Neuro-Oncology 14(12):1424–1431, 2012.
doi:10.1093/neuonc/nos257
Advance Access publication October 22, 2012

The molecular biology of WHO grade I astrocytomas

Nicholas F. Marko and Robert J. Weil

Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, Texas (N.F.M.); Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, Cleveland, Ohio (R.J.W.)

World Health Organization (WHO) grade I astrocytomas include pilocytic astrocytoma (PA) and subependymal giant cell astrocytoma (SEGA). As technologies in pharmacologic neo-adjuvant therapy continue to progress and as molecular characteristics are progressively recognized as potential markers of both clinically significant tumor subtypes and response to therapy, interest in the biology of these tumors has surged. An updated review of the current knowledge of the molecular biology of these tumors is needed. We conducted a Medline search to identify published literature discussing the molecular biology of grade I astrocytomas. We then summarized this literature and discuss it in a logical framework through which the complex biology of these tumors can be clearly understood. A comprehensive review of the molecular biology of WHO grade I astrocytomas is presented. The past several years have seen rapid progress in the level of understanding of PA in particular, but the molecular literature regarding both PA and SEGA remains nebulous, ambiguous, and occasionally contradictory. In this review we provide a comprehensive discussion of the current understanding of the chromosomal, genomic, and epigenomic features of both PA and SEGA and provide a logical framework in which these data can be more readily understood.

Keywords: astrocytoma, brain tumor, giant cell, pilocytic, subependymal.

The World Health Organization (WHO) classification system assigns a grade of I to 2 astrocytomas: pilocytic astrocytoma (PA) and subependymal giant cell astrocytoma (SEGA).[1] Although both are assigned the same grade, these tumors represent distinct molecular, histologic, and clinical entities, and their grading reflects only the absence of histologic evidence of malignant morphologic features. Because many cases of both tumors can be potentially cured with complete surgical resection,[1] interest in their molecular characteristics and pathophysiology was historically limited. However, clinical experience demonstrates that unfavorable anatomic locations may preclude complete resection and that incompletely resected lesions are at risk for progression or recurrence. In these circumstances, in particular, identification of molecular markers correlated with the risk for progression or recurrence and discovery of novel molecular targets for adjuvant therapies would be of particular value.

Interest in the biology of WHO grade I gliomas has surged as technologies in pharmacologic neo-adjuvant therapy have progressed and as molecular characteristics have been progressively recognized as potential markers of both clinically significant tumor subtypes and response to therapy. The past several years have seen rapid progress in the level of understanding of PA in particular, but the molecular literature regarding both PA and SEGA remains nebulous, ambiguous, and occasionally contradictory. The goal of this review is to provide a comprehensive discussion of the current understanding of the chromosomal, genomic, and epigenomic features of both PA and SEGA and to provide a logical framework in which these data can be more readily understood.

Pilocytic Astrocytoma

Overview

Brain tumors are the most common solid tumors in children,[2] with a prevalence of 9.5/100 000.[3] PA is the most frequent brain tumor in this age group,[4] comprising 23.5% of pediatric central nervous system (CNS) malignancies.[3,4] These tumors are phenotypically, histologically, and genotypically distinct from other low-grade gliomas.[5] They rarely progress to higher histologic grades, and anatomically favorable lesions can almost always be cured with gross total resection.[1] Despite

Received February 27, 2012; accepted August 23, 2012.

Corresponding Author: Nicholas F. Marko, MD; Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, FC7, Houston, TX 77030 (nmarko@gmail.com); Robert J. Weil, MD, Department of Neurosurgery, Cleveland Clinic, ND-40, 9500 Euclid Avenue, Cleveland, OH 44195.

some controversy,[6] current evidence suggests that PAs are monoclonal in origin.[7]

Chromosomal Abnormalities

Most PAs exhibit a normal karyotype.[8–14] Approximately 32% of PAs have some chromosomal abnormalities,[8,15,16] with gains of chromosomes 5, 7,[8,15] and possibly 8[15] being the most frequent. Other reported chromosomal gains in PAs include those of chromosomes 1, 3, 4, 6, 9–17, 19–22, and X,[1,8,9,12,13,15] while monosomies of chromosomes 7, 8, and 17 have also been reported.[17] Regional chromosomal abnormalities have also been reported in PAs, including gains of 1p, 2p, 4q–9q, and 13q and losses on 1p, 9q, 12q, and 19–22.[1,12,17,18] Subtelomeric duplication has been observed at 3pter, and gains have been demonstrated to involve 7qter, 12qter, 13cen, 19pter, and X/Yqter.[16] Subtelomeric deletion has been reported at 21qter, and losses involving 8pter and 20pter have also been observed.[16] Single chromosome abnormalities are more common in PAs from patients aged <15 years, while multiple abnormalities are more frequently observed in older PA patients.[1]

Genomic Abnormalities

Common glioma-associated genes.—Despite some early data to the contrary,[19] there is little contemporary evidence to suggest that PAs demonstrate consistent expression abnormalities or mutations in genes typically associated with WHO grade II gliomas. Specifically, differential expression or mutation of genes, including *TP53*, *PDGFA*, *PDGFRα*, *EGFR*, *IDH1*, and *IDH2*, has not been identified in molecular investigations of the PA genome.[1,20,21] This finding lends further support to the hypothesis that PAs represent a disease process that is molecularly and clinically distinct from their grade II counterparts.[5]

