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REVIEW

The molecular and cell biology of pediatric low-grade gliomas

Y-H Chen and DH Gutmann

Pilocytic astrocytoma (PA) is the most common glial cell tumor arising in children. Sporadic cases are associated with *KIAA1549:BRAF* fusion rearrangements, while 15–20% of children develop PA in the context of the neurofibromatosis 1 (NF1) inherited tumor predisposition syndrome. The unique predilection of these tumors to form within the optic pathway and brainstem (NF1-PA) and cerebellum (sporadic PA) raises the possibility that gliomagenesis requires more than biallelic inactivation of the *NF1* tumor suppressor gene or expression of the *KIAA1549:BRAF* transcript. Several etiologic explanations include differential susceptibilities of preneoplastic neuroglial cell types in different brain regions to these glioma-causing genetic changes, contributions from non-neoplastic cells and signals in the tumor microenvironment, and genomic modifiers that confer glioma risk. As clinically-faithful rodent models of sporadic PA are currently under development, *Nf1* genetically-engineered mouse (GEM) models have served as tractable systems to study the role of the cell of origin, deregulated intracellular signaling, non-neoplastic cells in the tumor microenvironment and genomic modifiers in gliomagenesis. In this report, we highlight advances in *Nf1*-GEM modeling and review new experimental evidence that supports the emerging concept that *Nf1*- and *KIAA1549:BRAF*-induced gliomas arise from specific cell types in particular brain locations.

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INTRODUCTION

Tumors of the nervous system are the leading cause of cancer-related death in children. Among the various histological subtypes, the most common brain tumor in the pediatric population is the WHO grade I pilocytic astrocytoma (PA).[1,2] These low-grade PAs are characterized by glial fibrillary acid protein (GFAP) expression and unique histologic features, including eosinophilic granular bodies and Rosenthal fibers.[3] Unlike their more malignant counterparts, they lack palisading necrosis, high mitotic indices or pronounced nuclear atypia. However, PAs may exhibit prominent microvascular proliferation and harbor large numbers of microglia. In addition, in distinction to adult gliomas, PAs most commonly arise in the optic pathway, brainstem and cerebellum.

Until recently, the only known genetic alteration associated with PA was mutational inactivation of neurofibromatosis 1 (*NF1*) tumor suppressor gene in children with the NF1 inherited tumor predisposition syndrome.[4,5] In these NF1-associated gliomas, biallelic inactivation of the *NF1* gene was observed, concomitant with the loss of *NF1* protein (neurofibromin) expression.[4,6] In the context of NF1, PAs predominate in the optic pathway (optic nerve, chiasm and post-chiasmatic radiations) in children younger than 7 years of age (Figures 1a and b).[7] While 75% of NF1-PAs are located within the optic pathway, 15–20% of these gliomas may also arise in the brainstem.[8]

In contrast to NF1-associated PA, sporadic PAs usually arise in the cerebellum (Figures 1c and d), but may also be located, with decreasing frequency, in the brainstem and optic pathway. When they form in the cerebellum, there is frequently a prominent cystic component, and surgical resection is usually curative. While *NF1* loss is not found in sporadic PAs,[5,9] converging evidence from numerous laboratories has revealed that the majority of non-NF1-associated PAs harbor a somatic rearrangement, in which the kinase domain of the *BRAF* gene is fused to an unknown gene (*KIAA1549*). This fusion event (*KIAA1549:BRAF* or *f-BRAF*) is most commonly detected in sporadic PAs arising in the cerebellum.[4,5,10,11]

The fact that these tumors do not progress to higher grade glial malignancies and exhibit a geographical predilection for specific midline brain regions raises the intriguing possibility that pediatric PAs are neurodevelopmental disorders. Similar to other neurodevelopmental diseases, these low-grade gliomas in children likely obey the rules that govern normal patterning in the developing central nervous system. In this review, we will discuss recent studies that collectively support a model in which pediatric gliomagenesis requires the confluence of numerous critical conditions that reflect activation of specific growth control pathways, the cell of origin, brain region-specific constraints and the local microenvironment.

