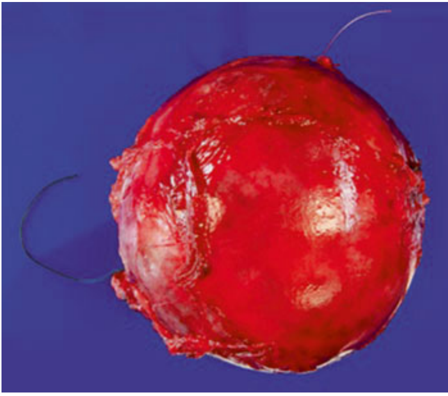

Fig. 16.8 Well-circumscribed, apparently encapsulated atypical neurofibroma

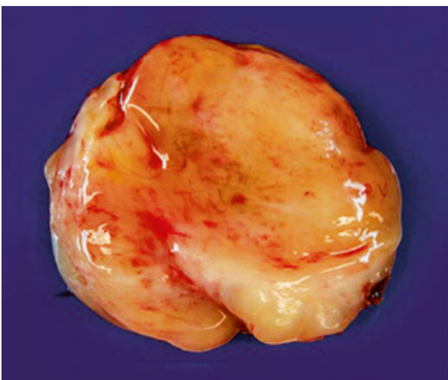

Fig. 16.9 Atypical neurofibroma shown in Fig. 16.8 with slightly gelatinous, yellow to white cut surfaces

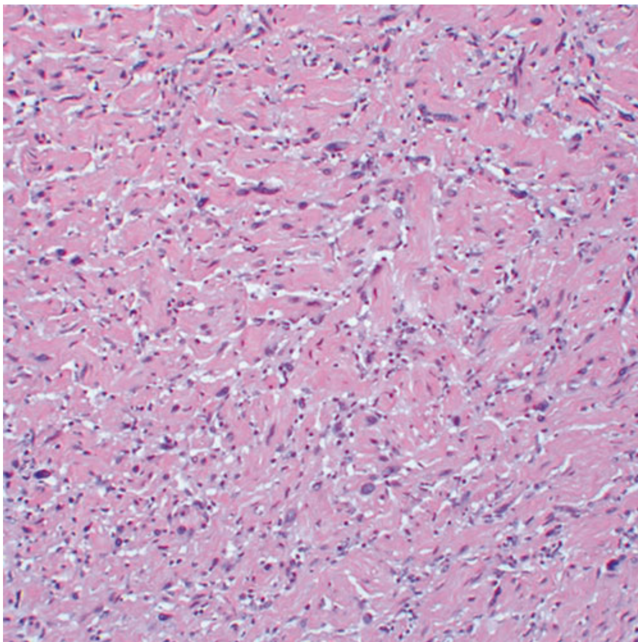

Fig. 16.10 Atypical neurofibroma with wavy collagenous stroma with increased cellularity, nuclear enlargement, and hyperchromasia; no mitotic activity was appreciated

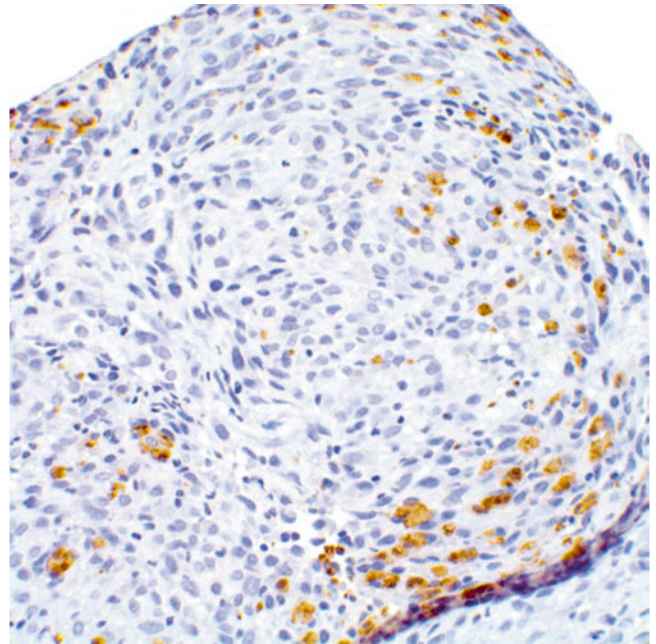

Fig. 16.11 Focal retention of S100β expression in high grade MPNST

transformation in neurofibromas, as may molecular alterations, including *EGFR* amplification [19].

The differential diagnosis of MPNST includes sarcomas, including adult-type fibrosarcoma, synovial sarcoma, rhabdomyosarcoma, leiomyosarcoma, dedifferentiated liposarcoma, and clear cell sarcoma. One useful distinction from benign Schwann cell tumors is the partial or complete loss of S100β expression in MPNST (Fig. 16.11). Conversely, isolated expression of S100β should not necessarily be diagnostic of MPNST, as S100β expression has been reported in leiomyosarcomas, rhabdomyosarcomas, and synovial sarcomas [6, 8, 18].

Synovial sarcoma, a high grade sarcoma of undetermined cell lineage, may occur in soft tissues in either biphasic (which includes a spindle cell component with interspersed glandular structures) or monophasic (spindle cell component only) forms. The monophasic variant may closely resemble MPNST, and may involve nerves or exhibit a plexiform growth pattern. Both synovial sarcoma and MPNST may show glandular differentiation. The only definitive histological feature used in the distinction of MPNST from synovial sarcoma is the presence of pleomorphic cells, not seen in synovial sarcoma (Fig. 16.12). Demonstration of *SS18-SSX1* or *SS18-SSX2* gene fusions, usually resulting from a characteristic X;18 translocation, may be required for definitive diagnosis of intraneural synovial sarcoma, as these gene fusions are limited to synovial sarcoma [8]. No specific chromosomal rearrangements in MPNST have been revealed by conventional cytogenetics, although a complex karyotype is characteristic (see below for details) [20].

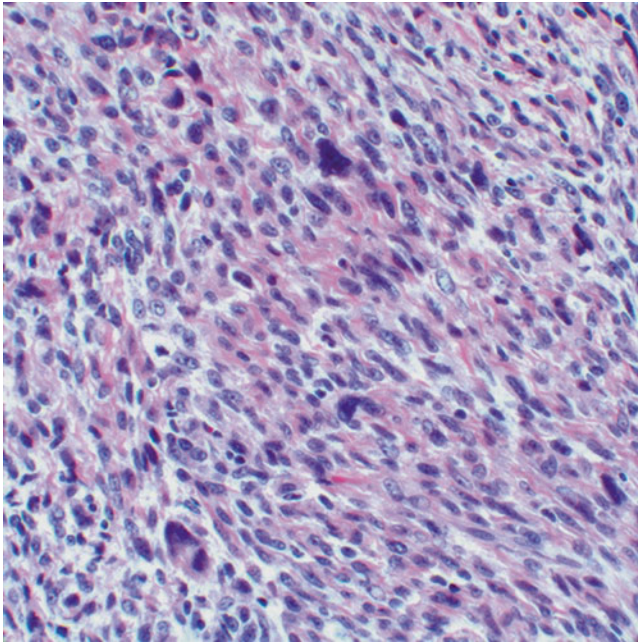

Fig. 16.12 High grade MPNST with eosinophilic stroma, elongated cells with vesicular nuclei, and cytological atypia

Epithelioid MPNST, a rare subtype of MPNST, is characterized by a predominance of large epithelioid cells. Epithelioid MPNSTs are more frequent in superficial sites and exhibit strong and usually diffuse expression of S100β protein [8]. The majority of MPNSTs arising within preexisting schwannomas, which occurs very rarely, are of epithelioid type [10]. The differential diagnosis of epithelioid MPNST includes epithelioid sarcoma, clear cell sarcoma, melanoma, and carcinoma. The absence of expression of melanocytic markers (MelanA, HMB45, MART-1) is useful in the differentiation of epithelioid MPNST from melanoma and clear cell sarcoma [8]. Absent cytokeratin expression distinguishes epithelioid MPNST from epithelioid sarcoma and carcinoma. Both epithelioid MPNST and epithelioid sarcoma may show loss of SMARCB1/INI1/BAF47 protein expression [21], a potential diagnostic pitfall in the consideration of rhabdoid tumor [6, 8, 13–15, 17, 18, 22].

Most MPNST are frankly high grade, aggressive tumors by histology and clinical behavior, and carry a dismal prognosis. Adverse prognosticators include truncal location, size >5 cm, incomplete resection, local recurrence, young age [7, 9], and high grade. According to some authors, histological grade is the most important prognostic factor for soft tissue sarcomas [13, 23] including MPNSTs. Past literature portended a worse prognosis for NF1-associated tumors as compared to sporadic MPNSTs [7, 24]. However, a large recent study indicates that while NF1 patients with MPNSTs demonstrate overall increased mortality compared to those patients with non-NF1-associated MPNSTs, decreased survival did not appear to be related to inherent tumor behavior [24].