Neurofibromatosis 1 mutations.—Approximately 30% of PAs arise in patients with neurofibromatosis type 1 (NF1), and 15%–20% of NF1 patients ultimately develop PAs of the optic pathways or other CNS structures.[22–25] PAs associated with NF1 appear to be molecularly distinct from sporadic PAs.[26,27] Most notably, they are characterized by loss of normal expression of the neurofibromin (*NF1*) gene on chromosome 17q11.2, through either deletion or mutation[28–30] (but not through epigenomic methylation[28]). The gene codes for a 13-kB mRNA that is translated into neurofibromin, a 327-kDa protein with a 360–amino acid region homologous to the catalytic domain of mammalian GTPase-activating protein (GAP).[30] This region, designated the NF1-GAP–related domain, is coded on exons 21–27a and is translated into a protein component homologous to other GAPs.[30] The GAPs, including neurofibromin, downregulate the activity of p21-Ras by stimulating its intrinsic GTPase activity. This promotes conversion of p21-Ras to its inactive (guanosine triphosphate–bound) form.[30–32] Loss of normal NF1 expression causes activation of the Ras pathway,[25,31] which is believed to play a role in PA oncogenesis.[1,23,30,31] NF1 abnormalities are unique to NF1-associated PAs and are usually not observed in sporadic versions of this tumor.[24,33–35] Additional abnormalities reported in NF1-associated PAs include $p16^{INK4a}$ deletion and loss of heterozygosity (LOH) of chromosome 10,[36] causing *PTEN* deletion.

BRAF mutations.—Although the *NF1* gene is not involved in sporadic (non-NF-associated) PAs, other Ras pathway abnormalities are believed to have an important pathophysiologic role in these tumors.[24] Recent gene expression analyses and genome-wide copy number analyses of sporadic PAs have identified frequent (53%–88%) focal chromosomal gains (~2 Mb) on chromosome 7q34, in the region of the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*)[35,37–44] gene, which appear to be highly (but not absolutely) specific to sporadic PAs.[45] These mutations are generally caused by a tandem duplication of this region, resulting in in-frame fusions of *BRAF* with the *KIAA1549* gene.[41,43,44,46] The 3 most common fusions are $KIAA1549^{ex16}$—$BRAF^{ex9}$ (13%–77% of PAs), $KIAA1549^{ex15}$—$BRAF^{ex9}$ (28% of PAs), and $KIAA1549^{ex16}$—$BRAF^{ex11}$ (5% of PAs), but other, infrequent fusions ($KIAA1549^{ex18}$—$BRAF^{ex10}$, $KIAA1549^{ex19}$—$BRAF^{ex9}$, and $KIAA1549^{ex18}$—$BRAF^{ex10}$) have also been reported.[25,40,43,44,46] The mechanisms underlying the formation of these specific fusion genes remain to be fully elucidated.[25]

Relatively little is known about *KIAA1549*, except that it is expressed in brain tissue and codes for a putative, multipass transmembrane protein.[46,47] The *KIAA154*—*BRAF* fusion gene products retain the transmembrane regions of *KIAA1549*, so they may be anchored to the cell membrane.[25,46] They also retain the *BRAF* kinase domain but lack the auto-inhibitory N-terminus[40] (which is the target of activated Ras[48]). The various fusion gene products are therefore presumed to function similarly,[46] exhibiting constitutive kinase activity that results in activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway,[44,46] which has been demonstrated in vitro.[43,44]

BRAF activation in PAs has also been identified outside of the context of *KIAA1549* or *FAM131B* fusion.[49] Activating mutations at or around the $BRAF^{V600E}$ "hot spot" site are suspected to be involved in other cancers and have been observed in various CNS malignancies.[49–51] $BRAF^{V600E}$ mutations in PAs have been observed to occur in 2 ways. The first is a T-to-A exchange at c.1799 (c.1799T>A). The second is an insertion of 3 base pairs coding for threonine either between positions 598 and 599 (c.1795_1796insCTA or c.1796_1797insTAC)[42] or between positions 599 and 600.[52] These mutations have been identified in as many as 9% of PAs, and they may be more common in nonpilocytic gliomas.[49] This mechanism represents an alternate pathway for *BRAF* and ERK/MAPK pathway activation in some PAs.

BRAF activation promotes clonogenic growth in neural progenitor cells,[53] and recent preclinical data using gene transfer of the $BRAF^{V600E}$ mutant gene kinase domain into neural progenitor cells of newborn mice suggest that constitutive *BRAF* activation may be sufficient to induce PA development in vivo.[54] Oncogene-induced senescence may subsequently play a role in the low-grade pathobiology of PAs,[53,55] although the details of this putative mechanism remain to be fully determined. A similar putative mechanism has been suggested for the *FAM131B—BRAF* fusion, a separate *BRAF* fusion gene formed through interstitial deletion of a region ~2.5 Mb on chromosome 7q34.[56] This mutation is not as well characterized as those involving *KIAA1549*.

Other Ras/ERK/MAPK pathway gene mutations.—While *BRAF* fusion genes are the most common genomic abnormalities affecting the Ras/ERK/MAPK pathway in sporadic PAs, other mutations causing activation of this pathway have also been described. An *SRGAP3—RAF1* fusion gene has been described, in which the auto-inhibitory domain of RAF1 is replaced in-frame by the beginning of the *SRGAP3* gene.[25,44] At least 2 versions of this fusion gene, $SRGAP3^{ex11}$—$RAF1^{ex8}$ and $SRGAP3^{ex12}$—$RAF1^{ex10}$, have been described.[25] *SRGAP3* (SLIT-ROBO Rho GTPase-activating protein 3) is more thoroughly characterized than *KIAA1549* and is known to be involved in neuronal migration, axonal branching, and neural development.[57,58] Unlike *KIAA1549—BRAF, SRGAP3—RAF1* does not code for a transmembrane domain but does contain a Fes/CIP4 (cell division control 42 protein–interacting protein 4) homology domain.[25] While this may imply a role in cytoskeletal activity,[59] the ultimate significance of this finding in PA remains unknown.[25]

Activating mutations of *KRAS* appear to be rare in PAs, although infrequent examples have been reported. The first mutation to be described was a single G13R mutation of *KRAS*, identified in a series of 21 sporadic PAs.[24] Since that time, a second *KRAS* mutation has been reported, this time a single G12A in a series of 25 PAs.[60] The actual extent of *KRAS* mutations in PAs are yet to be fully characterized, but these isolated reports lend further support for the putative role of activation of the Ras pathway in these tumors.

