Neuropathology and Applied Neurobiology (2012), **38**, 241–253 doi: 10.1111/j.1365-2990.2011.01230.x

Review: Low-grade gliomas as neurodevelopmental disorders: insights from mouse models of neurofibromatosis-1

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M. Thangarajh and D. H. Gutmann (2012) *Neuropathology and Applied Neurobiology* **38**, 241–253
Low-grade gliomas as neurodevelopmental disorders: insights from mouse models of neurofibromatosis-1

Over the past few years, the traditional view of brain tumorigenesis has been revolutionized by advances in genomic medicine, molecular biology, stem cell biology and genetically engineered small-animal modelling. We now appreciate that paediatric brain tumours arise following specific genetic mutations in specialized groups of progenitor cells in concert with permissive changes in the local tumour microenvironment. This interplay between preneoplastic/neoplastic cells and non-neoplastic stromal cells is nicely illustrated by the neurofibromatosis type 1-inherited cancer syndrome, in which affected children develop low-grade astrocytic gliomas. In this review, we will use neurofibromatosis type 1 as a model system to highlight the critical role of growth control pathways, non-neoplastic cellular elements and brain region-specific properties in the development of childhood gliomas. The insights derived from examining each of these contributing factors will be instructive in the design of new therapies for gliomas in the paediatric population.

Keywords: astrocytoma, cyclic AMP, microglia, mTOR, neurofibromin, tumour microenvironment

Introduction

The most common solid tumours in children are primary brain tumours, contributing 22% of all childhood neoplasms. Of the primary brain tumours, gliomas represent the most frequent pathologic type and the leading cause of cancer-related death in children [1–3]. While the overall survival of children with low-grade glioma is good compared with other types, there are often significant cognitive and neuroendocrine adverse effects following successful adjuvant therapy [4]. In this regard, many children experience declines in their IQ and school performance, difficulties with executive function (attention deficit), behaviour/social problems and hypothalamic/pituitary axis abnormalities [4–8].

Gliomas are a heterogeneous group of central nervous system (CNS) tumours and include astrocytomas, oligodendrogliomas and ependymomas. However, in children, the most common glioma is the WHO grade I pilocytic astrocytoma (PA) [9,10]. These tumours typically arise during the first two decades of life, without a clear sex predilection. PAs can occur sporadically or in the context of the neurofibromatosis type 1 (NF1)-inherited cancer syndrome. In either case, PAs usually affect midline structures, including the cerebellum, optic pathway and brainstem (Figure 1). On neuroimaging, PAs of the cerebellum appear as well-circumscribed and contrast-enhancing lesions, whereas those involving the optic pathway present as fusiform masses. Optic pathway gliomas (OPGs) can also exhibit prominent gadolinium contrast enhancement on magnetic resonance imaging.

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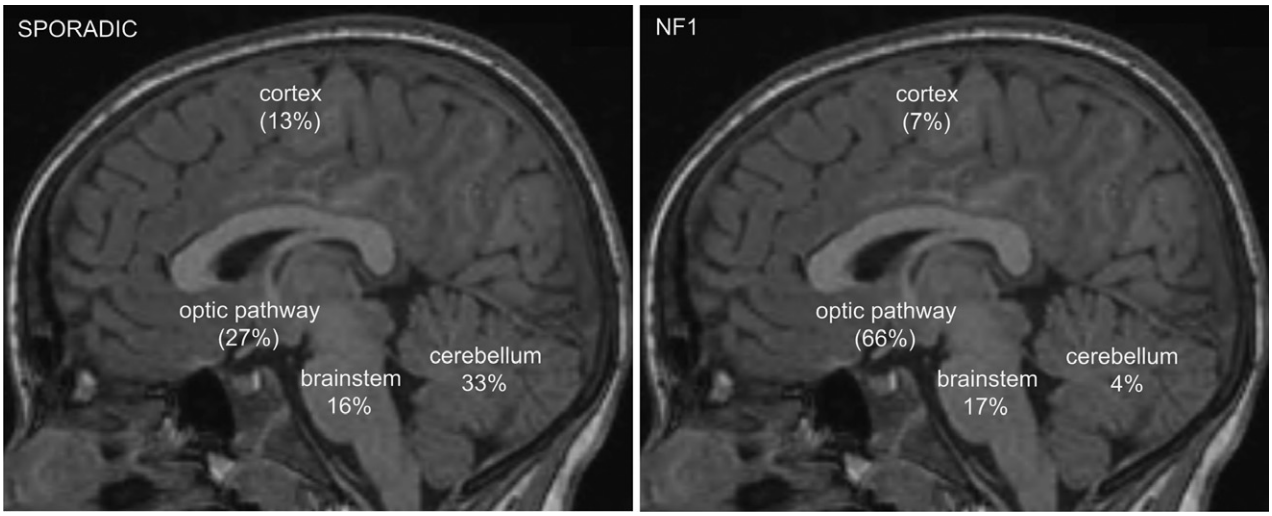

Figure 1. Location of sporadic and neurofibromatosis type 1 (NF1)-associated pilocytic astrocytoma (PA). Sagittal magnetic resonance images of the normal brain illustrate the most common locations for sporadic PA compared to NF1-associated PA. Whereas sporadic PAs tend to form in the cerebellum and optic pathway, NF1-associated PAs uncommonly arise in the cerebellum.

