Pediatric low-grade glioma: An overview of biology and molecular diagnostic/prognostic markers

David T. W. Jones

Background

Low-grade gliomas (LGG) account for more than 30 percent of all brain tumors in 0–14 year-olds, making them the most common central nervous system tumor in this age group (1). They typically display low proliferation rates and grow in a circumscribed manner, rarely progressing to higher malignancy grades (2). Surgery alone is the mainstay of current first-line treatment regimes, with five-year overall survival rates of ~95 percent (3). Despite this good prognosis, local recurrence is not uncommon even after a reported total resection, and can occur in 50–60 percent of cases where full surgical removal is not possible (3). Significant physical or psychosocial morbidity can be associated with both primary and recurrent tumor lesions, as well as the applied treatment modalities (4). A better understanding of both the basic biological mechanisms underlying this disease, as well as predictive markers of likely disease course, would, therefore, be of significant benefit for the rational introduction of additional targeted therapies. Furthermore, accurate diagnosis of pediatric gliomas is also centrally important to optimal patient management. A purely histological diagnosis of pilocytic astrocytoma, for example (the most common pediatric LGG), can be challenging. Regions reminiscent of high-grade astrocytoma, oligodendroglia or ependymoma can be seen, and features such as vascular proliferation and necrosis do not necessarily imply malignancy (2). Expanding the use of molecular diagnostic methods for these tumors could, therefore, be of significant value. Some of these key considerations, and future perspectives in the field, will be outlined here.

Discussion

The pre next-generation sequencing era

The advent of new, high-throughput sequencing technologies in recent years has rapidly expanded our knowledge on the fundamental mechanisms underlying many tumor types. Several studies made prior to this era, however, also provided significant insight into the biological workings of LGG. Most notable was the identification of a highly recurrent fusion between KIAA1549 and the BRAF oncogene on 7q34, leading to constitutive BRAF activation in pilocytic astrocytoma (PA; see (5), and (6) plus references therein). Additionally, multiple whole chromosome gains were seen to be a common feature of pilocytic astrocytomas in older children and adults, but rare in younger patients (7). Differences in the biology of tumors from different anatomic locations (possibly indicating distinct cells of origin) have also been...
described, with both gene expression and DNA methylation profiles varying by site (8–10) (Fig. 1). A possible explanation for the relatively indolent behavior of LGG was also proposed in two studies demonstrating that arrested cell division in response to activation of the mitogen-activated protein kinase (MAPK) pathway (a process known as oncogene-induced senescence, OIS) may act to limit the proliferative capacity of these tumors (11, 12). These represent just some of the aspects of LGG biology which still require a more detailed follow-up to understand their causes and implications.

The MAPK pathway revisited

Given the links between Neurofibromatosis Type 1 (NF1, an autosomal dominant disorder caused by mutations in NF1 – a negative regulator of Ras activity) and pilocytic astrocytoma (13), it has long been recognized that MAPK signaling plays a crucial role in this entity (reviewed in e.g. (6)). This link was cemented by the finding of recurrent BRAF alterations, as noted above. Only recently, however, has the full spectrum of MAPK pathway changes been recognized (Fig. 2). Two extensive genome sequencing studies detected several new alterations, such that essentially 100 percent of all PAs can be explained by an activating event in this pathway. These new findings included new changes in previously implicated genes (e.g. multiple new fusion partners of BRAF), but also novel targets that had not previously been recognized. Activating fusions involving the NTRK gene family and alterations of FGFR1 were each seen in ~5–10 percent of cases (mostly outside the cerebellum), expanding the types of MAPK aberration to include upstream receptor kinases (14, 15). FGFR1 activation involved either hotspot point mutations or a novel duplication of the whole kinase domain, resulting in

Fig. 1. Types of genetic alterations as well as other molecular variables in LGG differ according to anatomic location.
An update on molecular biology in pediatric LGG

Fig. 2. Frequency of MAPK pathway alterations in non-NF1 pilocytic astrocytoma, summarized from results presented in Jones, et al.(14) and Zhang, et al.(15).

constitutive signaling activity. Thus, pilocytic astrocytoma can now be considered as truly a single-pathway disease, with implications for potential targeted therapeutics.

**Non-pilocytic low-grade glioma**

The majority of molecular data available for pediatric LGG (including that described above) relates to pilocytic astrocytoma, since it is much more commonly diagnosed than the other subentities which fall under this bracket. Genomic sequencing has also recently shed light on other pediatric LGGs, however. For example, structural alterations of the MYB/MYBL1 oncogenes were found to be common in angiocentric glioma and diffuse astrocytoma (WHO grade II) (15, 16), while FGFR1 alterations were observed in a variety of low-grade histologies in addition to PA (15, 17). Further work is required to fully elucidate the tumorigenic mechanisms underlying these distinct subsets.

**Molecular-clinicopathologic correlates**

Whilst our understanding of the molecular alterations found in LGG has dramatically increased in recent years, there are still relatively limited data (particularly for clinical trial cohorts) as to how these alterations correlate with factors such as likelihood of tumor progression. It is known that the proportion of KIAA1549:BRAF fusion positive cases varies with tumor location and patient age (reviewed in (6)), but there are conflicting reports as to the
Pediatric Low-grade Gliomas: Silent but Still Deadly

prognostic value of this change (18, 19), and more work in this area is required. The diagnostic specificity of these changes is also not fully established. Whilst *BRAF* fusion is significantly more common in PA than any other entity, *BRAF* V600E can be seen in various gliomas (20). Choosing the optimum method for detecting these changes is important. The gold standard to be able to identify all possible classes of alteration that have previously been reported would be RNA sequencing, but cost and technical limitations may make this unfeasible in many centers. A combination of FISH, targeted gene sequencing and copy number profiling could be used to detect the majority of relevant changes.