Cytogenetics and Molecular Genetics

Primary among MPNST initiating mutations are mutations in the *NF1* gene. NF1 patients carry a constitutional mutation of the *NF1* tumor suppressor gene located on the long arm of chromosome 17 (17q11.2) [25], and mutation, or loss of the second allele, was found in 40 % of NF1 MPNST [26]. MPNST are particularly prevalent in NF1 patients whose constitutional mutations involve whole gene deletion, which can include contiguous genes that may contribute to tumor formation [27]. *NF1* mutations are also present in 41 % of sporadic MPNST [26], explaining why expression signatures and genomic changes overlap in NF1 and sporadic tumors [28–30]. Mutations in *RAS* or RAS pathway genes may also cause MPSNT tumor initiation in MPNST lacking *NF1* mutations; activating mutations in *N-RAS*(1/11), K-*RAS* (1/11) [31], and B-*RAF* (1/13 MPNST) [26] were identified in sporadic MPNST.

As discussed above, atypical neurofibromas represent an early stage in MPNST transformation from neurofibroma. Atypical neurofibromas (15/16) showed homozygous loss of the *CDKN2A* locus on chromosome 9p21.3 [17], and deletions of the *CDKN2A* locus are present in about 50 % of MPNSTs [16, 32]. The *CDKN2A* locus encodes two proteins: p16INK4A, which inhibits the cyclin-dependent kinases 4 (CDK4) and 6 (CDK6), and p14ARF, which inhibits the MDM2 ubiquitin ligase resulting in stabilization of tp53 [16, 32]. Mouse models support the importance of this locus in MPNST, as *Nf1*+/−; *Ink4a*/*Arf*−/− mice develop GEM-PNSTs resembling human MPNST [33].

Another tumor suppressor commonly inactivated in MPNST is TP53. An "inactivated p53-associated proliferation" gene expression signature was identified in 18/20 MPNST, and p53 inactivation caused downregulation of miR-34a, preventing MPNST cell apoptosis in tissue culture [34]. Estimates of MPNST with TP53 alterations (mutations or stabilized TP53) vary between 24 and 75 % [35–37], which is likely due to the variable sensitivity and specificity of different assays for assessing p53 expression and mutations, as well as intra-tumor heterogeneity [38–41]. TP53 stability can also be regulated through $p14^{ARF}$ so that if $p14^{ARF}$ is retained, TP53 is stabilized without TP53 mutation. While biallelic inactivation of the *TP53* locus is rare in MPNSTs [42] in mouse models, only complete loss of *tp53* and *Nf1* correlates with Genetically engineered mouse-Malignant Peripheral Nerve Sheath Tumor (GEM-MPNST) formation [43, 44].

The *PTEN* gene is an "off signal" for phosphoinositide 3-kinase (PI3K) signaling, and PTEN inactivation generally leads to activation of PI3K. Frequent monosomy of the *PTEN* locus was identified in MPNST without *PI3KCA* or *PTEN* mutations [31, 45]; *PTEN* methylation is detected in 45 % of MPNSTs, though not neurofibromas, and associated with early metastasis [46]. Co-deletion of *Nf1* and *Pte*n or expression of *RasG12D* or *EGFR* in combination with *Pten* deletion also resulted in GEM-PNST [47, 48]. In addition,

expression of the retinoblastoma (*RB*) tumor suppressor, a molecule that impedes cell cycle progression, is lost in 25 % of MPNSTs [49, 50].

As in most sarcomas, chromosomal gains, losses, and rearrangements in MPNSTs are numerous and variable [51], and MPNSTs commonly have hypodiploid or near-triploid karyotypes. Combined genomic somatic copy number alteration (CNA) and loss of heterozygosity (LOH) analysis on sets of neurofibromas and MPNSTs verified that recurrent or overlapping copy number variations (CNVs) or CNAs are absent from neurofibromas, while MPNSTs showed 232 CNAs (encompassing >2,900 genes) and more than 500 genes showed consistent LOH [52]. The microRNA miR-10b can target *NF1* messenger RNA [53]; in principal miRs that target NF1 might also contribute to NF1 tumorigenesis.

Amplification of the epidermal growth factor receptor gene *EGFR* is frequent in MPNST [45, 54]. Perrone et al. found that *EGFR* was amplified in all sporadic MPNST and half of NF1 MPNST [31]. EGFR overexpression was correlated with a worse prognosis in one study [55], but not in another [56]. No activating mutations in *EGFR* have been detected in MPNST. Ligands that activate the EGFR including transforming growth factor (TGF)α and heparin binding epidermal growth factor (HBEGF) are expressed in 90 % of MPNST, suggesting the presence of an autocrine loop in MPNST cells [45, 57]. In 15 % of a small series of MPNST, the amplicon including *PDGFRA*, *KIT*, and *VEGFR-2*/*KDR* was present. Among the three genes, *PDGFRA* is most frequently amplified [49, 58, 59] and rarely mutant [59]. Hepatocyte growth factor is expressed and its c-Met receptor is expressed in 82 % of MPNST, and the *MET* gene is amplified in MPSNT [49, 60]. Short hairpin RNAs targeting *MET* and XL184, a multi-kinase inhibitor targeting MET and VEGFR2, decreased MPNST tumor growth and metastasis in tumor xenografts [61]. While in vitro studies in cell lines support roles for these receptors in MPNST, several histology-specific clinical trials with agents targeting PDGFR, C-KIT, and EGFR were completed, all without achieving responses or meaningful disease stabilization as single agents [62–65]. Possibly blocking one or more of these receptors will be useful in combination with other therapeutic agents.

Gene Expression Profiling

Sporadic and NF1 MPNST are indistinguishable by transcriptome analysis [30]. Transcriptome analysis comparing Schwann cells to MPNST found that expression of markers of neural crest cells is a prominent theme in human MPNST cell lines and tumors [29, 66]. Neural crest markers include *SOX9* and *TWIST1*, which are dramatically upregulated in MPNST [29, 66–68]. MPNST cells are dependent on expression of these genes, as downregulation of *SOX9* caused cell death and downregulation of *TWIST1* decreased cell migration [29, 66]. Increased expression of the neural crest markers FOXD3, PAX7, SOX5, and AP-2α in MPNST compared to neurofibroma was described in a series of 34 MPNSTs [68]. The placodal markers *EYA*/*SIX* are also upregulated in MPNST cells and tumors [69], and shRNA to diminish *EYA4* expression prevented tumor formation and caused necrosis. EYAs are phophatases that could in principle be targeted therapeutically.

Whole genome microRNA analysis of MPNST tumors identified downregulation of 14 miRs, and upregulation of two (miR-210 and miR-339-5p) [70]. There was no overlap with serum microRNAs in MPNST patients [71]. Serum miR expression distinguished patients with MPNST from those without MPNST. The authors identified miR-24 as upregulated in NF1 and MPNST, and MiR-214 and miR-801 as upregulated in serum of individuals with sporadic or NF1-related MPNST. The sensitivity (0.820) and specificity (0.844) of a three miR panel to identify NF1 MPNST supports a potential role in helping to diagnose MPNST and/or as a possible indicator of response to therapy [71].

On two-dimensional gel analysis of proteins, MPNST most closely resembled synovial sarcoma and clear cell carcinoma [72]. For this reason, a goal remains to identify the markers that distinguish MPNST from these tumors, and from surrounding neurofibroma. Several markers, each analyzed in relatively few tumors, may distinguish neurofibromas from MPNST. These include Tenascin-C and NNAT [73]; Cathepsin K [74]; and markers of an angiogenic switch: SMA, vWF, VEGF, and VEGF receptors Flt1 and Flk1 [75]. Many growing or atypical neurofibromas and MPNST stained positive for CD10 [76]. Some neurofibromas and MPNST express hTERT [77].

Prognostic Stratification

Complete surgical resection is the only known curative MPNST therapy, and predicts favorable prognosis in all MPNST patients [23, 78, 79]. In addition, survival is significantly better in female versus male MPNST patients [80, 81]. Gain/amplification of the CDK4 gene on chromosome 12q14.1 and upregulation of the FOXM1 gene on chromosome 12p13.3 were significant independent predictors of poor survival in 87 MPNST patients [82]. Chromosomal losses of 10q and Xq and gain of 16p were also associated with reduced MPNST patient survival [28]. In a large series, 93 % of MPNST showed positive staining for phospho-MEK, while about half expressed phospho-S6K, phospho-mTOR and/or phospho-AKT, and immunoreactivity toward all three mTOR pathway markers predicted significantly worse outcomes than in patients with tumors negative for the three markers [83]. Intriguingly, a single nucleotide polymorphisms in the microRNA biogenesis pathway gene *DROSHA* (rs1991401) significantly increased MPNST risk in NF1 patients, while SNPs in *AGO2* and

GEMIN4 in this pathway decreased risk [71]. To date, none of these indicators have been used to stratify patients for clinical trials.