Pilocytic astrocytomas occasionally infiltrate adjacent CNS parenchyma, especially when they involve the optic pathways. Involvement of the subarachnoid space and leptomeningeal invasion are also not uncommon and do not necessarily indicate aggressive or malignant behaviour [10]. Malignant transformation is exceedingly rare, and some PAs have been reported to regress without medical intervention [11].

Histologically, PAs have a biphasic architecture with both cystic components and solid cellular areas (Figure 2**A**,**B**). Characteristically, they contain Rosenthal fibres and eosinophilic granular bodies, but typically do not harbour necrosis. Tumour cells express glial fibrillary acidic protein (GFAP), indicative of their glial (astrocytic) histogenesis (Figure 2**C**,**D**). Mitotic activity is usually low, and the growth fraction as estimated by Ki-67 immunolabelling is less than 1% [12]. While these tumours are low-grade neoplasms, they are vascular and can exhibit microvascular proliferation.

Genetic alterations in paediatric low-grade gliomas

Until recently, the most common genetic alteration associated with PAs was mutational inactivation of the *NF1* tumour suppressor gene. However, this mutation is only found in NF1-associated gliomas, and has not been reported as a causative molecular change in sporadic (non-NF1) paediatric low-grade gliomas [13–15]. The *NF1* gene product, neurofibromin, functions as a negative regulator of the p21-RAS proto-oncogene [16], such that loss of neurofibromin in NF1-associated gliomas results in increased RAS activation and cell growth [17] (Figure 3). The finding that RAS activation is observed in NF1-associated PA prompted several laboratories to determine whether mutational *RAS* activation accounted for sporadic PA tumorigenesis. However, this specific genetic alteration is a rare event in sporadic PA, observed in only 2–3% of cases [18,19].

Using converging genomic and gene expression strategies, recent work has culminated in the identification of signature mutations in the *BRAF* gene as a common mutation in sporadic PA [20–25]. BRAF is a brain RAF kinase molecule containing an amino terminal structure and a carboxyl terminal kinase domain. The amino terminal region is thought to regulate the activity of the kinase domain, such that the removal of this amino terminal domain results in unchecked BRAF kinase activity and increased mitogen-activated protein kinase (MAPK) mitogenic signalling. While *BRAF*-activating mutations, as seen in melanoma [26], are not typically observed in sporadic PA, genetic rearrangements result in the creation of a fusion molecule in which a portion of the *KIAA1549* gene is fused to the kinase domain of the *BRAF* gene [20–25]. Two-thirds of PAs are associated with this novel *KIAA1549:BRAF* gene fusion, which results in constitutive activation of the MAPK signalling pathway [27] (Figure 4). In addition, the presence of this

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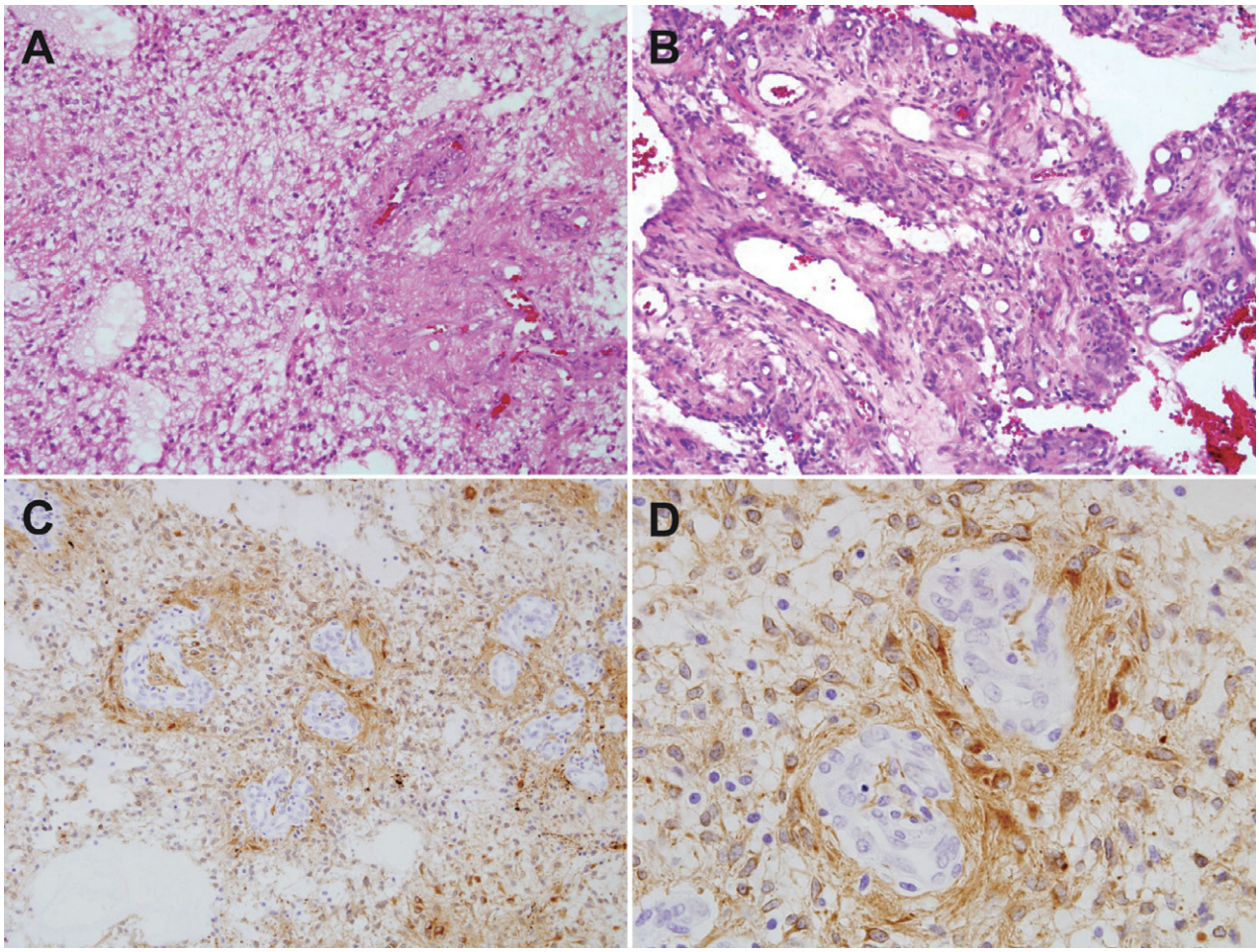