Finally, mutations in the *PTPN11* gene may be associated with PAs. *PTPN11* codes for a positive regulator of the Ras pathway, and mutations of this gene are associated with Noonan's syndrome.[61,62] At least 3 cases of PA have been reported in this clinical context.[63–65]

Other genomic abnormalities.—Additional genomic abnormalities involving at least 800 genes[66] uniquely associated with PAs have been reported, although with considerably less consistency than those specifically discussed above. Common CNS tumor-associated mutations, including those of *p53*, *p16*, *IDH1*, and *IDH2*, are rarely reported in PAs.[67,68] Conversely, overexpression of a series of immune system–related genes uncommon among other gliomas, including *SOCS3*, *HLA-DRα*, *HLA-DPB1*, and *A2M*, has been reported in PAs.[69] Overexpression of the homeobox-interacting protein kinase–2 (*HIPK2*) gene, which (like *BRAF*) localizes to chromosome 7q34, has been associated with PA,[37,70] as has overexpression among the extracellular matrix–associated genes *MATN2, TIMP1*, and *TIMP4*.[71,72] Unique expression patterns of additional genes and gene products associated with the extracellular matrix, including the *tenascin-R* gene[73] and the tenascin-R[73] and galectin-3 proteins,[74] have also been reported in PAs. These genes and gene products may have roles in PA growth,[37] although confirmatory evidence is necessary.

Complex transcriptional networks associated with PAs are in the early stages of investigation,[66] and additional research will be necessary to achieve consensus on the identity and the activity of these networks in PA. Signaling-associated genes in the *ErbB* family, including *ErbB4*, *ErbB3*, and its regulator, *Sox10*, also demonstrate distinct expression patterns in PA.[75,76] Expression of genes associated with vascular proliferation, including vascular endothelial growth factor (*VEGF*) and its receptors, *VEGFR-1* (*FLT1*) and *VEGFR-2* (*KDR*), has been identified in PAs, although data regarding expression levels remain inconsistent, and the functional significance of these findings is unresolved.[77–79] Stem cell–related factors, including Stem Cell Factor and its receptor Kit, have been identified in the endothelial cells of PAs,[77] but their role in tumor initiation or growth is unknown. Hedgehog pathway activation has also been observed in a small series of PAs,[80] but the functional significance of this finding remains uncertain. Even more cryptic are the functional roles of recently identified associations between chromosomal loss and gene underexpression in PAs, including loss of 7q11.23 corresponding to underexpression of *BCL7B*, 12q24.33 loss corresponding to underexpression of *BCL7A*, 9p21.2-p23 loss corresponding to underexpression of *SH3GL2*, 17q21.31 loss corresponding to underexpression of *TUBG2* and *CNTNAP1*, and 10q26.3 loss corresponding to underexpression of *DRD1IP*.[81]

Epigenomic Abnormalities

Epigenomic data regarding PAs are currently limited. No consistent evidence for gene hypermethylation in the molecular biology of PAs has yet been reported, and general cytosine–phosphate–guanine hypomethylation (relative to normal brain) has actually been observed among PAs.[82] Micro(mi)RNA expression profiles are beginning to be investigated in PAs, and overexpression has been reported in miR-432, -29a, -138, -299–5p, and 34a, while underexpression has been observed in miR-93, -135a, -129, -135b, and -106b.[83] Additionally, recent data suggest that somatic mitochondrial (mt) mutations may be common (84%) among patients with PAs, and at least 34 unique mtDNA mutations have been identified.[84] Additionally, PA is the first tumor identified with discordance between alternative lengthening

of telomeres–associated promyelocytic leukemia body length and terminal restriction fragment length.[85] This finding may suggest unique methods of telomere maintenance in PAs, but further investigation is necessary.

Clinical Correlations

The anatomic location of sporadic PAs appears to correlate with their underlying molecular abnormalities.[26] Homozygous *p16* deletion is more common in PAs of the midbrain, brain stem, and spine than in cortical or cerebellar PAs, while *BRAF* gene rearrangements are more common in cerebellar than in noncerebellar tumors.[35,86] Conversely, the $BRAF^{V600E}$ mutation in PAs is strongly associated with an extracerebellar tumor location.[49]

Various correlations among genotypes and clinical phenotypes have been suggested in PAs. *BRAF* fusions may be associated with favorable prognosis, although the supporting data are somewhat nonspecific.[87] Apolipoprotein-D expression appears correlated with relatively non-infiltrative PA phenotypes,[88] while underexpression of *ALDH1L1* has been suggested as a marker of aggressive PA subtypes.[89] Additionally, LOH of 17p13 appears correlated with increased risk for recurrence of cerebellar PAs.[86]

In vitro and in vivo studies suggest that BRAF and MAPK/ERK kinase (MEK) inhibitors may be potentially effective therapies for some molecular subtypes of PAs.[41,90,91] Additionally, recent investigations have suggested that a subset of *BRAF*-mutated PAs may be amenable to targeted therapies based on these putative mechanisms of molecular pathophysiology.[92] Such findings are helping to drive current research in adjuvant therapy for low-grade gliomas, including the MEK inhibitor AZD6244,[91] which is currently the subject of a pediatric phase 1 clinical trial (NCT01386450).