Treatment of MPNSTS

Only complete MPNST surgical resection has been shown to be curative, and remains the cornerstone of therapy, but is rarely feasible due to tumor location or nerve association [23, 78, 79, 84, 85]. Radiotherapy is commonly used for local control in inoperable or incompletely resected MPNSTs, but when used as primary treatment, high doses of radiation are needed (median 50 Gy) [3]. The role of chemotherapy for adult and pediatric soft tissue sarcomas, including MPNSTs, is controversial. Only doxorubicin, dacarbazine, and ifosfamide are agents consistently associated with response rates of 20 % or more in patients with soft tissue sarcomas [23, 86, 87], and the combination of ifosfamide and doxorubicin has produced response rates as high as 46 % in these tumors [87, 88]. The response rate of MPNSTs to chemotherapy is unknown. Some investigators have suggested that they have intermediate chemosensitivity, less responsive than synovial sarcoma, but more responsive than refractory diseases such as alveolar soft part sarcoma [86]. However, recently others have questioned whether MPNSTs are at all chemosensitive [84]. Carli et al. summarized the 25-year experience of pediatric MPNSTs in German and Italian Groups [3]. The patients described encompass a span of three decades and were treated on standard sarcoma protocols. First, response to ifosfamide was significantly better than to cyclophosphamide (65 % vs. 17 %). Second, while chemotherapy increased overall and event-free survival over no chemotherapy, the 5-year overall survival for patients with unresectable and metastatic MPNST remained approximately 30 %. It may be that the addition of targeted agents to chemotherapy will improve response rate, and potentially improve outcome without undue morbidity.

Molecular Signaling Pathways

NF1 is an off signal for Ras GTPases [89]. Therefore, *NF1* loss activates signaling pathways downstream of Ras-GTP, and the Raf-MEK-ERK and mTOR-S6K-Akt pathways have been explored as potential therapeutic targets (Fig. 16.13). Targeting MEK with PD0325901 in a xenograft and in a genetically engineered mouse model transiently delayed MPNST growth, correlating with suppression of tumor vasculature and tumor cell proliferation [90, 91]. Using rapamycin or its analog RAD001 to target the mTOR/S6K pathway also transiently blocked MPNST growth in xenografts and a mouse model [92–94]. This effect of rapamycin was converted to cytotoxicity in combination with agents that promote proteotoxic/endoplasmic reticulum (ER) stress in a genetically engineered mouse model [95]. Based on these data, combinatorial clinical trials are being considered.

We have chosen to omit discussion of a host of studies focusing on effects in MPNST cell lines, pending confirmation of significant effects in in vivo model systems. In xenografts, hyaluronan oligomers suppressed drug transporter activity and inhibited growth of MPNST tumor growth, with synergy between oligomers and doxorubicin [96]. The effect of 4-hydroxytamoxifen on K-Ras degradation and MPNST cell autophagy correlated with decreased MPNST growth [57, 97]. Inhibition of Aurora kinases using MLN2036 caused prolonged MPNST growth arrest in the G2/M phase of the cell cycle [98]. The combination of histone deacetylase inhibitor PC-24791 (which promotes autophagy) and autophagy blockade with chloroquine abrogated MPNST xenograft growth and promoted cell apoptosis, although the durability of the response is not known [99]. Blocking STAT3 with FLLL32 or shSTAT3 prevented growth of MPNST xenografts but did not arrest growth of established tumors [100]. Whether these xenograft studies will translate to effects in immune-competent models or clinical trials remains to be tested.

An exciting recent development is a new link between MPNST and β-catenin signaling. Transposon-based

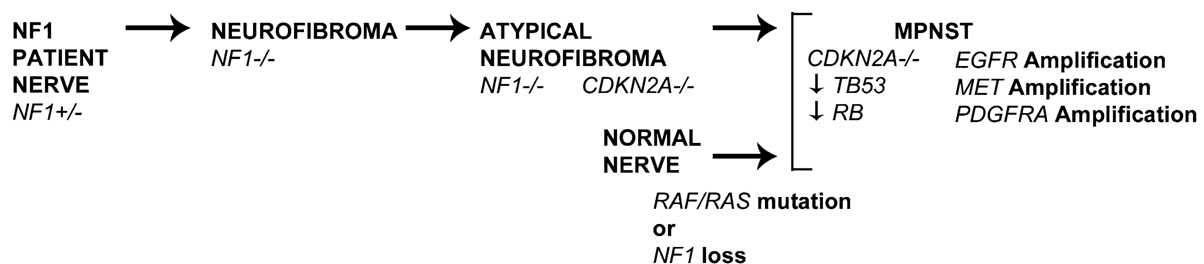

Fig. 16.13 Schematic illustration of some of the multiple genetic changes believed to contribute to NF1-related and sporadic MPNST. In NF1 patients, benign neurofibromas form when *NF1* haploinsufficient cells in the Schwann cell lineage lose remaining functional *NF1*. Subsequent progression toward MPNST is via an atypical neurofibroma intermediate, and is associated with loss of the tumor suppressor gene CDKN2A. MPNST also show mutation of additional tumor suppressor genes and amplification of several growth factor receptors. The bottom row shows that mutations in RAS genes, RAF genes, and *NF1* were recently identified in sporadic MPNST

mutagenesis screens identified many components of the β-catenin signaling pathway as potential driver mutations in MPNST [101, 102]. Strong evidence also supports the role for autocrine CXCL12 and CXCR4 signaling upstream of β-catenin [103]. Both blockade of CXCR4 with AMD3100 (which is already in clinical trials in other cancers) and treatment of MPNST cells with shβ-catenin decreased cell proliferation and MPNST tumor growth [102, 103].

Molecular Targeted Therapies

The outcome for patients with relapsed unresectable MPNST remains poor. Therapy options remain particularly limited in patients with NF-1 in the face of the increased risk of therapy-related monosomy 7 myelodysplastic syndrome and leukemia in NF-1 patients who had previously received alkylator-based chemotherapy or radiotherapy for solid tumors [104]. As elucidated above, several effector pathways have been interrogated in order to find a cure for resistant MPNST. Ohishi et al. analyzed the cytotoxic effects of imatinib mesylate blockade of PDGFRβ using six human MPNST cell lines [105]. They found that imatinib mesylate effectively suppressed cell growth in vitro at concentrations within the therapeutic range in three of the six human MPNST cell lines. In two of these three, imatinib mesylate-sensitive cell lines, imatinib mesylate also significantly suppressed tumor growth in a xenograft model. Others have seen similar results with the second-generation tyrosine kinase inhibitor nilotinib [106].

Another group identified bone morphogenetic protein 2 (BMP2) expression as neurofibromin regulated but independent of NRAS and MEK1/2. BMP2 belongs to the TGF-β superfamily and functions as a morphogen required for the development of lung, heart, and central nervous system. Overexpression of BMP2 promotes malignancy-related attributes such as migration and invasion and is found in NF1-related malignant tumors [107]. Inhibition of BMP2 signaling by the small molecule LDN-193189 or by BMP2 short hairpin RNA (shRNA) decreased the motility and invasion of Nf1-deficient MPNST cells in vitro.

Pigment epithelium-derived factor (PEDF) can induce differentiation and inhibit angiogenesis in several tumors, including MPNST. Demestre et al. determined that PEDF inhibited proliferation and augmented apoptosis in S462 MPNST cells in vitro, and suppressed MPNST tumor burden in a nude mouse model, mainly due to inhibition of angiogenesis [108]. These results demonstrate the inhibitory effects of PEDF on the growth of human MPNST via induction of anti-angiogenesis and apoptosis, and suggest a potential novel approach for future therapy against MPNST.

Chau et al. recently described a novel small chemical compound, Compound 21 (Cpd21) that inhibits tumor cell growth [109]. Cpd21 inhibits growth of all available in vitro models of MPNST and human MPNST cell lines, while remaining nontoxic to normally dividing Schwann cells or mouse embryonic fibroblasts by delaying the cell cycle, thereby leading to cellular apoptosis. While too early to determine if these findings will be replicable and transferable to treating human patients, Cpd21 certainly has potential as a novel chemotherapeutic agent.