Figure 2. Neurofibromatosis type 1 (NF1)-associated pilocytic astrocytoma (PA). Histopathological analysis of a typical PA reveals both solid cellular areas and cystic regions (**A**) as well as areas with microvascular proliferation (**B**). The neoplastic glial cells are immunopositive for glial fibrillary acidic protein (GFAP) expression, a marker of astroglial lineage cells (**C**,**D**). Magnification = × 200 (**A**–**C**) and × 600 (**D**). Images were kindly provided by Dr Robert E. Schmidt, Neuropathology Division, School of Medicine, Washington University.

signature mutation is more commonly seen in cerebellar PAs [28], where it confers a threefold increase in progression-free survival [29].

Based on the discovery of the *KIAA1549:BRAF* mutational event in sporadic PAs, several reports have described other fusion rearrangements involving *BRAF*, including *FAM131B-BRAF* [30]. Similarly, oncogenic RAF1 rearrangements have also been reported in some sporadic PAs [31].

Interestingly, RAF expression alone in the brains of genetically engineered mice does not result in glioma formation, unless coupled with protein kinase-B (Akt) activation or Arf tumour suppressor gene loss [32]. Similarly, activated BRAF expression alone is insufficient for gliomagenesis, but does result in glioma development when combined with Ink4a/Arf loss [33]. In contrast, a recent report demonstrated that somatic retroviral delivery of mutationally activated *BRAF* into the brains of newborn mice results in the development of astrocytomas, with histological features matching the human disease [34]. Future mouse modelling will be required to reconcile these differences in experimental outcomes as well as to determine how deregulated BRAF function and deregulated MAPK signalling, as seen following *KIAA1549:BRAF* expression, result in glioma formation.

NF1-associated PA

NF1 is an autosomal dominant cancer predisposition syndrome affecting one in 3000 individuals worldwide. While

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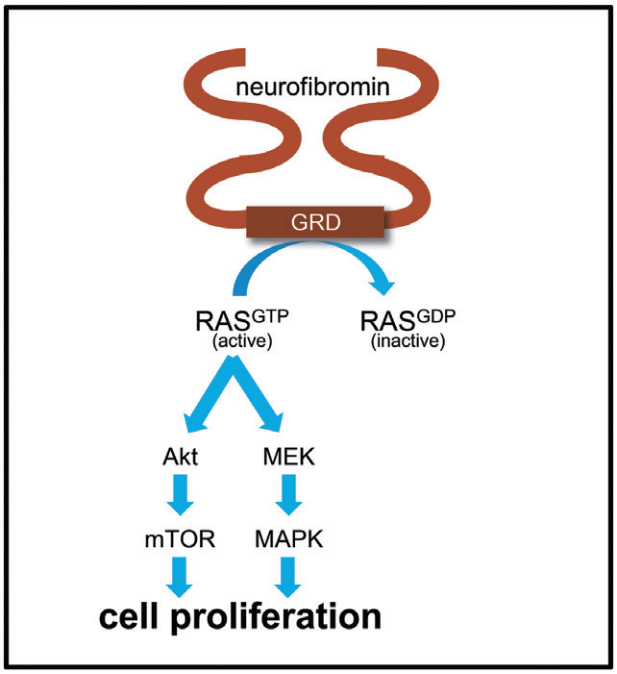

Figure 3. Neurofibromin is a negative regulator of cell proliferation. Neurofibromin functions as a glial cell tumour suppressor protein by accelerating the conversion of the RAS proto-oncogene from its active GTP-bound state to its inactive GDP-bound state. Loss of neurofibromin expression, as seen in neurofibromatosis type 1-associated gliomas, leads to increased RAS-GTP levels and hyperactivation of downstream Akt/mTOR signalling pathways to result in higher levels of cell proliferation. Akt, protein kinase-B; GDP, guanosine diphosphate; GRD, GTPase activating domain; GTP, guanosine triphosphate; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin.