Subependymal Giant Cell Astrocytoma

SEGAs are histologically benign tumors of the subependymal region that occur almost exclusively in the context of the tuberous sclerosis complex (TSC).[1] SEGA is the most common CNS tumor in TSC patients, with subependymal nodules occurring in 88%–95% of TSC patients and progressing to SEGA in 6%–14%.[1,93] Notwithstanding, sporadic cases of SEGA have been reported outside of the setting of TSC. Once thought to represent exclusively cases of mosaicism in otherwise subclinical and undiagnosed TSC patients, sporadic cases representing rare but legitimate instances of SEGAs attributable to de novo somatic mutations are becoming increasingly accepted as possible.[94]

Regardless of its pathologic origin, SEGA remains an uncommon tumor. Its rarity, the small patient population in which it tends to occur, and the fact that anatomically favorable lesions can be potentially cured with surgical resection have limited the available data regarding the molecular biology of SEGAs. Much of what is known or suspected regarding the pathogenesis of SEGA is therefore extrapolated from molecular investigations of TSC in general.

More than 85% of TSC patients have inactivating mutations of the *TSC1* or *TSC2* gene.[95] *TSC1* (9q34) encodes the protein hamartin,[96] while *TSC2* (16p13) encodes the protein tuberin.[97] These proteins heterodimerize and modulate the activity of the mammalian target of rapamycin (mTOR) complex, a serine-threonine kinase involved in regulation of cell growth and proliferation in response to energy supply and hypoxia.[93,98] Deficiencies of hamartin or tuberin lead to constitutive mTOR activation and subsequently to unregulated cellular growth.[99,100]

CNS-specific investigations of this process remain in their early stages, but it is generally hypothesized that abnormalities in *TSC1*, *TSC2*, or their gene products in subependymal astrocytes result in constitutive activation of the mTOR pathway and that these are the molecular abnormalities responsible for SEGA tumorigenesis. This hypothesis is supported by identification of *TSC1* and *TSC2* mutations with accompanying underexpression of hamartin and tuberin in tissue from human SEGAs.[101] Additionally, preclinical data from an astrocyte-specific *TSC1* knockout mouse model[102] demonstrate increased astrocyte proliferation in vitro and in vivo,[102,103] further supporting the putative role for abnormal mTOR signaling in SEGA. Subsequent investigations using this model have identified increased expression of vimentin and brain lipid binding protein, which may suggest that underexpression of *TSC1* results in developmentally immature astrocytic phenotypes that may be prone to unconstrained proliferation.[103] More recently, animal and human studies have revealed in SEGAs overexpression of genes for known mTOR pathway modulators, including epidermal growth factor and its receptor (*EGF* and *EGFR*), hepatocyte growth factor and its receptor (*HGF* and *c-Met*), and *VEGF* and its modulator (*HIF-1α*).[104] Differential expression of other genes putatively involved in mTOR pathway regulation, including *ANXA1*, *GPNMB*, *LTF*, *RND3*, *S100A11*, *SFRP4*, and *NPTX1*, has also been reported in genomic studies of human SEGAs.[105] Accordingly, mTOR inhibitors have demonstrated some clinical efficacy against SEGA.[106] Positive immunostaining for Bax has also been reported in SEGA, but the functional significance of this finding remains unknown.[107] Unlike other astrocytomas, chromosomal copy number abnormalities have not been observed in SEGA,[2,18] although multiple subtelomeric chromosomal abnormalities have been reported in data from a single tumor sample,[16] and LOH at 16p and 21q has been observed.[14]

Conclusion

Both PA and SEGA represent areas of active molecular and translational research, and these investigations offer the potential for novel prognostic and therapeutic strategies for these lesions. While they are both histologically "benign" lesions, their clinical courses can be complex and life threatening, so additional investigations

are needed to further clarify the pathophysiology of these tumors and to identify potential novel therapeutic targets. The neuro-oncology community remains optimistic that the coming years will see meaningful progress on both of these fronts.

Funding

N. F. M. is supported in part by a grant from the American Association of Neurological Surgeons' William P. VanWagenen Fellowship program and by an education and training grant from the Neurosurgery Research and Education Foundation (NREF). R. J. W. is supported in part by the Melvin Burkhardt Chair in Neurosurgical Oncology and by the Karen Colina Wilson research endowment within the Brain Tumor and Neuro-oncology Center at the Cleveland Clinic Foundation.

Conflict of interest statement. None declared.