Perhaps the most promising is the work done on the Ras/Raf/MEK/ERK signaling pathway in MPNST. Jessen et al. showed that the MEK inhibitor, PD0325901, had a robust, yet transient, in vivo effect on survival in MPNST xenografts, possibly due to effects on tumor vasculature [91]. Others have reported dramatic response in a patient with resistant *BRAF* V600E mutated MPNST to the second-generation B-Raf enzyme inhibitor, Vemurafenib [110]. There are many new BRAF and MEK inhibitors still to be investigated in this tumor type. In addition, due to the multiplicity of Ras effectors and complexity of negative feedback regulation, therapeutic strategies against more aggressive Ras-related tumors are likely to include combinations of compounds that target multiple points in the Ras signaling network [91].

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17 Meningiomas

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Meningiomas are brain tumors originating from meningeal coverings of the brain and spinal cord. Meningiomas are the most common intracranial tumors, with an incidence estimated at approximately 7.7/100,000 [1]. Meningiomas are predominantly tumors of the elderly, with a clear increase of incidence after the age of 65 years [1]. Among children, meningiomas are exceedingly rare (0.4–4.1 % of all pediatric tumors) [2]. However, pediatric meningiomas are an interesting subgroup because a high proportion is associated with germline alterations in the neurofibromatosis type 2 (*NF2*) gene and the diagnosis of a meningioma in a child therefore may represent the first clinical manifestation of NF2 [3]. Another hallmark of meningiomas is the preferential affection of women with a female:male ratio of 3.5:1 [4]. Other risk factors include ionizing radiation [5], presence of diabetes mellitus and arterial hypertension, and possibly smoking [6, 7]. In contrast, the use of mobile phones is not associated with increased meningioma development [8]. Radiation-induced meningiomas tend to present with aggressive histological features and are characterized by a more aggressive clinical course including frequent tumor recurrence [9]. Epidemiological data points to genetic susceptibilities to develop radiation-induced meningioma [10]. In children, radiation-induced meningiomas are often multiple on first presentation, and rare histological subtypes are encountered more frequently [11].

About 90 % of meningiomas can be found in the cranial meninges, while 10 % occur in the spinal meninges. Meningiomas may occur at multiple sites; in about 1 % multiple meningiomas are associated with NF2, while 4 % of cases are unrelated to NF2 [12]. Interestingly, individuals with a first-degree relative suffering from meningioma have a threefold higher risk of developing a tumor, suggesting underlying hereditary conditions [13]. Hereditary meningiomas in adults are again highly associated with *NF2* (see below), and at least 50–75 % of NF2 patients develop meningiomas during their lifetime [14]. Meningioma development in other familial tumor syndromes is uncommon. Few cases have been reported in the setting of Gorlin syndrome [15], Cowden syndrome [16], Li-Fraumeni syndrome [17], and multiple endocrine neoplasia type 1 (MEN1) [18].

Histopathology

Meningiomas are thought to originate from arachnoidal cap cells, which form the outer layer of the arachnoid mater and the arachnoid villi, the latter being responsible for cerebrospinal fluid (CSF) drainage into the dural sinuses and veins. Arachnoidal cap cells can appear normally as a single fibroblast-like cell layer, or as epithelioid nests consisting of several layers. With age, the arachnoidal cap cell clusters become increasingly prominent, forming whorls and psammoma bodies identical to those found in meningiomas. Based on cytological and functional similarities to meningioma cells, arachnoidal cap cells are favored as the most likely cell of origin [19]. Embryonically, the meninges at the skull base are derived from the mesoderm, while telencephalic meninges are neural crest derived [20].

As the neoplastic counterpart of cap cells, meningiomas display both mesenchymal and epithelial-like features. This is reflected by the histopathological appearance of the most frequent meningioma subtypes. Among the group of WHO (*World Health Organization*) grade I meningiomas which comprises about 80 %, meningothelial, fibrous, or mixed (transitional) tumors displaying both epithelial and mesenchymal characteristics are the dominating subtypes (Fig. 17.1a, b; Table 17.1). Interestingly, there is a preponderance of specific intracranial sites affected by meningiomas in association with certain histopathological subtypes. Meningothelial (epithelial) meningiomas are prone to develop at the skull base, while fibroblastic meningiomas are more likely to occur at the convexity of the brain [21, 22]. If the location is related to the grade of malignancy, the proportion of grade II/grade III meningiomas at the convexity or with parasagittal location is much higher than at the skull base, where grade I meningiomas

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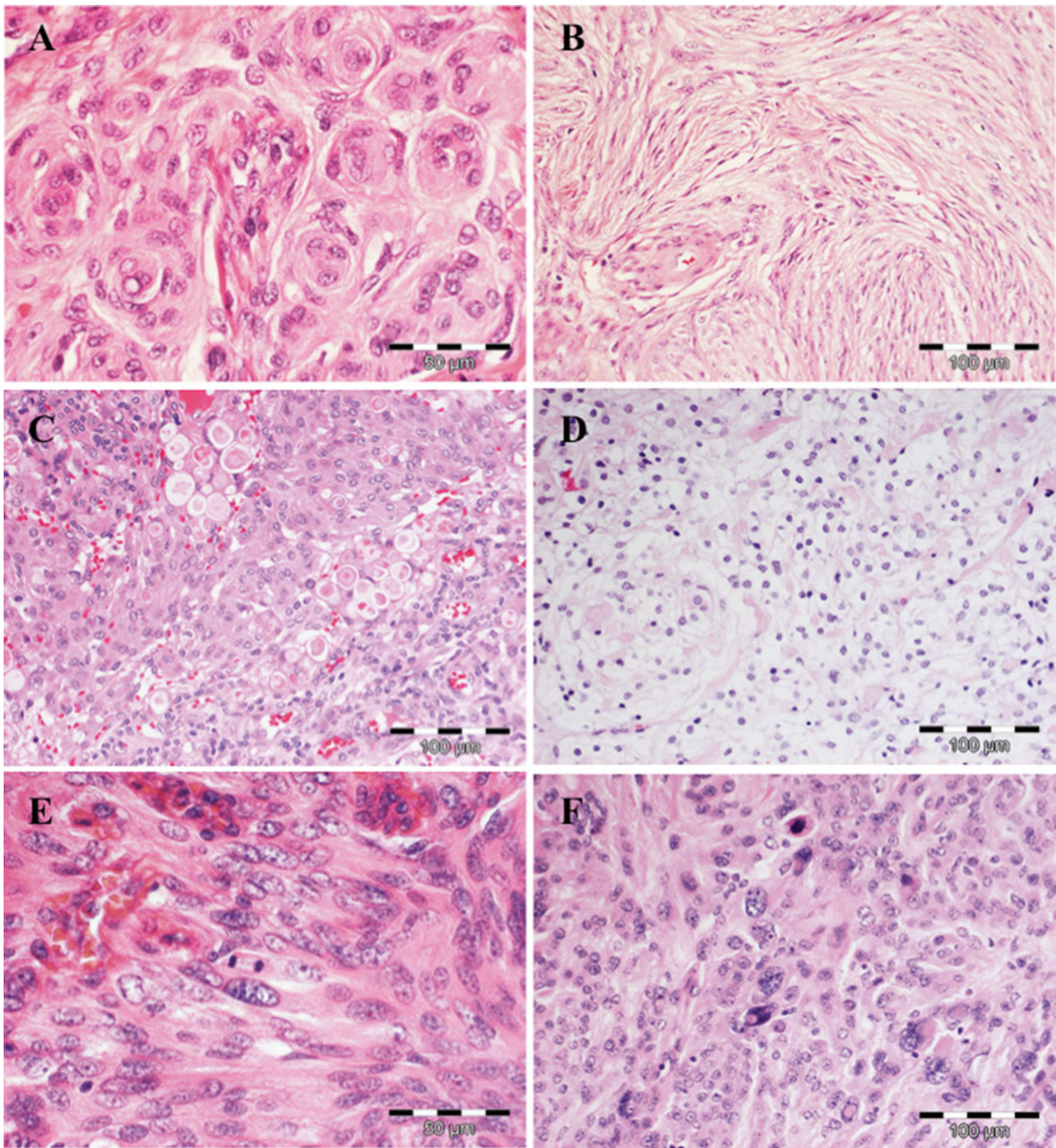

Fig. 17.1 Histopathological features of meningiomas. (**a**) Typical meningothelial meningioma WHO grade I with whorl formation. (**b**) Fibroblastic meningioma WHO grade I with spindle-shaped nuclei and fascicular growth. (**c**) Secretory meningioma WHO grade I with numerous inclusions. (**d**) Clear cell meningioma WHO grade II. (**e**) Atypical meningioma WHO grade II with increased nuclear polymorphisms and marked mitotic activity. (**f**) Anaplastic meningioma WHO grade III characterized by highly pleomorphic tumor cells and lack of typical meningioma features

dominate [23]. Rare meningioma variants designated as WHO grade I comprise psammomatous (calcified), angiomatous, secretory, microcystic, lymphoplasmacyte-rich, and metaplastic forms. Some variants, such as the secretory meningioma (Fig. 17.1c), have been recently related to characteristic molecular alterations (see below).