GROWTH CONTROL PATHWAYS

With the identification of the *NF1* gene and *f-BRAF* fusion event, it now becomes possible to determine how PA growth is regulated. The *NF1* gene product, neurofibromin is a large cytoplasmic protein (220–250 kDa) that contains three differentially-spliced exons (9a, 23a and 48a)[12] (Figure 2a). While the precise function of these alternatively spliced products has not been clearly elucidated to date, they likely reflect tissue-specific or differentiation-regulated properties of neurofibromin.[13–17] Inspection of the predicted protein sequence of neurofibromin reveals a 300 amino acid domain with similarity to proteins that negatively regulate RAS proto-oncogene activity. These GTPase-activating proteins accelerate the conversion of active GTP-bound

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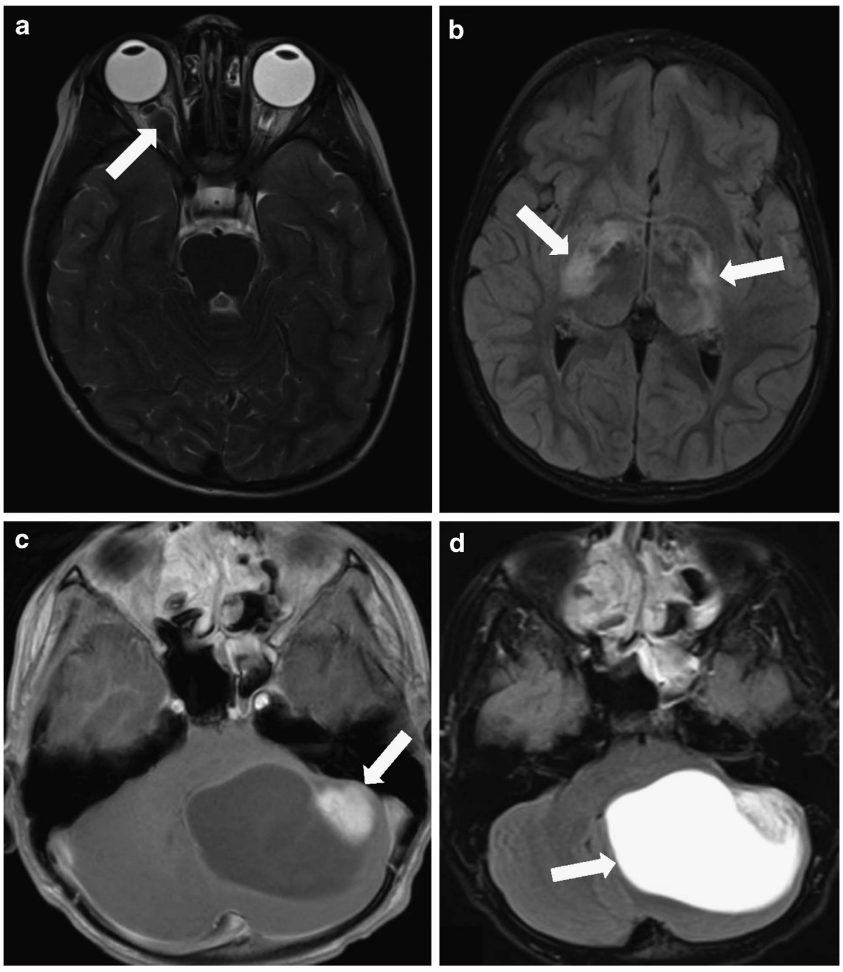

Figure 1. PAs in children. (**a**) Optic pathway glioma in a child with NF1 predominantly affecting the right optic nerve (arrow). (**b**) Optic pathway glioma in a child with NF1 involving the post-chiasmatic optic radiations (arrow). (**c, d**) Large left cerebellar PA with a nodular component (arrow, panel **c**) and a significant cystic component (arrow, panel **d**).