References

1. Louis D, Ohgaki H, Wiestler O, Cavenee W, eds. WHO Classifiation of Tumors of the Central Nervous System. Lyon, France: IARC; 2007.
2. Rickert CH, Paulus W. Epidemiology of central nervous system tumors in childhood and adolescence based on the new WHO classification. *Childs Nerv Syst.* 2001;17(9):503–511.
3. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004–2007. 2011. http://www.cbtrus.org/2011-NPCR-SEER/WEB-0407-Report-3-3-2011.pdf. Accessed 25 January 2012.
4. Dunham C. Pediatric brain tumors: a histologic and genetic update on commonly encountered entities. *Semin Diagn Pathol*. 2010;27(3):147–159.
5. Marko NF, Prayson RA, Barnett GH, Weil RJ. Integrated molecular analysis suggests a three-class model for low-grade gliomas: a proof-of-concept study. *Genomics*. 2010;95(1):16–24.
6. Ransom DT, Ritland SR, Kimmel DW, et al. Cytogenetic and loss of heterozygosity studies in ependymomas, pilocytic astrocytomas, and oligodendrogliomas. *Genes Chromosomes Cancer*. 1992;5(4):348–356.
7. Payton JE, Schmidt J, Yu J, Lusis EA, Watson MA, Gutmann DH. Genome-wide polymorphism analysis demonstrates a monoclonal origin of pilocytic astrocytoma. *Neuropathol Appl Neurobiol*. 2011;37(3):321–325.
8. Jones DT, Ichimura K, Liu L, Pearson DM, Plant K, Collins VP. Genomic analysis of pilocytic astrocytomas at 0.97 Mb resolution shows an increasing tendency toward chromosomal copy number change with age. *J Neuropathol Exp Neurol*. 2006;65(11):1049–1058.
9. Sanoudou D, Tingby O, Ferguson-Smith MA, Collins VP, Coleman N. Analysis of pilocytic astrocytoma by comparative genomic hybridization. *Br J Cancer*. 2000;82(6):1218–1222.
10. Shlomit R, Ayala AG, Michal D, et al. Gains and losses of DNA sequences in childhood brain tumors analyzed by comparative genomic hybridization. *Cancer Genet Cytogenet*. 2000;121(1):67–72.
11. Schrock E, Blume C, Meffert MC, et al. Recurrent gain of chromosome arm 7q in low-grade astrocytic tumors studied by comparative genomic hybridization. *Genes Chromosomes Cancer*. 1996;15(4):199–205.
12. Roberts P, Chumas PD, Picton S, Bridges L, Livingstone JH, Sheridan E. A review of the cytogenetics of 58 pediatric brain tumors. *Cancer Genet Cytogenet*. 2001;131(1):1–12.
13. Bhattacharjee MB, Armstrong DD, Vogel H, Cooley LD. Cytogenetic analysis of 120 primary pediatric brain tumors and literature review. *Cancer Genet Cytogenet.* 1997;97(1):39–53.
14. Wong K, Tsang YTM, Chang Y, Lau CC. Genome-wide allelic imbalance analysis of pediatric gliomas by high-density single nucleotide polymorphic allele (SNP) array. *Neuro-Oncol*. 2005;7(3):344–344.
15. White FV, Anthony DC, Yunis EJ, Tarbell NJ, Scott RM, Schofield DE. Nonrandom chromosomal gains in pilocytic astrocytomas of childhood. *Hum Pathol*. 1995;26(9):979–986.
16. Grau E, Balaguer J, Canete A, et al. Subtelomeric analysis of pediatric astrocytoma: subchromosomal instability is a distinctive feature of pleomorphic xanthoastrocytoma. *J Neuro-Oncol*. 2009;93(2):175–182.
17. Wemmert S, Romeike BF, Ketter R, Steudel WI, Zang KD, Urbschat S. Intratumoral genetic heterogeneity in pilocytic astrocytomas revealed by CGH-analysis of microdissected tumor cells and FISH on tumor tissue sections. *Int J Oncol*. 2006;28(2):353–360.
18. Ward SJ, Karakoula K, Phipps KP, et al. Cytogenetic analysis of paediatric astrocytoma using comparative genomic hybridisation and fluorescence in-situ hybridisation. *J Neuro-Oncol*. 2010;98(3):305–318.
19. Hayes VM, Dirven CM, Dam A, et al. High frequency of TP53 mutations in juvenile pilocytic astrocytomas indicates role of TP53 in the development of these tumors. *Brain Pathol*. 1999;9(3):463–467.
20. Watanabe T, Nobusawa S, Kleihues P, Ohgaki H. IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol*. 2009;174(4):1149–1153.
21. Capper D, Weissert S, Balss J, et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathol*. 2010;20(1):245–254.
22. Listernick R, Louis DN, Packer RJ, Gutmann DH. Optic pathway gliomas in children with neurofibromatosis 1: consensus statement from the NF1 Optic Pathway Glioma Task Force. *Ann Neurol*. 1997;41(2):143–149.
23. Gutmann DH, Donahoe J, Brown T, James CD, Perry A. Loss of neurofibromatosis 1 (NF1) gene expression in NF1-associated pilocytic astrocytomas. *Neuropathol Appl Neurobiol*. 2000;26(4):361–367.
24. Sharma MK, Zehnbauer BA, Watson MA, Gutmann DH. RAS pathway activation and an oncogenic RAS mutation in sporadic pilocytic astrocytoma. *Neurology*. 2005;65(8):1335–1336.
25. Tatevossian RG, Lawson AR, Forshew T, Hindley GF, Ellison DW, Sheer D. MAPK pathway activation and the origins of pediatric low-grade astrocytomas. *J Cell Physiol*. 2010;222(3):509–514.
26. Sharma MK, Mansur DB, Reifenberger G, et al. Distinct genetic signatures among pilocytic astrocytomas relate to their brain region origin. *Cancer Res*. 2007;67(3):890–900.
27. Tchoghandjian A, Fernandez C, Colin C, et al. Pilocytic astrocytoma of the optic pathway: a tumour deriving from radial glia cells with a specific gene signature. *Brain*. 2009;132(Pt 6):1523–1535.
28. Ebinger M, Senf L, Wachowski O, Scheurlen W. No aberrant methylation of neurofibromatosis 1 gene (NF1) promoter in pilocytic astrocytoma in childhood. *Pediatr Hematol Oncol*. 2005;22(1):83–87.

