The molecular biology of WHO grade I astrocytomas

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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). 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, 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, with a prevalence of 9.5/100 000. PA is the most frequent brain tumor in this age group, comprising 23.5% of pediatric central nervous system (CNS) malignancies. These tumors are phenotypically, histologically, and genotypically distinct from other low-grade gliomas. They rarely progress to higher histologic grades, and anatomically favorable lesions can almost always be cured with gross total resection. Despite
some controversy, current evidence suggests that PAs are monoclonal in origin.

**Chromosomal Abnormalities**

Most PAs exhibit a normal karyotype. Approximately 32% of PAs have some chromosomal abnormalities, with gains of chromosomes 5, 7, and 13q and losses on 1p, 9q, 12q, and 19q. 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 19q. Subtelomeric duplication has been observed at 3p, and gains have been demonstrated to involve 7qter, 12qter, 13cen, 19pter, and X/Yqter. Subtelomeric deletion has been reported at 21qter, and losses involving 8pter and 20pter have also been observed. Single chromosome abnormalities are more common in PAs from patients aged <15 years, while multiple abnormalities are more frequently observed in older PA patients.

**Genomic Abnormalities**

**Common glioma-associated genes.**—Despite some early data to the contrary, 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 outside of the context of NF1, and 15%–20% of NF1 patients ultimately have NF1 abnormalities are unique to NF1-associated PAs and are usually not observed in sporadic versions of this tumor. Additional abnormalities reported in NF1-associated PAs include p16 deletion and loss of heterozygosity (LOH) of chromosome 10, 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. Recent gene expression analyses and genome-wide copy number analyses of sporadic PAs have identified frequent focal chromosomal gains (50%–80%) on chromosome 7q34, in the region of the v-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene, which appears to be highly (but not absolutely) specific to sporadic PAs. These mutations are generally caused by a tandem duplication of this region, resulting in in-frame fusions of BRAF with the KIAA1549 gene. The 3 most common fusions are KIAA1549ex16—BRAFex9 (13%–77% of PAs), KIAA1549ex15—BRAFex9 (28% of PAs), and KIAA1549ex16—BRAFex11 (5% of PAs), but other, infrequent fusions (KIAA1549ex18—BRAFex10, KIAA1549ex19—BRAFex9, and KIAA1549ex18—BRAFex10) have also been reported. The mechanisms underlying the formation of these specific fusion genes remain to be fully elucidated.

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

**BRAF** activation in PAs has also been identified outside of the context of KIAA1549 or FAM131B fusion. Activating mutations at or around the BRAFV600E “hot spot” site are suspected to be involved in other cancers and have been observed in various CNS malignancies. The second 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) or between positions 599 and 600. These mutations have been identified in as many as 9% of PAs, and they may be more common in nonpilocytic gliomas. 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, and recent preclinical data using gene transfer of the BRAFVE600E 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. Oncogene-induced senescence may subsequently play a role in the low-grade pathobiology of PAs, 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 \( \sim 2.5 \text{ Mb} \) on chromosome 7q34. 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—RAFI fusion gene has been described, in which the auto-inhibitory domain of RAF1 is replaced in-frame by the beginning of the SRGAP3 gene. At least 2 versions of this fusion gene, SRGAP3ex11—RAFIex8, and SRGAP3ex12—RAFIex10, have been described. 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.

Unlike KIAA1549—BRAF, SRGAP3—RAFI does not code for a transmembrane domain but does contain a Cys/CIP4 (cell division control 42 protein–interacting protein 4) homology domain. While this may imply a role in cytoskeletal activity, the ultimate significance of this finding in PA remains unknown.

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. Since that time, a second KRAS mutation has been reported, this time a single G12A in a series of 25 PAs. 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. At least 3 cases of PA have been reported in this clinical context.

**Other genomic abnormalities.—** Additional genomic abnormalities involving at least 800 genes 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. 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.

Overexpression of the homeobox-interacting protein kinase 2 (HIPK2) gene, which (like BRAF) localizes to chromosome 7q34, has been associated with PA, as has overexpression among the extracellular matrix–associated genes MATN2, TIMP1, and TIMP4. Unique expression patterns of additional genes and gene products associated with the extracellular matrix, including the tenascin-R gene and the tenascin-R and galectin-3 proteins, have also been reported in PAs. These genes and gene products may have roles in PA growth, although confirmatory evidence is necessary.

Complex transcriptional networks associated with PAs are in the early stages of investigation, 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 PAs.

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. Stem cell–related factors, including Stem Cell Factor and its receptor Kit, have been identified in the endothelial cells of PAs, but their role in tumor initiation or growth is unknown. Hedgehog pathway activation has also been observed in a small series of PAs, 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 DRD11P.

**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. 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. 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. Additionally, PA is the first tumor identified with discordance between alternative lengthening
of telomeres—associated promyelocytic leukemia body length and terminal restriction fragment length. 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. 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. Conversely, the BRAFV600E mutation in PAs is strongly associated with an extracerebellar tumor location.

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. Apolipoprotein-D expression appears correlated with relatively non-infiltrative PA phenotypes while underexpression of ALDH1L1 has been suggested as a marker of aggressive PA subtypes. Additionally, LOH of 17p13 appears correlated with increased risk for recurrence of cerebellar PAs.

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. 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. Such findings are helping to drive current research in adjuvant therapy for low-grade gliomas, including the MEK inhibitor AZD6244, which is currently the subject of a pediatric phase 1 clinical trial (NCT01386450).

Subependymal Giant Cell Astrocytoma

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. TSC1 (9q34) encodes the protein hamartin, while TSC2 (16p13) encodes the protein tuberin. 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. Deficiencies of hamartin or tuberin lead to constitutive mTOR activation and subsequently to unregulated cellular growth.

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 astrocytomas 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. Additionally, preclinical data from an astrocyte-specific TSC1 knockout mouse model demonstrate increased astrocyte proliferation in vitro and in vivo, 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. 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). 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. Accordingly, mTOR inhibitors have demonstrated some clinical efficacy against SEGA. Positive immunostaining for Bax has also been reported in SEGA, but the functional significance of this finding remains unknown. Unlike other astrocytomas, chromosomal copy number abnormalities have not been observed in SEGA, although multiple subtelomeric chromosomal abnormalities have been reported in data from a single tumor sample, and LOH at 16p and 21q has been observed.

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

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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.

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References