**Future Directions**

A number of important challenges remain in the field, which would have the potential to positively impact on patient care and clinical outcomes if successfully addressed. Firstly, a lot remains to be elucidated about the precise functions and downstream effects of the different genetic alterations that are seen in low-grade glioma, and how they converge on a phenotype of being relatively benign but frequently recurring. Outside of typical MAPK pathway alterations, the role of multiple trisomies, of anatomically distinct cellular origins, and of oncogene-induced senescence are just a few of the areas which require further investigation. Also, whilst the genome of pediatric LGG is now relatively well understood, very few studies have looked at broader aspects of the transcriptome (non-coding RNAs, splice variants etc.) or at the multiple facets of the epigenome which combine to modulate cellular behavior. All of these have the potential to point towards specific vulnerabilities in the functioning of LGG, and thus to suggest novel therapeutic strategies.

Several novel treatment options are already being pursued in early-phase trials (e.g. MEK inhibitors, see NCT01386450 and NCT01089101 at clinicaltrials.gov), and it will be important to fully investigate the reasons for response or resistance to such agents. The cautionary tale of the failure of Sorafenib for LGG treatment due to paradoxical pathway activation shows that companion biology will be key to making the most of such trials (21).

Trying to determine biological factors which may predict the most likely clinical course for each individual patient is also of particular importance. For example, why do some incompletely resected tumors progress rapidly after surgery, while others remain stable for 10 years or more? Why is it that infants with LGG have a notably inferior outcome compared with slightly older children? What biological factors can predict response to chemo- or radiotherapy, or how likely a tumor is to show leptomeningeal dissemination?

Finally, it is likely that molecular diagnostics will play an increasing role in the classification of pediatric low-grade gliomas. Certain molecular alterations (such as *BRAF* fusion) may aid in pointing to one particular diagnosis amongst related entities, and it is also to be expected that molecular subgroups of LGG will at some point be defined by global gene expression or methylation profiles, in a similar manner to their higher grade counterparts (reviewed in (22)). To what extent these groups mimic the histological distinctions drawn in the current WHO classification, and what will be the best method to identify them, remains to be seen.

Interestingly, however, LGG may be one of the areas in which either molecular or morphological grouping might start to take less of a role in terms of clinical trial stratification,
which may rather be based on precise genotyping of molecular alterations. It is easy to envision (and to be encouraged), for example, that a trial could be based on the presence of a BRAF V600E mutation (common to several gliomas), rather than on microscopic appearance.

References
1. Ostrom QT, et al. Alex's Lemonade Stand Foundation infant and childhood primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. Neuro Oncol 2015;16(Suppl 10): x1–36.
2. Louis DN, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007;114:97–109.
3. Stokland T, et al. A multivariate analysis of factors determining tumor progression in childhood low-grade glioma: A population-based cohort study (CCLG CNS9702). Neuro Oncol 2010;12:1257–68.
4. Armstrong GT, et al. Survival and long-term health and cognitive outcomes after low-grade glioma. Neuro Oncol 2011;13:223–34.
5. Jones DTW, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 2008;68:8673–7.
6. Jones DTW, et al. MAPK pathway activation in pilocytic astrocytoma. Cell Mol Life Sci 2012;69:1799–811.
7. Jones DTW, et al. 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;65:1049–58.
8. Lambert SR, et al. Differential expression and methylation of brain developmental genes define location-specific subsets of pilocytic astrocytoma. Acta Neuropathol 2013;126:291–301.
9. Sharma MK, et al. Distinct genetic signatures among pilocytic astrocytomas relate to their brain region origin. Cancer Res 2007;67:890–900.
10. Tochogandjian A, 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):1523–35.
11. Jacob K, et al. Genetic aberrations leading to MAPK pathway activation mediate oncogene-induced senescence in sporadic pilocytic astrocytomas. Clin Cancer Res 2011.
12. Raabe EH, et al. BRAF activation induces transformation and then senescence in human neural stem cells: A pilocytic astrocytoma model. Clin Cancer Res 2011;17:3590–9.
13. Listernick R, Charrow J, Gutmann DH. Intracranial gliomas in neurofibromatosis type 1. Am J Med Genet 1999;89:38–44.
14. Jones DTW, et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. Nat Genet 2013;45:927–32.
15. Zhang J, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet 2013;45:602–12.
16. Ramkisson LA, et al. Genomic analysis of diffuse pediatric low-grade gliomas identifies recurrent oncogenic truncating rearrangements in the transcription factor MYBL1. Proc Natl Acad Sci U S A 2013, 110:8188–93.
17. Gessi M, et al. FGFR1 mutations in Rosette-forming glioneuronal tumors of the fourth ventricle. J Neuropathol Exp Neurol 2014;73:580–4.
18. Cin H, et al. Oncogenic FAM131B-BRAF fusion resulting from 7q34 deletion comprises an alternative mechanism of MAPK pathway activation in pilocytic astrocytoma. Acta Neuropathol 2011;121:763–74.
19. Hawkins C, et al. BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. Clin Cancer Res 2011.
20. Schindler G, et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthisastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. Acta Neuropathol 2011;121:397–405.
21. Karajannis MA, et al. Phase II study of sorafenib in children with recurrent or progressive low-grade astrocytomas. Neuro Oncol 2014;16:1408–16.
22. Sturm D, et al. Pediatric and adult glioblastoma: Multiform (sp)genomic culprits emerge. Nat Rev Cancer 2014;14:92–107.