29. Cichowski K, Jacks T. NF1 tumor suppressor gene function: narrowing the GAP. *Cell*. 2001;104(4):593–604.
30. Oguzkan S, Terzi YK, Cinbis M, Anlar B, Aysun S, Ayter S. Molecular genetic analyses in neurofibromatosis type 1 patients with tumors. *Cancer Genet Cytogenet*. 2006;165(2):167–171.
31. Lau N, Feldkamp MM, Roncari L, et al. Loss of neurofibromin is associated with activation of RAS/MAPK and PI3-K/AKT signaling in a neurofibromatosis 1 astrocytoma. *J Neuropathol Exp Neurol*. 2000;59(9):759–767.
32. Xu GF, O'Connell P, Viskochil D, et al. The neurofibromatosis type 1 gene encodes a protein related to GAP. *Cell*. 1990;62(3):599–608.
33. Wimmer K, Eckart M, Meyer-Puttlitz B, Fonatsch C, Pietsch T. Mutational and expression analysis of the NF1 gene argues against a role as tumor suppressor in sporadic pilocytic astrocytomas. *J Neuropathol Exp Neurol*. 2002;61(10):896–902.
34. Kluwe L, Hagel C, Tatagiba M, et al. Loss of NF1 alleles distinguish sporadic from NF1-associated pilocytic astrocytomas. *J Neuropathol Exp Neurol*. 2001;60(9):917–920.
35. Jacob K, Albrecht S, Sollier C, et al. Duplication of 7q34 is specific to juvenile pilocytic astrocytomas and a hallmark of cerebellar and optic pathway tumours. *Br J Cancer*. 2009;101(4):722–733.
36. Tada K, Kochi M, Saya H, et al. Preliminary observations on genetic alterations in pilocytic astrocytomas associated with neurofibromatosis 1. *Neuro-Oncol*. 2003;5(4):228–234.
37. Yu J, Deshmukh H, Gutmann RJ, et al. Alterations of BRAF and HIPK2 loci predominate in sporadic pilocytic astrocytoma. *Neurology*. 2009;73(19):1526–1531.
38. Hargrave D. Paediatric high and low grade glioma: the impact of tumour biology on current and future therapy. *Br J Neurosurg*. 2009;23(4):351–363.
39. Bar EE, Lin A, Tihan T, Burger PC, Eberhart CG. Frequent gains at chromosome 7q34 involving BRAF in pilocytic astrocytoma. *J Neuropathol Exp Neurol*. 2008;67(9):878–887.
40. Sievert AJ, Jackson EM, Gai X, et al. Duplication of 7q34 in pediatric low-grade astrocytomas detected by high-density single-nucleotide polymorphism-based genotype arrays results in a novel BRAF fusion gene. *Brain Pathol*. 2009;19(3):449–458.
41. Pfister S, Janzarik WG, Remke M, et al. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest*. 2008;118(5):1739–1749.
42. Jones DT, Kocialkowski S, Liu L, Pearson DM, Ichimura K, Collins VP. Oncogenic RAF1 rearrangement and a novel BRAF mutation as alternatives to KIAA1549:BRAF fusion in activating the MAPK pathway in pilocytic astrocytoma. *Oncogene*. 2009;28(20):2119–2123.
43. Jones DT, Kocialkowski S, Liu L, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res*. 2008;68(21):8673–8677.
44. Forshew T, Tatevossian RG, Lawson AR, et al. Activation of the ERK/MAPK pathway: a signature genetic defect in posterior fossa pilocytic astrocytomas. *J Pathol*. 2009;218(2):172–181.
45. Lawson AR, Tatevossian RG, Phipps KP, et al. RAF gene fusions are specific to pilocytic astrocytoma in a broad paediatric brain tumour cohort. *Acta Neuropathol*. 2010;120(2):271–273.
46. Jeuken JW, Wesseling P. MAPK pathway activation through BRAF gene fusion in pilocytic astrocytomas: a novel oncogenic fusion gene with diagnostic, prognostic, and therapeutic potential. *J Pathol*. 2010;222(4):324–328.
47. Nagase T, Kikuno R, Nakayama M, Hirosawa M, Ohara O. Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. *DNA Res*. 2000;7(4):273–281.
48. Tran NH, Wu X, Frost JA. B-Raf and Raf-1 are regulated by distinct autoregulatory mechanisms. *J Biol Chem*. 2005;280(16):16244–16253.
49. Schindler G, Capper D, Meyer J, et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extracerebellar pilocytic astrocytoma. *Acta Neuropathol*. 2011;121(3):397–405.
50. Nicolaides TP, Li H, Solomon DA, et al. Targeted therapy for BRAFV600E malignant astrocytoma. *Clin Cancer Res*. 2011;17(24):7595–7604.
51. Schiffman JD, Hodgson JG, VandenBerg SR, et al. Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. *Cancer Res*. 2010;70(2):512–519.
52. Eisenhardt AE, Olbrich H, Roring M, et al. Functional characterization of a BRAF insertion mutant associated with pilocytic astrocytoma. *Int J Cancer*. Dec 28 2010;129(9):2297–2203.
53. Raabe EH, Lim KS, Kim JM, et al. BRAF activation induces transformation and then senescence in human neural stem cells: a pilocytic astrocytoma model. *Clin Cancer Res*. 2011;17(11):3590–3599.
54. Gronych J, Korshunov A, Bageritz J, et al. An activated mutant BRAF kinase domain is sufficient to induce pilocytic astrocytoma in mice. *J Clin Invest*. 2011;121(4):1344–1348.
55. Jacob K, Quang-Khuong DA, Jones DT, et al. Genetic aberrations leading to MAPK pathway activation mediate oncogene-induced senescence in sporadic pilocytic astrocytomas. *Clin Cancer Res*. 2011;17(14):4650–4660.
56. Cin H, Meyer C, Herr R, et al. Oncogenic FAM131B-BRAF fusion resulting from 7q34 deletion comprises an alternative mechanism of MAPK pathway activation in pilocytic astrocytoma. *Acta Neuropathol*. 2011;121(6):763–774.
57. Wong K, Ren XR, Huang YZ, et al. Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit-Robo pathway. *Cell*. 2001;107(2):209–221.
58. Soderling SH, Guire ES, Kaech S, et al. A WAVE-1 and WRP signaling complex regulates spine density, synaptic plasticity, and memory. *J Neurosci*. 2007;27(2):355–365.
59. Yang Y, Marcello M, Endris V, et al. MEGAP impedes cell migration via regulating actin and microtubule dynamics and focal complex formation. *Exp Cell Res*. 2006;312(12):2379–2393.
60. Janzarik WG, Kratz CP, Loges NT, et al. Further evidence for a somatic KRAS mutation in a pilocytic astrocytoma. *Neuropediatrics*. 2007;38(2):61–63.
61. Brasil AS, Pereira AC, Wanderley LT, et al. PTPN11 and KRAS gene analysis in patients with Noonan and Noonan-like syndromes. *Genet Test Molec Biomarkers*. 2010;14(3):425–432.
62. Ko JM, Kim JM, Kim GH, Yoo HW. PTPN11, SOS1, KRAS, and RAF1 gene analysis, and genotype-phenotype correlation in Korean patients with Noonan syndrome. *J Hum Genet*. 2008;53(11–12):999–1006.
63. Fryssira H, Leventopoulos G, Psoni S, Kitsiou-Tzeli S, Stavrianeas N, Kanavakis E. Tumor development in three patients with Noonan syndrome. *Eur J Pediatr*. 2008;167(9):1025–1031.
64. Sanford RA, Bowman R, Tomita T, De Leon G, Palka P. A 16-year-old male with Noonan's syndrome develops progressive scoliosis and deteriorating gait. *Pediatr Neurosurg*. 1999;30(1):47–52.
65. Schuettpelz LG, McDonald S, Whitesell K, et al. Pilocytic astrocytoma in a child with Noonan syndrome. *Pediatr Blood Cancer*. 2009;53(6):1147–1149.
66. Deshmukh H, Yu J, Shaik J, et al. Identification of transcriptional regulatory networks specific to pilocytic astrocytoma. *BMC Med Genomics*. 2011;4:57.