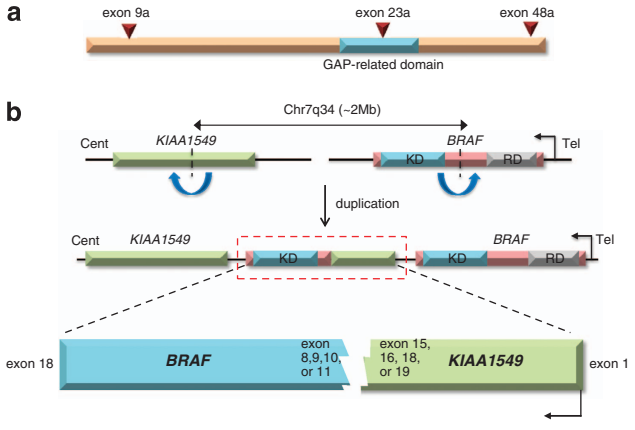

Figure 2. (**a**) Structure of the *NF1* gene product, neurofibromin. Neurofibromin is a 2818 amino acid protein containing three alternatively spliced exons (9a, 23a and 48a), which reflect tissue-specific or differentiation-regulated RNA splicing events. The GTPase-activating proteins-related domain of neurofibromin functions as a negative RAS regulator. (**b**) Schematic representation of the predicted structure of human *KIAA1549:BRAF* fusion proteins. Numerous distinct rearrangements have been described involving different exons from *KIAA1549* and *BRAF*. RD, regulatory domain; KD, kinase domain.

RAS to its inactive GDP-bound form, thus inactivating RAS, and reducing RAS-mediated growth signaling.[18–20] In this respect, loss of neurofibromin expression results in increased RAS activity.[20,21]

Recently, array-based comparative genomic hybridization identified a low-level copy number gain of the *BRAF* gene in a large portion of PAs.[11,22] Further studies demonstrated that this gain was due to a tandem duplication at 7q34 with subsequent fusion between the 5′ end of the *KIAA1549* gene and the 3′ end of the *BRAF* gene (Figure 2b).[10,11,22,23] The resulting fusion transcripts contain the amino terminus of the *KIAA1549* protein and the *BRAF* kinase domain, but lack the auto-regulatory domain of *BRAF*, resulting in constitutive BRAF kinase activity and downstream activation of the mitogen-activated protein kinase signaling pathway. In addition to *KIAA1549:BRAF*, other somatic mutations have also been reported in PA, including rare oncogenic *Ras* or *BRAF* mutations,[24–28] as well as *SRGAP3:RAF1*[26] and *FAM131B:BRAF*[29] gene fusions, which similarly result in increased mitogen-activated protein kinase activation.

In many cell types, RAS activation leads to increased signaling through RAS downstream effector proteins, including PI3-Kinase (PI3K)/AKT and RAF/MEK (Figure 3). To define how neurofibromin loss regulates glial cell proliferation downstream of RAS, a proteomic approach was utilized. Using wild-type and *Nf1*-deficient mouse brain astrocytes, mass spectrometry revealed increased expression of ribosomal proteins involved in protein translation following neurofibromin loss.[30] As predicted from the