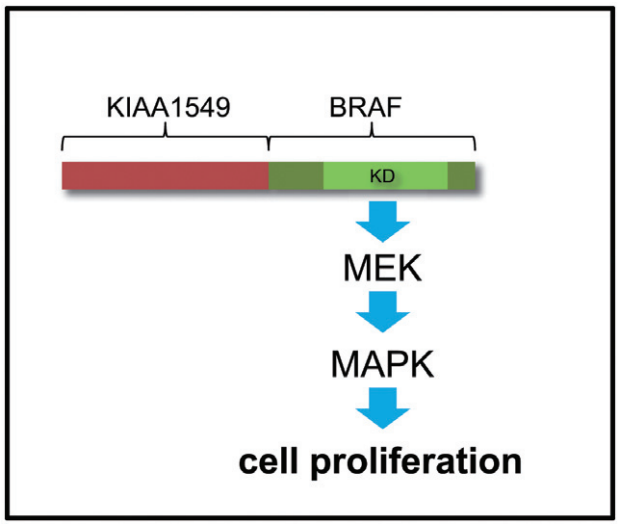

Figure 4. *BRAF* fusion mutations predominate in sporadic pilocytic astrocytoma. A novel fusion of the *KIAA1549* and *BRAF* genes results in increased BRAF kinase function. Deregulated BRAF kinase activity leads to increased mitogen-activated protein kinase (MAPK) pathway signalling, leading to higher levels of cell proliferation. KD, kinase domain; MEK, mitogen-activated protein kinase kinase.

pigmentary abnormalities (café-au-lait macules, skin-fold freckling and iris Lisch nodules) are common in affected individuals [35], 15–20% of children with NF1 develop low-grade gliomas (PAs) of the optic pathway and brainstem (Figure 5). NF1-associated OPGs usually present at a younger age than sporadic OPGs. NF1-associated gliomas are typically detected by 4–5 years of age and rarely grow after age 10 years, whereas sporadic PAs are commonly first diagnosed in the second decade of life. In children with NF1, PAs form anywhere between the optic globe and the optic radiations, although the anterior optic pathway (optic nerves and chiasm) is most frequently involved. Infiltration of the post-chiasmal optic radiations is found in 10% of individuals with NF1-associated OPG, and is usually associated with more aggressive clinical behaviour [36].

Whereas sporadic OPG usually affects only one optic nerve, it is common to encounter bilateral optic nerve involvement in children with NF1. NF1-associated OPGs can be large and exhibit prominent contrast enhancement on magnetic resonance imaging. However, neither the size nor degree of contrast enhancement correlates with the clinical behaviour of the tumour. In this regard, the clinical course of NF1-associated OPGs tends to be more indolent compared to their sporadic counterparts. While 15% of patients with NF1 develop OPGs [37,38], only one-third of children require treatment for clinical progression (visual loss, endocrinopathy). First-line treatment in the majority of cases is carboplatin/vincristine chemotherapy. Response rates range from 30% to 50%, and as many as 70% of children exhibit no further tumour growth while on treatment [39–42].

NF1 gene function

The *NF1* gene is a large tumour suppressor gene located on chromosome 17q11.2 and encodes a 220–250 kDa protein called neurofibromin [43–45]. Neurofibromin is expressed by numerous different cell types, including neurones, Schwann cells, oligodendrocytes, adrenal medulla, muscle and skin [46–48]. Based on its predicted protein sequence, neurofibromin was hypothesized to function primarily as a negative regulator of the p21-RAS proto-oncogene [49,50]. A small portion of the neurofibromin