About 20 % of meningiomas belong to the group of atypical WHO grade II tumors. Atypical meningiomas have been

Table 17.1. Histopathological subtypes and grading of meningiomas in relation to genetic alterations and preferred sites affected by the tumor.

Histological subtype	WHO Grade	Molecular characteristics	Preferred site#	References
Meningothelial meningioma	I	(*NF2*), *TRAF7*, *AKT1*^{E17K}	Skull base	[43, 59, 60]
Fibroblastic meningioma	I	*NF2*	Convexity	[21]
Transitional (mixed) meningioma	I	*NF2*, *AKT1*^{E17K}	Convexity	[60]
Psammomatous meningioma	I	*NF2*	Spinal	[46, 59]
Angiomatous meningioma	I	?	–	
Microcystic meningioma	I	?	–	
Secretory meningioma	I	*KLF4/TRAF7*	–	[44, 59, 63]
Lymphoplasmacyte-rich meningioma	I	?	–	
Metaplastic meningioma	I	?	–	
Chordoid meningioma	II	?	–	
Clear cell meningioma	II	*SMARCE1*	Spinal	[74]
Atypical meningioma	II	*NF2*, *TRAF7*, *AKT1*^{E17K}	–	[59, 60]
Brain-invasive meningioma	II	?	–	
Papillary meningioma	III	?	–	
Rhabdoid meningioma	III	?	–	
Anaplastic meningioma	III	*NF2*	–	[43]

increasingly recognized in the last few years, mainly due to a shift in histopathological diagnosis from grade I to grade II meningiomas [24]. Atypical meningiomas are characterized by histopathological features indicating aggressiveness, including increased mitotic activity, nuclear atypia, and overall malignant biology (Fig. 17.1e, Table 17.1). Indeed, patients suffering from grade II meningiomas have a roughly eightfold increased risk of recurrence compared to benign WHO grade I tumors, and a slightly, but statistically significantly increased risk of mortality compared with age- and sex-matched controls. Within the group of atypical meningiomas, special attention is necessary for meningiomas characterized by brain invasion. It is now widely accepted that these patients are prone to increased risk of tumor recurrence, but the molecular mechanisms driving brain invasion are not well understood so far. Malignant meningiomas WHO grade III are rare, accounting for only 1–2 % of all meningiomas, but are associated with considerable risk of death from disease, with the average survival being less than 2 years [25–27]. While in atypical meningiomas the characteristic histopathological features of meningiomas (i.e., whorls, psammoma bodies) are at least focally present, malignant WHO grade III meningiomas sometimes completely lack any morphological hint of a meningeal origin and do require extensive immunohistochemical investigations to confirm the origin (Fig. 17.1f).

Cytogenetics and Molecular Genetics

Grade I Meningiomas

In 1967, Zang and Singer described loss of chromosome 22 in meningiomas [28]. This was the first report of genetic alterations in meningiomas, and chromosome 22 alterations are still by far among the most frequent findings in these tumors. Subsequently, a gene on chromosome 22 responsible for the hereditary tumor syndrome neurofibromatosis type 2 (NF2) was identified [29, 30]. Although bilateral vestibular schwannomas are the hallmark of the disorder, the majority of NF2 patients develop multiple meningiomas, implying a role for the *NF2* gene in meningioma development [31]. Indeed, several groups reported allelic losses of chromosome 22 including the *NF2* region in more than 50 % of sporadic meningiomas [32–35]. In meningiomas with allelic losses (LOH, *loss of heterozygosity*) at the *NF2* locus, point mutations in the remaining allele were found in a significant fraction of sporadic meningiomas, suggesting a complete inactivation of the gene [36, 37]. *NF2* mutations commonly result in a truncated, nonfunctional protein product. Aberrant promoter methylation in a fraction of tumors may represent an alternative mode of *NF2* inactivation in meningiomas [38, 39], or increased calpain-mediated proteolysis of merlin (also named schwannomin), the protein product of the *NF2* gene [40]. If the *NF2* gene is intact, promoter methylation is absent [41]. Merlin has significant sequence homology to members of the Ezrin/Radixin/Moesin (ERM) family of proteins, which link various cell adhesion receptors to the cortical actin cytoskeleton [42]. In keeping with *NF2* inactivation, protein expression of merlin is commonly reduced in meningiomas [37]. The frequency of *NF2* inactivation is roughly equal among different WHO grades, suggesting that it represents an important initiation rather than progression-associated alteration [26, 43–45]. Interestingly, differences in the frequency of *NF2* alterations have been noted based on variant histology, with higher rates in fibroblastic, transitional, and psammomatous than in meningothelial or secretory grade I meningiomas [39, 43, 46, 47]. Thus, *NF2* alterations appear to play a preferential role in the mesenchymal-like pathology. Accordingly, patients with non-NF2 familial multiple meningiomas are more likely to develop meningothelial tumors [48]. An association between

the *NF2* gene and location has also been reported such that tumors of the convexity are more likely to harbor *NF2* alterations than anterior cranial-based tumors [21]. Excluding NF2 patients, about 4–10 % of meningioma patients experience multiple tumors [49]. Recurrent meningiomas often appear to have spread discontinuously along the dura. This has raised the question about the clonal origin of multiple meningiomas. Using clonality markers, it was demonstrated that *NF2*-mutated tumors within a patient are of clonal origin [48, 50, 51]. Somatic mosaicism is another issue in NF2 patients presenting with multiple meningiomas. Somatic mosaicism is caused by postzygotic mutations in the early stage of embryo development and results in only a subpopulation of normal cells carrying the constitutional mutation [52]. About a third of NF2 patients is affected by somatic mosaicism, which is associated with a milder phenotype [53]. Approximately 8 % of multiple meningiomas may be caused by mosaic *NF2* [54]. In contrast, patients with neurofibromatosis type 1 (NF1) only rarely present with meningiomas [55], and *NF1* gene mutations are absent in anaplastic meningiomas, suggesting that *NF1* alterations are not involved in meningioma development and/or progression [45].

Based on the clearly established role of *NF2* in meningiomas, it could be demonstrated that *Nf2* inactivation in leptomeningeal cells of conditional *Nf2* knockout mice (*Nf2*$^{flox/flox}$) by Cre-recombinase injection is sufficient to induce meningiomas [56]. Transorbital Cre-recombinase injection led to meningioma development in 29 % of mice, while subdural injection was efficient in 19 % of the animals. More interestingly, most of these tumors recapitulated the meningothelial, fibroblastic, or transitional subtype of human meningiomas, and tumors were characterized by reduced merlin expression. The knowledge concerning the mechanisms driving the development of the main histopathological subtypes among grade I meningiomas could be recently expanded by generating a mouse model with inactivation of meningeal *NF2* by using the prostaglandin D2 synthase (*PGDS*) gene promoter. PGDS is a specific marker of arachnoidal cells [57]. It was demonstrated that *Nf2* inactivation in PDGS-positive meningeal progenitor cells was capable to give rise to both meningothelial and fibroblastic meningiomas in 38 % of mice [58]. Moreover, it could be demonstrated that only during a critical pre- and perinatal time frame, *NF2* inactivation in mice led to the development of meningiomas. Surprisingly, additional knockout of *p16Ink4A* or *Tp53* did not result in an increase of meningioma frequency or aggressiveness in mice, but predisposed for the development of osteosarcomas and malignant peripheral nerve sheath tumors in these animals.

Besides the inactivation of *NF2*, few other recurrent genetic alterations have been identified in benign meningiomas, and these findings are largely based on recent whole genome-sequencing approaches. Three papers published in 2013 reported that four genes are affected in a small fraction of meningiomas: *TRAF7*, *KLF4*, *AKT1*, and *SMO* [44, 59, 60]. Interestingly, all reports emphasized the relation of these alterations to both tumor localization and *NF2* status. Moreover, the high frequency of *NF2* alterations was confirmed with inactivation in 43 % of tumors [44]. Probably the most important new mutation identified is related to the *AKT1* (*v-akt murine thymoma viral oncogene homolog 1*) gene. All three reports described a hotspot mutation (p.Glu17Lsy), also named *AKT1*^{E17K} mutation. This somatic mutation occurs in breast, ovarian, and colorectal cancers [61, 62]. The mutation activates *AKT1* due to pathological location to the plasma membrane, with subsequent growth factor-independent activation of the PI3K/Akt signaling pathway [61]. In meningioma, this mutation was found nearly exclusively in 7–12 % of WHO grade I meningiomas but was (exceptionally) rare in grade II meningiomas and absent in grade III meningiomas, respectively [59, 60]. Moreover, the *AKT1*^{E17K} mutation was predominantly found in the meningothelial or transitional subtype of grade I tumors, and meningiomas harboring the *AKT1*^{E17K} mutation were of *NF2* wildtype. The exact biological role, however, of the *AKT1*^{E17K} mutation for both tumor initiation and potential target for treatment using Akt inhibitors, remain to be determined.