67. Cheng Y, Pang JC, Ng HK, et al. Pilocytic astrocytomas do not show most of the genetic changes commonly seen in diffuse astrocytomas. *Histopathology*. 2000;37(5):437–444.
68. Korshunov A, Meyer J, Capper D, et al. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. *Acta Neuropathol*. 2009;118(3):401–405.
69. Huang H, Hara A, Homma T, Yonekawa Y, Ohgaki H. Altered expression of immune defense genes in pilocytic astrocytomas. *J Neuropathol Exp Neurol*. 2005;64(10):891–901.
70. Deshmukh H, Yeh TH, Yu J, et al. High-resolution, dual-platform aCGH analysis reveals frequent HIPK2 amplification and increased expression in pilocytic astrocytomas. *Oncogene*. 2008;27(34):4745–4751.
71. Rorive S, Lopez XM, Maris C, et al. TIMP-4 and CD63: new prognostic biomarkers in human astrocytomas. *Mod Pathol*. 2010;23(10): 1418–1428.
72. Sharma MK, Watson MA, Lyman M, et al. Matrilin-2 expression distinguishes clinically relevant subsets of pilocytic astrocytoma. *Neurology*. 2006;66(1):127–130.
73. El Ayachi I, Baeza N, Fernandez C, et al. KIAA0510, the 3′-untranslated region of the tenascin-R gene, and tenascin-R are overexpressed in pilocytic astrocytomas. *Neuropathol Appl Neurobiol*. 2010;36(5): 399–410.
74. Camby I, Belot N, Rorive S, et al. Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration. *Brain Pathol*. 2001;11(1):12–26.
75. Addo-Yobo SO, Straessle J, Anwar A, Donson AM, Kleinschmidt-Demasters BK, Foreman NK. Paired overexpression of ErbB3 and Sox10 in pilocytic astrocytoma. *J Neuropathol Exp Neurol*. 2006;65(8): 769–775.
76. Zeng N, Liu L, McCabe MG, Jones DT, Ichimura K, Collins VP. Real-time quantitative polymerase chain reaction (qPCR) analysis with fluorescence resonance energy transfer (FRET) probes reveals differential expression of the four ERBB4 juxtamembrane region variants between medulloblastoma and pilocytic astrocytoma. *Neuropathol Appl Neurobiol*. 2009;35(4):353–366.
77. Puputti M, Tynninen O, Pernila P, et al. Expression of KIT receptor tyrosine kinase in endothelial cells of juvenile brain tumors. *Brain Pathol*. 2010;20(4):763–770.
78. Sikkema AH, de Bont ES, Molema G, et al. Vascular endothelial growth factor receptor 2 (VEGFR-2) signalling activity in paediatric pilocytic astrocytoma is restricted to tumour endothelial cells. *Neuropathol Appl Neurobiol*. 2011;37(5):538–548.
79. Leung SY, Chan AS, Wong MP, Yuen ST, Cheung N, Chung LP. Expression of vascular endothelial growth factor and its receptors in pilocytic astrocytoma. *Am J Surg Pathol*. 1997;21(8):941–950.
80. Rush SZ, Abel TW, Valadez JG, Pearson M, Cooper MK. Activation of the Hedgehog pathway in pilocytic astrocytomas. *Neuro-Oncol*. 2010;12(8):790–798.
81. Potter N, Karakoula A, Phipps KP, et al. Genomic deletions correlate with underexpression of novel candidate genes at six loci in pediatric pilocytic astrocytoma. *Neoplasia*. 2008;10(8):757–772.
82. Uhlmann K, Rohde K, Zeller C, et al. Distinct methylation profiles of glioma subtypes. *Int J Cancer*. 2003;106(1):52–59.
83. Birks DK, Barton VN, Donson AM, Handler MH, Vibhakar R, Foreman NK. Survey of MicroRNA expression in pediatric brain tumors. *Pediatr Blood Cancer*. 2011;56(2):211–216.
84. Lueth M, Wronski L, Giese A, et al. Somatic mitochondrial mutations in pilocytic astrocytoma. *Cancer Genet Cytogenet*. 2009;192(1):30–35.
85. Slatter T, Gifford-Garner J, Wiles A, et al. Pilocytic astrocytomas have telomere-associated promyelocytic leukemia bodies without alternatively lengthened telomeres. *Am J Pathol*. 2010;177(6):2694–2700.
86. Horbinski C, Hamilton RL, Nikiforov Y, Pollack IF. Association of molecular alterations, including BRAF, with biology and outcome in pilocytic astrocytomas. *Acta Neuropathol*. 2010;119(5):641–649.