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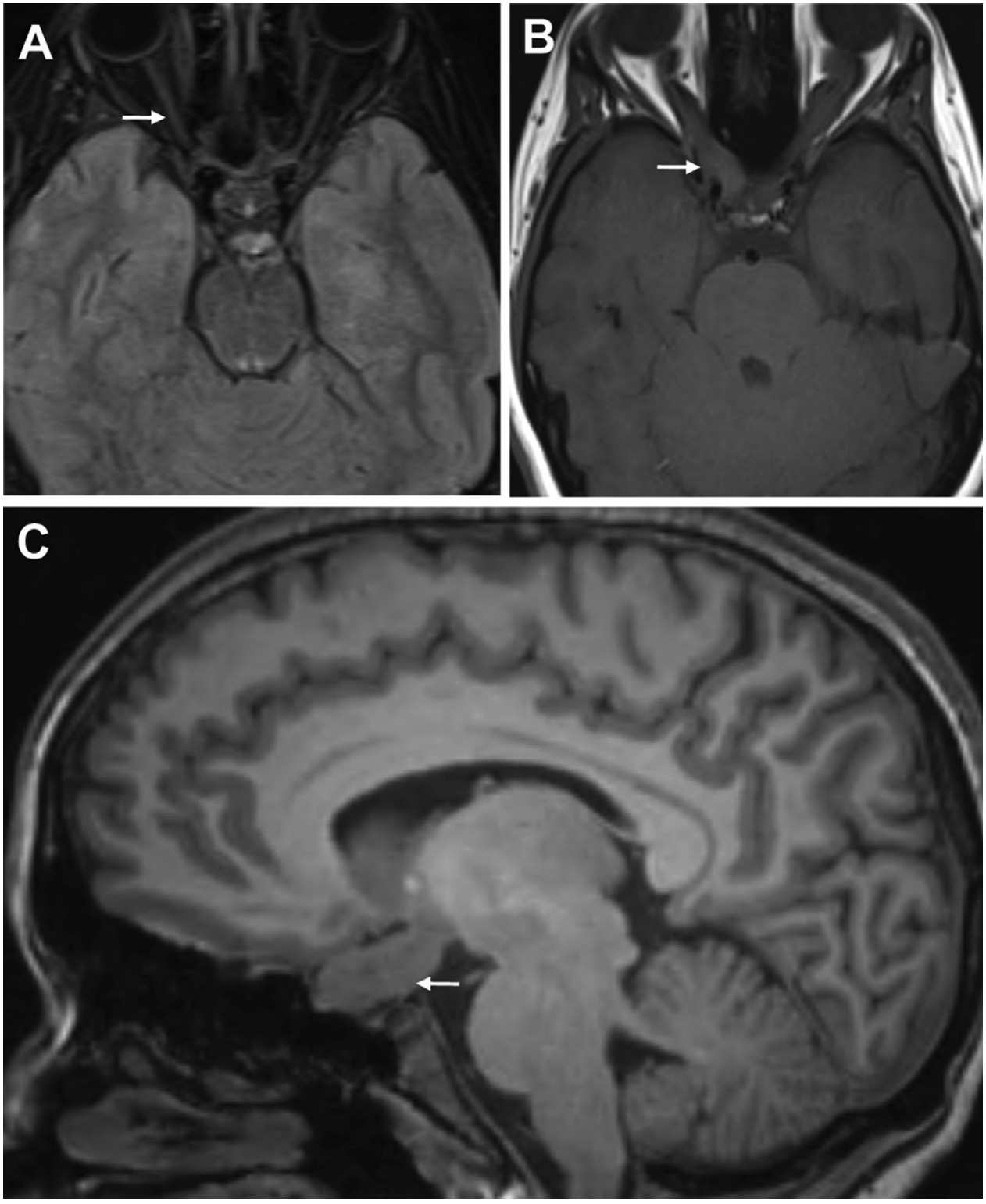

Figure 5. Neurofibromatosis type 1-associated optic glioma. (**A**) A representative normal optic nerve (child without an optic glioma) is shown by axial magnetic resonance imaging (arrow). (**B**) Fusiform enlargement of the optic nerve is evident in a child with a large right optic pathway glioma (arrow). (**C**) The extent of the optic pathway glioma, which includes the optic chiasm, is shown on the sagittal magnetic resonance image (arrow).

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protein shares sequence similarity with a family of molecules called GTPase-activating protein (GAPs) that function to inactivate RAS and reduce RAS-mediated growth signalling. Increased RAS activation leads to increased cell proliferation, and oncogenic transformation in some cell types, supporting the notion that neurofibromin negative growth regulation operates through RAS inhibition. In this regard, loss of neurofibromin in NF1-associated tumours is associated with increased levels of RAS activation [51–53].

Downstream RAS effectors relevant to neurofibromin growth regulation in neuroglial cells include Akt, mammalian target of rapamycin (mTOR) and MAPK. While BRAF regulates MAPK signalling, neurofibromin primarily regulates Akt/mTOR signalling in astrocytes *in vitro* and *in vivo* [54]. mTOR is a serine/threonine kinase involved in the phosphorylation of several downstream targets, and is important in ribosomal biogenesis [55].The Akt/mTOR pathway is hyperactivated in *Nf1*−/− astrocytes, as evidenced by increased activity (and phosphorylation) of the ribosomal S6 protein [54,56]. In OPG mouse models and in human PA, phosphorylated S6 expression is detectable in astrocytes. *Nf1*−/− astrocytes exhibit increased proliferation *in vitro*, which is blocked by rapamycin, a pharmacological inhibitor of mTOR [54]. The finding that mTOR inhibition blocks *Nf1*-deficient glial cell growth prompted preclinical genetically engineered mouse (GEM) studies aimed at determining whether rapamycin might be a suitable targeted therapy for NF1-associated glioma.