The *AKT1*^{E17K} mutation was also found in about 65 % of meningiomas that harbor a mutation in the *TRAF7* (*TNF receptor-associated factor 7*) gene [59]. The *TRAF7* gene is located on chromosome 16p13. It encodes a proapoptotic protein which interacts with multiple signaling pathways. *TRAF7* mutations are mutually exclusive of *NF2* mutations and occur in about 24 % of meningiomas [59]. *TRAF7* mutations are present in 93–100 % of secretory meningiomas but also in meningothelial and atypical meningiomas [63]. In addition, meningiomas with *TRAF7* mutations are almost always characterized by the mutation K409Q in the gene for the transcription factor *KLF4* (*Kruppel-like factor 4*). *KLF4* gene, located on chromosome 9q, is involved in both transcriptional activation and repression, and both oncogenic activation and tumor suppression have been reported [64]. The combined *TRAF7/KLF4* mutation is highly characteristic for secretory meningiomas (Fig. 17.1c) [59, 63], providing a molecular marker for this grade I subtype that is characterized clinically by extensive peritumoral edema formation [65]. In contrast to *TRAF7*, *KLF4* mutations are absent in other meningioma subtypes [63]. KLF4 is known to co-regulate the bradykinin B2 receptor. Activation of bradykinin B2 mediates the formation of brain edema and may be targeted by specific antagonists [66]. Meningiomas with *TRAF7/KLF4* mutations are predominantly located at the medial/lateral skull base. Interestingly, based on retained merlin staining, an *NF2*-independent molecular background of secretory meningiomas was already suggested earlier [67].

Another non-*NF2*-associated mutation of meningiomas located at the skull base affects the *SMO* (Smoothened) gene

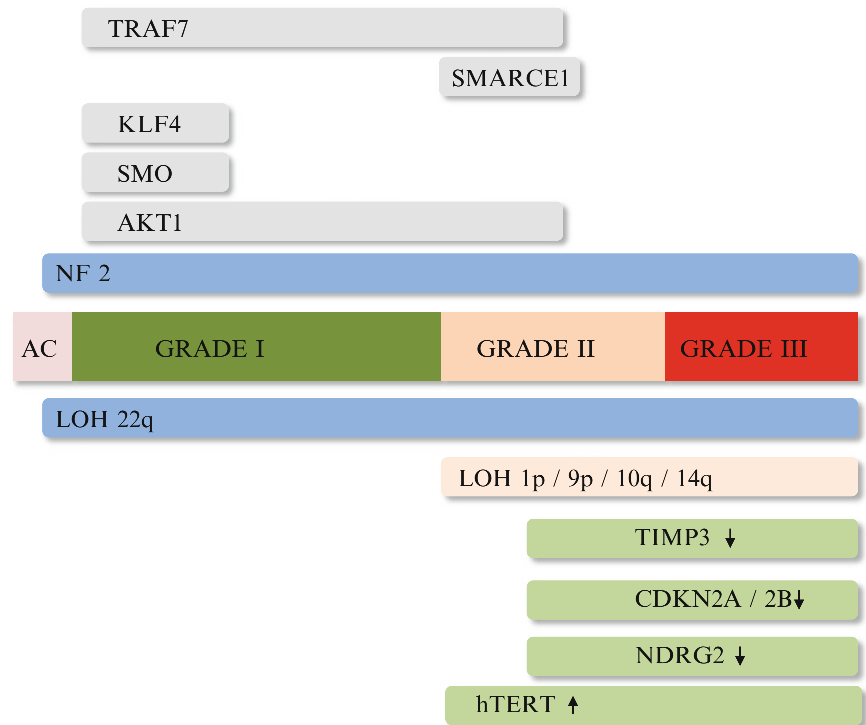

FIG. 17.2 Genetic alterations associated with meningioma development and progression. Bar lengths represent relative frequency of alterations in the given WHO group. See text for abbreviations. *AC* arachnoidal cap cell

which is a member of the hedgehog signaling pathway. *SMO* mutations occur in 4–5 % of grade I meningiomas and are restricted to the medial anterior skull base near the midline. Interestingly, *SMO* mutations are not only exclusive of *NF2* but also of *AKT1* and *TRAF7*/*KLF4* mutations, respectively [44, 59]. Although *SMO* affects only a minority of tumors, alterations of the hedgehog signaling pathway had been already reported in meningiomas. A family characterized by increased risk for meningiomas including multiple meningiomas with absent *NF2* mutations was described, in which meningiomas at different sites were associated with a mutation in the *SUFU* (*suppressor of fused homolog* [*Drosophila*]) tumor suppressor gene with dysregulated hedgehog signaling [68]. *SUFU* mutations have been found in meningiomas from patients with Gorlin syndrome [69].

While all of these mutations are mutually exclusive of *NF2* alterations, other relevant genetic alterations have been found in association with chromosome 22 in meningiomas. One interesting gene is *SMARCB1* (also named *INI1*/*hSNF5*/*BAF47*). *SMARCB1* is located on chromosome 22, and alterations are frequently found in pediatric malignant rhabdoid tumors. Screening a large group of sporadic meningiomas, including a fraction with LOH at chromosome 22, revealed that *SMARCB1* mutations occur with low frequency and might be cooperating with *NF2* mutations, because tumors harboring both *SMACRB1* and *NF2* mutations were identified [70, 71]. Meningiomas with SMARCB1 mutation are preferentially localized to the falx cerebri [72]. However, familial multiple meningiomas in non-NF2 patients are not associated with germline *SMARCB1* mutations [73].

In families with multiple spinal meningiomas and without *NF2* mutations, a loss-of-function mutation in the *SMARCE1* gene was recently identified [74]. *SMARCE1* is located on chromosome 17q21 and encodes for a 57-kDa subunit if the SWI/SNF complex which is involved in the regulation of chromatin structure by nucleosome remodeling. Loss of SMARCE1 expression was evident in immunohistochemical staining, suggesting a tumor suppressor mode of action similar to *SMARCB1*. Interestingly, the mutation only affected spinal meningiomas with histological features of clear-cell meningioma (Fig. 17.1d). This genetic alteration may be a hallmark of non-NF2 familial spinal meningiomas.

High-Grade (Malignant) Meningiomas

Meningiomas are generally thought to progress from low-grade to high-grade tumors, although this is not always easy to demonstrate clinically [75]. Histologically, progression from grade I to grade II can be confirmed in 17–38 % and from grade I/II to grade III in 54–70 %, respectively [76, 77]. At the cytogenetic level, a stepwise acquisition of chromosomal gains and losses during meningioma progression has been proposed (Fig. 17.2). As mentioned before, allelic losses at 22q12.2 (*NF2*) are regarded as an early event, and mouse models suggest that a restricted time window exists for loss of *NF2* to have tumorigenic potential [56, 58]. Comparing *NF2*-mutated and *NF2*-wildtype human meningiomas, overall chromosomal alterations are less frequent in *NF2*-wildtype compared to *NF2*-mutated tumors regardless of the histological grading, respectively [45]. This clearly indicates a greater chromosomal instability in meningiomas

with *NF2* inactivation. This chromosome 22q-associated chromosomal instability has been suggested to be related to the tumor suppressor gene CHEK2, which is located near the *NF2* gene, but additional evidence will be needed to support this hypothesis [78].

The merlin protein belongs to the protein 4.1 family, with members linking membrane protein to the cytoskeleton. One gene of the protein 4.1 family has been suspected to be implicated in meningioma biology is *DAL1* with its gene product protein 4.1B. Allelic losses at 18p11.3 have been reported with frequencies between 20 and 70 % [79, 80]. Reduced protein 4.1B expression was found in about 60 % of meningiomas regardless of histological grade, suggesting protein 4.1B loss as another early event in meningioma pathogenesis [79, 81]. This is supported by the observation that nearly all tumors with *DAL-1* LOH have simultaneous *NF2* LOH [80]. Pediatric meningiomas also frequently show genetic losses of *DAL1* [3]. Interestingly, mice lacking *DAL1* do not develop tumors [82], suggesting *DAL1* alterations as early progression-associated rather than initiation steps. However, the mutational frequency of *DAL1* is low in meningiomas, indicating epigenetic inactivation as a more likely mode of gene inactivation [83]. In patients with multiple meningiomas, *DAL1* gene mutations were found in both tumor and paired blood samples, suggesting substantial differences between sporadic single and multiple meningioma patients with respect to *DAL1* [48].