87. Hawkins C, Walker E, Mohamed N, et al. BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res*. 2011;17(14):4790–4798.
88. Hunter S, Young A, Olson J, et al. Differential expression between pilocytic and anaplastic astrocytomas: identification of apolipoprotein D as a marker for low-grade, non-infiltrating primary CNS neoplasms. *J Neuropathol Exp Neurol*. 2002;61(3):275–281.
89. Rodriguez FJ, Giannini C, Asmann YW, et al. Gene expression profiling of NF-1-associated and sporadic pilocytic astrocytoma identifies aldehyde dehydrogenase 1 family member L1 (ALDH1L1) as an underexpressed candidate biomarker in aggressive subtypes. *J Neuropathol Exp Neurol*. 2008;67(12):1194–1204.
90. Jones DT, Gronych J, Lichter P, Witt O, Pfister SM. MAPK pathway activation in pilocytic astrocytoma. *Cell Molec Life Sci: CMLS*. 2012;69(11):1799–1811.
91. Kolb EA, Gorlick R, Houghton PJ, et al. Initial testing (stage 1) of AZD6244 (ARRY-142886) by the Pediatric Preclinical Testing Program. *Pediatr Blood Cancer*. 2010;55(4):668–677.
92. Huillard E, Hashizume R, Phillips JJ, et al. Cooperative interactions of BRAFV600E kinase and CDKN2A locus deficiency in pediatric malignant astrocytoma as a basis for rational therapy. *Proc Natl Acad Sci USA*. 2012;109(22):8710–8715.
93. Turner SG, Peters KB, Vredenburgh JJ, Desjardins A, Friedman HS, Reardon DA. Everolimus tablets for patients with subependymal giant cell astrocytoma. *Expert Opin Pharmacother*. 2011;12(14): 2265–2269.
94. Ichikawa T, Wakisaka A, Daido S, et al. A case of solitary subependymal giant cell astrocytoma: two somatic hits of TSC2 in the tumor, without evidence of somatic mosaicism. *J Mol Diagn*. 2005;7(4): 544–549.
95. Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med*. 2006;355(13):1345–1356.
96. van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science*. 1997;277(5327):805–808.
97. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell*. 1993;75(7):1305–1315.
98. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev*. 2004;18(16):1926–1945.
99. Chan JA, Zhang H, Roberts PS, et al. Pathogenesis of tuberous sclerosis subependymal giant cell astrocytomas: biallelic inactivation of TSC1 or TSC2 leads to mTOR activation. *J Neuropathol Exp Neurol*. 2004;63(12): 1236–1242.
100. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J*. 2008;412(2):179–190.
101. Jozwiak S, Kwiatkowski D, Kotulska K, et al. Tuberin and hamartin expression is reduced in the majority of subependymal giant cell astrocytomas in tuberous sclerosis complex consistent with a two-hit model of pathogenesis. *J Child Neurol*. 2004;19(2):102–106.

102. Uhlmann EJ, Wong M, Baldwin RL, et al. Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. *Ann Neurol*. 2002;52(3):285–296.

103. Ess KC, Uhlmann EJ, Li W, et al. Expression profiling in tuberous sclerosis complex (TSC) knockout mouse astrocytes to characterize human TSC brain pathology. *Glia*. 2004;46(1):28–40.

104. Parker WE, Orlova KA, Heuer GG, et al. Enhanced epidermal growth factor, hepatocyte growth factor, and vascular endothelial growth factor expression in tuberous sclerosis complex. *Am J Pathol*. 2011;178(1):296–305.

105. Tyburczy ME, Kotulska K, Pokarowski P, et al. Novel proteins regulated by mTOR in subependymal giant cell astrocytomas of patients with tuberous sclerosis complex and new therapeutic implications. *Am J Pathol*. 2010;176(4):1878–1890.

106. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010;363(19):1801–1811.

107. Kim SK, Wang KC, Cho BK, et al. Biological behavior and tumorigenesis of subependymal giant cell astrocytomas. *J Neuro-Oncol*. 2001;52(3):217–225.