In addition to RAS regulation, neurofibromin positively controls intracellular cyclic adenosine monophosphate (cAMP) levels [57,58]. While neurofibromin loss in NF1-associated neuroglial cells leads to increased RAS and RAS pathway activation, reduced neurofibromin expression results in attenuated intracellular cAMP levels. Consistent with this prediction, *Nf1*−/− astrocytes have decreased basal levels of cAMP [58,59]. Numerous cytokines function by binding G protein-coupled receptors and control cell growth and survival in a cAMP-dependent fashion [60]. One of these cytokines, CXCL12 (or stroma-derived factor-1-alpha) is highly expressed along the optic nerve in young children and mice [59]. CXCL12 normally increases cAMP levels in wild-type astrocytes to result in increased programmed cell death (apoptosis). In contrast, CXCL12 treatment of *Nf1*-deficient astrocytes leads to inappropriate cell survival [59]. This observation suggests that therapies targeting cAMP may have utility in the treatment of NF1-associated glioma.

Determinants of gliomagenesis

The unique predilection for NF1-associated gliomas to form in the optic pathway in young children raises the intriguing possibility that gliomagenesis requires more than *NF1* gene inactivation in glial progenitor cells. Several clinical observations support the requirement for other conditions for glioma development in children with NF1. First, while sporadic PAs commonly form in the cerebellum, this is an unusual location for NF1-associated PA. Second, NF1-associated PAs arise in younger children and are frequently less clinically aggressive. Third, the majority of children with NF1 do not develop gliomas, despite having a genetic susceptibility.

The underlying explanations for the pattern of gliomagenesis in NF1 are likely to reflect: (i) differences in susceptibilities of preneoplastic neuroglial cell types in the CNS; (ii) contributions from non-neoplastic cells and signals in the tumour microenvironment; and (iii) genomic modifiers that confer glioma risk.

Neuroglial populations respond differently to *NF1* gene inactivation

The notion that neuroglial cells (astrocytes and astroglial progenitors) from different regions of the CNS are distinct has recently gained traction. Elegant studies by Richard Gilbertson and colleagues have demonstrated that another glial neoplasm (ependymoma) is comprised of separate tumour types based on their cell of origin and genetic background [61]. Based on these findings, it is possible that NF1-associated PAs are distinct from their sporadic counterparts, not only with respect to causative mutation (*BRAF* activation *vs. NF1* gene loss), but also cell of origin. In this regard, the molecular signature of NF1-associated PA was found to be distinct from that seen in sporadic PA [62].

The contribution of cellular heterogeneity to the pattern of gliomagenesis in NF1 is supported by recent studies demonstrating that mouse astrocytes and neural stem cells from different brain regions differ in their ability to proliferate in response to *Nf1* gene inactivation. While astrocytes from the mouse optic nerve and brainstem express neurofibromin, neocortical astrocytes have significantly lower levels of *Nf1* gene expression [63]. These differences in neurofibromin expression account for the response of these distinct astroglial cell populations to *Nf1* gene inactivation: astrocytes from the brainstem and optic

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nerve increase their proliferation *in vitro* and *in vivo* following *Nf1* gene loss, while those from the neocortex fail to increase their growth [63].

In addition, neurofibromin dictates the proliferation of neural stem cells and gliogenesis in a brain region-specific manner [64]. Mouse neural stem cells from the brainstem increase their proliferation *in vitro* and their capacity to form differentiated glial cells *in vivo* following *Nf1* gene inactivation. In contrast, *Nf1*-deficient mouse neural stem cells from the cortex exhibit no significant increase in proliferation or glial differentiation relative to their wild-type counterparts. This cell autonomous difference in neural stem cell behaviour does not reflect neurofibromin expression levels, but rather the expression of one component of the mTOR complex, rictor, which specifies Akt activation.

Collectively, these results demonstrate that neuroglial cells from different brain regions respond uniquely to neurofibromin loss and that these differences may partly determine why gliomas form in the optic pathway and brainstem of children with NF1.

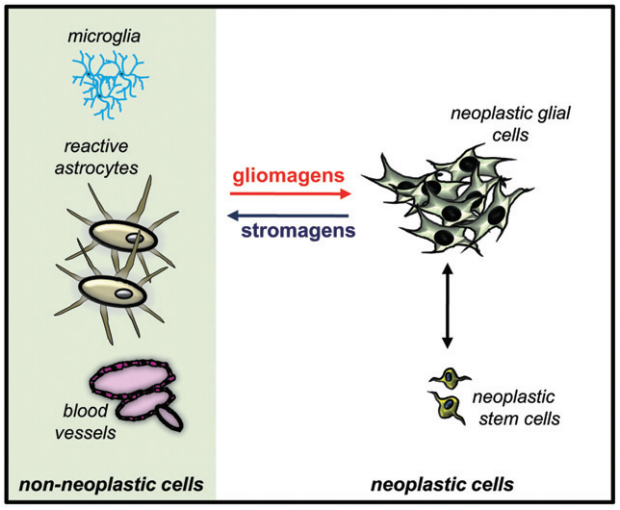

Figure 6. Gliomagenesis requires the interplay between neoplastic and non-neoplastic cellular elements. Paediatric glioma formation ensues following cancer-causing genetic mutations in susceptible preneoplastic cells (glial cells or stem cells), leading to increased glial cell proliferation. These glial lineage cells elaborate molecules ('stromagens') that recruit non-neoplastic cells (microglia, blood vessels and reactive astrocytes) to form a microenvironment permissive for tumour evolution. The non-neoplastic cells in the tumour microenvironment in turn produce additional molecules ('gliomagens') that further promote neoplastic glial cell growth, neoplastic transformation and tumour progression.