Losses of 1p, 6q, 10q, 14q, 18q, as well as gains of 1q, 9q, 12q, 15q, 17q, and 20q have been proposed as important events in meningioma progression and recurrence [44, 84–88], and especially 1p and 14q loss are associated with meningioma progression [89–91]. The number of aberrations in 1p, 14q, and 22q correlates with meningioma cell proliferation index as determined by MIB1 immunohistochemical labeling, and also correlates with tumor growth and recurrence [92]. In grade I meningiomas, recurrent losses of 1p, 7p, 14p, and 19, as well as gains of chromosome 5 and 20 can be found [44]. Reduced expression of genes located on chromosome 1p, 6q, and 14q is a feature of recurrent meningiomas [93]. Of note, 1p loss is commonly found in tumors located at the convexity, but is rare in skull base or spinal meningiomas [23]. Moreover, 1p loss is associated with meningioma recurrence shorter overall survival [94]. Losses of 6q, 9p, 13 and 14 are exclusively found in highly proliferating meningiomas [95]. Loss of 18q is preferentially detected in women [91]. Comparing de novo atypical meningiomas and transformed atypical or anaplastic meningiomas, both groups share chromosome 14 and 22 alterations, while losses at chromosome 1, 10, and 18 are restricted to the progressive tumors [76]. Radiation-induced aggressive meningiomas show cytogenetic aberrations on chromosome 1p, 6q, and 22 [9].

Some of these chromosomal alterations have been associated with specific genes. Besides the *NF2* gene on chromosome 22, another tumor suppressor important for meningioma progression is the *TIMP3* (tissue inhibitor of metalloproteinase 3) gene located on 22q12. Hypermethylation of the *TIMP3* promoter was found in 17 % of benign, 22 % of atypical, and 67 % of anaplastic meningiomas and was exclusively associated with allelic loss on 22q12 [96] but seems to be not related to overall survival [94]. Comprehensive genomic studies also identified TIMP3 as a gene with significantly reduced expression in grade III compared to grade I meningiomas [97, 98]. TIMP3 protein inhibits matrix metalloproteinases, suggesting that epigenetic inactivation of TIMP3 by promoter hypermethylation might favor aggressive invasive tumor growth. TIPM3 has additional tumor suppressor activity, and in vitro overexpression of TIMP3 reduces tumor growth and induces apoptosis [99]. However, recently *TIMP3* hypermethylation was reported as not associated with tumor recurrence with no significant effect on overall survival [94].

Among other relevant candidate genes, alterations on 9p21 have been found to represent losses of the tumor suppressor genes *CDKN2A* (*p16*$^{\mathrm{INK4a}}$), *p14*$^{\mathrm{ARF}}$, and *CDKN2B* (*p15*$^{\mathrm{INK4b}}$) in meningiomas [45, 100]. In anaplastic grade III meningiomas, deletions of *CDKN2A*/*CDKN2B* are associated with poorer survival [101]. However, the frequency of *CDKN2A*/*2B* promoter methylation appears to be low [100, 102, 103] and unrelated to the histological grade or risk of tumor recurrence [94].

In mouse models, deletion of *Cdkn2a*, together with *Nf2* inactivation, results in increased meningioma frequency, as well as development of grade II or grade III meningiomas, proving that loss of *CDKN2A* and *CDKN2B* is essential to generate aggressive meningiomas [104]. Interestingly, in this study the rate of pure meningothelial proliferation induced by inactivation of *Nf2* (50 %) dropped to 9 % in mice with combined *NF2*/*Cdkn2ab*, suggesting an accelerated tumorigenesis. Analysis of the *PATCH* (*Patched*) gene on chromosome 9q22 as an alternative candidate gene revealed only one mutation among nine meningiomas [105].

Amplification of the *S6 kinase* gene region on chromosome 17q23 is present in malignant meningiomas [106, 107], making the mTOR signaling pathway attractive for meningioma therapy [108]. The 14q32 region has been implicated in meningioma progression due to the maternally expressed gene 3 (*MEG3*) which has antiproliferative activity in meningiomas. *MEG3* encodes a noncoding RNA, and aggressive meningiomas show allelic losses, promoter hypermethylation, and reduced expression of MEG3 compared to normal arachnoidal cells [109, 110]. The important role of chromosome 14q loss was underlined by a study which identified *NDRG2* as a gene commonly inactivated in meningioma progression. *NDRG2* was found to be downregulated in anaplastic meningiomas, as well as in a small subset of lower-grade meningiomas and atypical meningiomas with aggressive clinical behavior. Recurrent meningiomas have also reduced NDRG2 expression levels. The reduced expression of NDRG2 is associated with promoter hypermethylation [111, 112].

LOH at chromosome 1p has been linked to few genes in meningioma. The *CDKN2C* gene was found to be deleted or mutated [100, 113]. Another candidate gene inactivated on 1p is the *TP73* gene, which was found to be aberrantly methylated in meningiomas [38]. However, these alterations are present in only a small fraction of tumors, leaving the relevant genes on chromosome 1p involved in progressive meningioma to be determined.

Hormone receptors, i.e., progesterone receptor (PR) and estrogen receptor (ER) are expressed in about 90 % and 40 % of meningiomas, respectively. Atypical and anaplastic meningiomas are characterized by a reduced incidence of ER or PR positivity, suggesting a progression-associated mechanism of hormone receptor loss [114]. Reduced expression of PR has been demonstrated in associated with increased recurrence rates and unfavorable prognosis [115]. ER-negative but PR-positive meningiomas have increased cytogenetic abnormalities on chromosome 14 and 22 [116], and meningiomas lacking PR have a higher rate of chromosome 22q loss than tumors with retained PR expression [117].

In normal cells, ends of chromosomal DNA strands are equipped with specialized DNA strands called telomeres. Telomeres are shortened during mitosis, thus limiting the life cycle of a cell. Telomerase is a reverse transcriptase using an RNA template encoded by the *hTR* gene to generate telomeric DNA. Therefore, maintenance of telomere length by telomerase activity is a prerequisite for continuous growth of tumor cells. The catalytic subunit of human telomerase is called hTERT. Telomerase activity has been reported to be another important mechanism of relevant for meningioma progression. Telomerase activity is rare in benign meningiomas, but is frequently detected in atypical and anaplastic meningiomas [118, 119]. Moreover, telomerase activity correlates with hTERT expression in meningiomas [120, 121]. Papillary meningiomas (WHO grade III) have higher hTR expression levels than atypical (grade II) or benign grade I meningiomas [122]. Clinically, telomerase activity is associated with shorter progression-free survival time [119]. Recurrent meningiomas have higher immunohistochemical hTERT expression levels compared to nonrecurrent tumors [123].

Recently, *hTERT* promoter mutations was found at high incidence exclusively in patients with meningiomas undergoing malignant histological progression (28 %), associated with a marked increase in TERT expression. *TERT* promoter mutations were found in both the lowest and the highest grade tumors [124].

DNA methylation and inactivation of gene promoters, resulting in reduced gene expression and function, represent additional mechanisms relevant to tumor growth, including meningiomas. The *O^6-methylguanine-DNA methyltransferase* (*MGMT*) promoter, which is frequently hypermethylated in malignant gliomas and associated with sensitivity to alkylating agents such as temozolomide [125], is rarely hypermethylated in meningiomas [126, 127]. In line with this observation, temozolomide has not shown any clinical activity in patients with meningiomas [128].

Recently, microRNAs (miRNAs) have been intensively studied in various tumor entities including meningiomas. Downregulation of miRNA-29c-3p, miRNA-219-5p, and miRNA-145 were found to be downregulated in aggressive meningiomas [129, 130]. Moreover, miRNA-145 expression could be clearly associated with meningioma cell invasion [130]. The miRNA-335 was attributed to meningioma cell proliferation targeting the Rb1 signaling pathway [131]. The miRNA-200a, which is downregulated in meningiomas, directly interacts with E-cadherin and the beta-catenin signaling pathway [132]. Regarding prognosis, high expression of miR-190a and low expression of miR-29c-3p and miR-219-5p was shown to be associated with increased recurrence rates in meningioma patients [129].