The contribution of the tumour microenvironment to glioma formation and growth

Studies in other cancers have revealed an important role for the tumour microenvironment in promoting tumour formation and growth [65–67]. The need for a permissive microenvironment in NF1-associated gliomagenesis is nicely illustrated by *Nf1* GEM modelling experiments. Mice lacking *Nf1* gene expression in astroglial progenitors alone do not form brain tumours [68], whereas *Nf1*+/– mice lacking *Nf1* gene expression in these same astroglial progenitors develop optic gliomas [69,70]. *Nf1*+/– mice have reduced neurofibromin expression in every cell of their body and brain, similar to children born with NF1. The requirement for reduced *Nf1* gene expression in the non-neoplastic cells of the brain supports a model in which gliomagenesis involves the productive interplay between *Nf1*+/– stromal cells and *Nf1*–/– preneoplastic/neoplastic cells (Figure 6).

Because *Nf1* loss in astroglial progenitors must be coupled with reduced *Nf1* gene expression in non-neoplastic (*Nf1*+/–) cells, *Nf1*+/– stromal cells must produce key growth/survival factors that facilitate *Nf1* OPG formation. In both human NF1-associated OPGs and *Nf1* GEM OPGs, there are numerous other cell types present, including non-neoplastic microglia, endothelial cells and reactive astrocytes. Microglia appear early in the evolving *Nf1* mouse OPG [71], which suggests that they may be important stromal cells relevant to glioma formation and growth. These immune system-like cells comprise as many as 30% of the cells in human NF1-associated PAs [72] and produce numerous growth factors and cytokines [73,74].

The importance of microglia to NF1-associated optic glioma growth is underscored by several experimental observations. First, *Nf1*+/–, but not wild-type, microglia increase the proliferation of *Nf1*–/– astrocytes *in vitro* [75]. Second, soluble factors made by *Nf1*+/– microglia increase the proliferation of *Nf1*-deficient astrocytes *in vitro* [75]. Third, inhibition of microglia function attenuates *Nf1* mouse OPG proliferation *in vivo*. In these experiments, blocking microglial activation with the antibiotic minocycline reduces *Nf1* OPG growth [75]. Similarly, inhibiting the signalling pathway responsible for *Nf1*+/– microglial function results in decreased *Nf1* OPG proliferation *in vivo* [76]. Finally, genetic ablation of microglia leads to reduced optic nerve glioma proliferation *in vivo* [72]. Collectively, these observations establish microglia

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as one of the key stromal cell types that drives glioma growth.

Based on *in vitro* experiments demonstrating that *Nf1*+/− microglia produce soluble factors that increase *Nf1*-deficient astrocyte growth, we sought to identify these glioma-promoting molecules (gliomagens). Two such gliomagens have been studied, meningioma-expressed antigen-5 (MGEA5) and CXCL12. *Nf1*+/− microglia express higher levels of MGEA5, a member of the hyaluronidase family of enzymes, than their wild-type counterparts. While the role of MGEA5 and related hyaluronidase molecules in NF1-associated gliomagenesis has not been fully elucidated, hyaluridonase treatment of *Nf1*-deficient astrocytes increases their proliferation, and inhibition of hyaluronidase function ameliorates the growth advantage conferred by *Nf1*+/− microglia [75]. Additional studies will be required to clarify the role of this interesting molecule as a gliomagen in the formation and growth of NF1-associated gliomas.

In addition, *Nf1*+/− microglia produce two to threefold more CXCL12 than wild-type microglia. The importance of CXCL12 to gliomagenesis is underscored by several observations. First, CXCL12 increases the survival of *Nf1*−/− astrocytes in a cAMP-dependent fashion, but leads to increased apoptosis in wild-type astrocytes [59]. Second, pharmacological inhibition of cAMP degradation using a phosphodiesterase-4 (PDE4) inhibitor, Rolipram, attenuates *Nf1* mouse OPG growth *in vivo* [77]. Third, lentiviral expression of CXCL12 in the forebrain of *Nf1* OPG mice results in low-penetrance glioma formation [78]. To more effectively lower cyclic AMP levels and mimic the effect of CXCL12 on *Nf1*-deficient astroglial cells, lentiviral delivery of PDE4 into the forebrains of *Nf1* optic glioma mice results in a greater incidence of glioma formation [77].

Together, these findings suggest that gliomagenesis and continued tumour growth require interactions between non-neoplastic and neoplastic cell types. We envision a model in which *Nf1*-deficient preneoplastic glial progenitor cells produce molecules that attract microglia and other non-neoplastic cell types (stromagens). These stromagens create a microenvironment that supports glioma formation. In turn, the attracted *Nf1*+/− stromal cells (microglia) elaborate gliomagens (growth factors and cytokines) that facilitate further *Nf1*-deficient astroglial cell proliferation/survival and permit neoplastic transformation. Each of these factors represents a potential target for stroma-directed therapies that aim to inhibit the formation of a permissive tumour microenvironment or block the tumour-promoting activity of stroma-derived gliomagens.

Genomic determinants of gliomagenesis

As 80–85% of children with NF1 do not develop OPGs while the vast majority (90–95%) of *Nf1* genetically engineered mice harbouring identical genetic changes do, it is likely that subtle genomic alterations dictate which children with NF1 will develop a brain tumour. In experimental *Nf1* mouse models, the specific genetic background greatly influences glioma development. Mice heterozygous for mutations in the *Nf1* and *Trp53* (p53) tumour suppressor genes (*Nf1*+/−; *Trp53*+/− *cis*; NP*cis* mice) develop brain gliomas at a much higher frequency on the B6/C57BL/6J (B6) genetic background compared to the 129S4/SvJae (129S4) background [79–81]. These findings support the existence of epigenetic or polymorphic differences that potentially confer resistance or susceptibility to gliomagenesis. These modifier loci could function by changing: (1) the expression or function of genes important for glial progenitor behaviour; (2) neurofibromin growth regulation; or (3) chemokine function or microglia activity, which separately or collectively impact on glioma formation or growth. While the modifier genes have yet to be elucidated, genomic polymorphisms in these loci might identify which children with NF1 are at highest risk for the development of OPG. The identification of single nucleotide polymorphisms predictive of glioma risk would positively impact on the management of children with NF1, and would allow clinicians to stratify children from an early age into clinically relevant subgroups for surveillance and potential treatments.

Implications for paediatric glioma-targeted therapies

The management approaches for PA in children currently involve surgery and genotoxic chemotherapy. Surgical resection is often a first-line therapy for cerebellar and hemispheric PAs, but is associated with significant morbidity when tumours are located in deeper structures (brainstem) or in the optic pathway. For these tumours, chemotherapy or radiotherapy is recommended. While radiotherapy is curative, its use for paediatric glioma is problematic, owing to the high incidence of cognitive and neuroendocrine adverse effects that result from

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irradiating the developing brain. Moreover, radiation is contraindicated in children with NF1; following brain irradiation, there is an increased frequency of secondary high-grade glioma and moyamoya disease [82,83].

Chemotherapy with carboplatin and vincristine is usually well tolerated and achieves good disease control [40,42,84]. However, antineoplastic drugs cause DNA damage not only in cancer cells, but may also impact other dividing cell populations in the developing brain. For this reason, more targeted therapies are actively being sought for this patient population.

With the availability of robust preclinical mouse models of *Nf1* OPG, it becomes possible to identify and evaluate alternative therapeutic strategies. Using *Nf1* OPG mice, previous studies have shown that treatment with a genotoxic alkylating agent, temozolomide, results in decreased OPG volumes, reduced glioma proliferation and increased glioma cell death [85]. These GEM strains have subsequently been used to evaluate both neoplastic and non-neoplastic cell-directed therapies. In this regard, treatments that target the deregulated RAS/mTOR [85] and cyclic AMP [77] signalling pathways in the *Nf1*-deficient astroglial cells reduce *Nf1* OPG proliferation. Similarly, therapies that inhibit non-neoplastic microglia function also attenuate OPG growth in these *Nf1*-mutant mice *in vivo* [72,75,76].

Another use of *Nf1* genetically engineered mice is the development of therapies that restore visual function. Previous studies have revealed that improvement in visual function infrequently accompanies the successful chemotherapeutic treatment of NF1-associated glioma [41]. Insights into the cellular and molecular basis for optic nerve dysfunction have been partly revealed by the analysis of *Nf1* OPG mice. In these studies, reduced visual evoked potentials are seen early during tumour development and are associated with axonal damage and retinal ganglion cell death [86,87]. *Nf1*+/− neurones exhibit increased apoptosis, which reflects reduced levels of intracellular cAMP [88]. Raising cAMP levels with the PDE4 inhibitor (Rolipram) during tumour formation resulted in reduced retinal ganglion cell apoptosis [88], suggesting a potential neuroprotective adjuvant strategy for managing OPGs in children with NF1.

Conclusion

Advances in genomic technologies, small-animal modelling and molecular biology discovery methods offer unprecedented opportunities to develop personalized treatments for children with low-grade gliomas. It is possible that future therapies will incorporate genetic/genomic diagnostics to identify patients at high risk for glioma development and progression, as well as specific glioma subtypes most likely to respond to targeted therapies. Similarly, the use of robust low-grade glioma GEM strains facilitates the identification of treatments with efficacy against particular molecular subgroups of paediatric low-grade glioma. These next-generation treatments might target specific cell types or gliomagens in the tumour microenvironment in combination with standard or biologically based antineoplastic therapies. This ecosystems approach could reduce the toxicity of current treatments on the developing brains of children. Finally, the use of adjuvant neuroprotective strategies has the potential to decrease secondary neurocognitive declines and partially restore vision in affected children.

Acknowledgements

We thank Dr Robert Schmidt (Division of Neuropathology, School of Medicine, Washington University) for the photomicrographs shown in Figure 2.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Received 22 August 2011
Accepted after revision 9 October 2011
Published online Article Accepted on 28 October 2011

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