Gene Expression Profiling

The study of gene expression in tumors in general, and meningiomas in particular can be driven by various questions. Besides the detection of genes under- or overexpressed in meningiomas compared to non-tumoral meningeal tissue, unraveling of gene expression changes with increasing grade of malignancy is a key question to be addressed. Additional questions relate to differences between primary and recurrent meningiomas, including progressive meningiomas, or to differences between meningioma locations (spinal versus intracranial tumors). Finally, differences in gene expression depending on the primary genetic driver, predominantly *NF2*, are of high interest in meningiomas.

Starting from the earliest studies in 2002, a number of genes have been described to be associated with higher grades of malignancy in meningioma. In general, genes related to the insulin signaling pathway (IGF2, IGFBP-3, IGFBP-7, AKT3), the MAPK pathway, cell adhesion pathways, extracellular matrix remodeling-associated genes, Notch signaling, and the wingless (wnt) pathway were identified as overexpressed in high-grade meningiomas [98, 133–138]. In contrast, malignant meningiomas have been shown to have reduced expression of TGF-beta signaling components, TIMP3 and KCNMA1 [95, 97]. Moreover, the loss of NDRG2 as a feature of anaplastic meningiomas was identified by an Affymetrix U133A/B GenChip microarray [111]. Regardless of WHO grading, meningiomas can be separated into a "low-proliferative" and "high-proliferative" group based on the combination of gene expression profiling and array comparative genomic hybridization (aCGH). This is especially interesting because atypical meningiomas WHO grade II can fall in one or another of these group, while all grade I meningiomas are low proliferative and all grade III meningiomas are high proliferative [95]. Gene expression profile also differs between infiltrative and non-infiltrative meningiomas [139]. Combining data from gene expression profiling, copy number

alterations, and clinicopathological information, five meningioma subgroups can be defined: while group 1 is characterized by benign histology and the absence of chromosomal losses, group 5 contains mainly grade II and grade III meningiomas and displays a high number of chromosomal losses. Interestingly, the designated group 3 contained meningiomas with all grades of malignancy but clustered especially with recurrent meningiomas. Group 2 and 4 consisted mainly of grade I or grade II/III meningiomas with variable degree of chromosomal losses, respectively. This study also showed for the first time that gene expression between meningiomas is highly variable in general [91].

One initial study reported different gene expression profiles between histological subtypes among grade I meningiomas. Additionally, an increased prevalence of *NF2* alterations in transitional/fibroblastic meningiomas was confirmed [47]. Compared to meningothelial meningiomas, fibroblastic meningiomas have a unique gene expression signature with differences in the genes BMPR1B, RAMP1, DMD, as well as genes involved in extracellular matrix remodeling like MMP-2 and Tenascin-C [98, 140]. Moreover, the expression profiles from infiltrative and non-infiltrative meningiomas appear to be different [139]. Moreover, there is an upregulation of genes related to the PI3K/AKT and TGF-beta signaling pathways in fibroblastic meningioma [138]. Different expression profiles between spinal and intracranial meningiomas have been reported, showing overexpression of transcription factors involved in cell proliferation and differentiation in spinal meningiomas [141].

Interestingly, the cytogenetically well-characterized prognostic groups, i.e., loss of chromosome 22 or deletion of 1p and 14q, can be matched with specific tumor-related gene expression profiles, and the expression profiles are more closely related to patient outcome than purely histology [142, 143]. In recurrent meningiomas, loss of chromosome 1p, 6q, and 14q is related to downregulation of genes involved in several pathways such as Notch, TGF-beta, WNT, PDGF, and PPAR signaling, as well as in cell cycle control and oxidative phosphorylation [93, 144]. Increased expression of Topoisomerase-2alpha in grade II meningiomas identified by gene expression analyses was found to be associated with reduced overall survival compared to patients with low Topoisomerase-2alpha expression levels [97]. Relapsing grade I meningiomas have reduced leptin receptor (LEPR) and cyclin-dependent kinases regulatory subunit 2 gene (CKS2) expression, and the C/L-index was proposed to define meningioma patients at risk for tumor relapse [145]. Altered expression of genes regulating tumor metabolism was identified as another risk factor influencing recurrence of histologically benign meningiomas [146]. Radiation-induced meningiomas, in contrast, do not appear to have a gene expression profile that can be distinguished from spontaneous meningiomas [147].

Prognostic Stratification

One of the strongest factors influencing tumor recurrence and overall prognosis is the histological tumor grading according to the WHO criteria [27]. High MIB-1 labeling index is another marker of poor prognosis [148]. Losses of 1p and 14q represent important steps for meningioma progression and, therefore, have poor prognostic implication [89–91, 94, 149–151]. Patients with tumor size over 50 mm and combined loss of 1p and 14q have been shown to represent a subgroup at high risk for early relapse [152]. Furthermore, relapse-free survival is negatively associated with male gender, presence of brain edema, intraventricular and anterior cranial base tumor location, age below 55 years, and tumor size larger than 50 mm [153]. Loss of progesterone receptor expression also indicates an unfavorable prognosis [115].

Molecular Signaling Pathways

Molecular signaling pathways, including those involved in mitogenic signal transduction, have been studied intensively in meningiomas. Nearly all of the growth factor receptors/kinases known to be involved in tumor growth have been described to be expressed in meningiomas, including epidermal growth factor receptor (EGFR), platelet-derived growth factor beta receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and insulin-like growth factor receptor (IGFR) [154–157]. Activation of these receptors by their cognate ligands drives intracellular signaling cascades involved in a plethora of cellular functions. Mitogenic signals of EGFR and PDGFR are usually transduced by activation of the Ras-Raf-Mek-MAPK pathway. It was demonstrated that this signaling pathway is indeed activated in meningiomas [158, 159]. The PI3K-AKT/protein kinase B—p70 signaling pathway is another important mediator of growth-favoring signals in meningiomas [108, 159, 160]. The mTOR signaling pathway is of relevance for *NF2* mutant meningiomas, as well as meningiomas with other mechanisms of mTOR pathway activation, such as *S6K* gene amplification [106, 107]. Merlin (*NF2*) is a negative regulator of the mTORC1 kinase complex, and constitutive activation of mTORC1 signaling is present in meningioma cells from NF2 patients [161, 162]. Other signaling pathways shown to be activated in meningiomas include the phospholipase A2-arachidonic acid-cyclooxygenase pathway [163, 164] and the PLC-gamma1-PKC pathway [159, 165]. The TGF-beta–SMAD signaling pathway represents an inhibitory mechanism, and TGF-beta, as well as the TGF-beta receptor, are expressed in meningiomas [166–168].

Molecular Targeted Therapies

Due to the lack of efficacy of conventional chemotherapy in meningiomas, targeting signaling pathways by novel inhibitors offers therapeutic opportunities. These approaches are based on the characterization of signaling pathways and their growth factor receptors in meningiomas and meningioma cells. Indeed, some clinical trials have been performed already, however, with limited success thus far. The PDGF alpha/beta inhibitor imatinib mesylate was tested in a phase II study and was well tolerated, but had no significant activity [169]. Combining imatinib with hydroxyurea, a substance with a long history in meningioma chemotherapy, in recurrent or progressive meningiomas showed only very modest activity [170]. Gefitinib and erlotinib, both inhibitors of EGFR, were evaluated in recurrent meningiomas but failed to have significant anti-meningioma activity [171]. Inhibition of angiogenesis by targeting VEGF has been studied in a few retrospective series with encouraging results, but phase II trials have not been performed [172]. The tyrosine kinase inhibitor sunitinib, targeting both the VEGF and PDGF system, was studied in recurrent meningiomas, achieving disease stabilization. The same holds true for the combined VEGF/PDGF inhibitor vatalanib (summarized in [173]). No therapeutic clinical studies have been performed to test mTOR-inhibitors such as temsirolimus or everolimus, although preclinical data supports this approach [108]. A pharmacokinetic/pharmacodynamics "phase 0" study to explore the activity of everolimus in human meningiomas in vivo is ongoing (ClinicalTrials.gov identifier NCT01880749), and may provide valuable insights into mTOR inhibition as a potential clinical strategy. As of today, however, no chemotherapy or molecular targeted therapies have proven to be clinically active in meningiomas, leaving surgery and radiation therapy as the only standard treatment options [174].

The recently identified, novel mutations and activated signaling pathways in subsets of meningiomas provide opportunities for future development of targeted therapies, but the appropriate selection of target populations will require access to routine molecular genetic testing. It is also hoped that the ongoing efforts of developing therapies inhibiting oncogenic signaling pathways that are activated by the loss of Merlin (*NF2*) will be of benefit not only to NF2 patients, but also to the large subset of patients with sporadic meningiomas driven by *NF2* loss [175, 176].

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