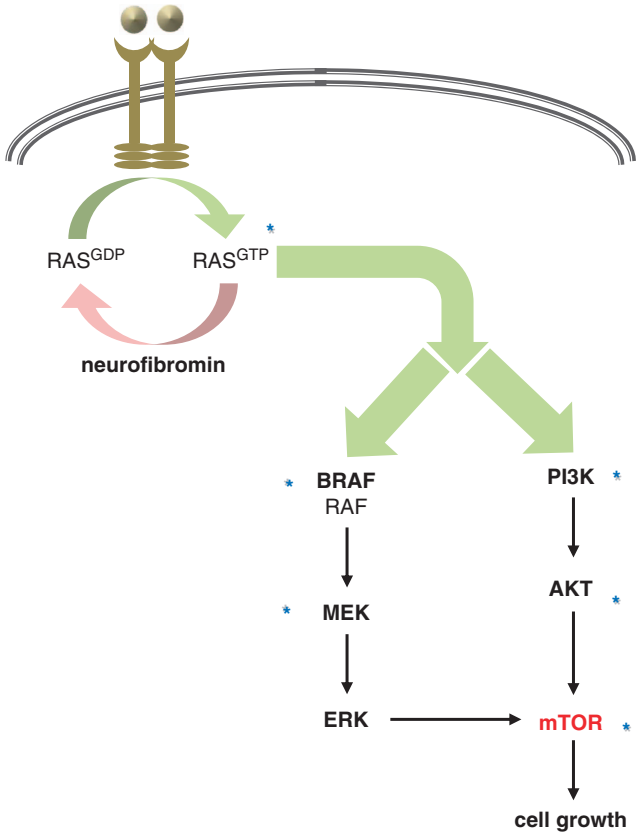

Figure 3. Regulation of neuroglial cell proliferation. Neurofibromin operates as a tumor suppressor protein by accelerating the conversion of the RAS proto-oncogene from its active, GTP-bound state to its inactive, GDP-bound state. Loss of neurofibromin expression, as seen in NF1-associated gliomas, results in increased RAS-GTP levels and hyperactivation of downstream RAS signaling pathways, to result in increased cell growth. RAS activation following neurofibromin loss leads to high levels of MEK/ERK and PI3K/AKT/mTOR activity. Deregulated BRAF kinase activity leads to increased MEK/ERK signaling to result in high levels of mTOR activation and increased cell growth. The asterisks denote the molecular targets for which clinically-available pharmacological inhibitors exist for the potential treatment of PAs in children.

elevated ribosomal protein expression in *Nf1*-deficient astrocytes, these cells also exhibited increased protein synthesis resulting from hyperactivation of the mammalian target of rapamycin (mTOR) protein. mTOR is a serine/threonine kinase, which directly controls protein synthesis and ribosomal biogenesis by modulating ribosomal S6-Kinase activity. Subsequent experiments demonstrated mTOR hyperactivation in *Nf1* mouse optic glioma and NF1-associated human PA tumors. In addition, a previous study showed that loss of neurofibromin expression was associated with PI3K-AKT signaling pathway activation in a single human NF1-associated PA.[6] The importance of PI3K/AKT and mTOR pathway activation to neurofibromin growth regulation in astrocytes and neural stem cells (NSCs) was further confirmed by pharmacological and genetic inhibition of mTOR function in *Nf1*-deficient astrocytes.[30,31] Relevant to the treatment of NF1-PA, treatment of *Nf1*-deficient astrocytes with the mTOR inhibitor, rapamycin, reduced their proliferation to wild-type levels and attenuated glioma growth in a mouse model of *Nf1* optic glioma.[32]

In contrast, deregulated BRAF activity is thought to increase cell growth in a MEK/mitogen-activated protein kinase-dependent manner. While MEK inhibition ameliorates the growth advantage conferred by *f-BRAF* expression, recent studies have demonstrated that MEK activation converges on a pathway shared with NF1-PA. In cerebellar NSCs expressing *f-BRAF*, increased MEK activation leads to mTOR hyperactivation (Figure 3). While *Nf1* loss in astrocytes activates mTOR in a PI3K/AKT-dependent manner,[33] *f-BRAF* expression in these NSCs results in increased mTOR activation by phosphorylation and inactivation of the tuberous sclerosis complex-2 protein (tuberin), leading to elevated Rheb-mediated mTOR signaling.[34] These exciting findings suggest that mTOR might be a central target for both sporadic and NF1-associated PA, and have prompted recent clinical studies using rapamycin analogs to treat these tumors.

CELL OF ORIGIN

Studies on malignant brain tumors, as well as other central nervous system tumor types (ependymoma, medulloblastoma (MB), meningioma) have demonstrated that the cell of origin and developmental stage matter greatly in the genesis of these cancers.[35–38] For example, intracranial and spinal ependymomas likely arise from radial glial cells,[38] which function as NSCs in the embryo and give rise to adult NSCs.[39] Further evidence has revealed that *Ink4a/Arf-/-* NSCs with amplified *EPHB2* from the embryonic cerebrum, but not from other regions or from adults, form ependymomas resembling human supratentorial ependymoma.[40] Similarly, distinct subgroups of MB are characterized by activation of different signaling pathways, including sonic hedgehog and WNT.[41] While the desmoplastic subtype of MB harboring mutations in the sonic hedgehog pathway likely arises from granule neuron precursors,[42] the classic subtype with aberrant WNT pathway signaling derives from mossy-fiber neuronal precursors in the dorsal brainstem.[36]

The cell of origin for malignant glioma remains controversial, as these cancers can arise from numerous cell types, ranging from lateral subventricular zone (SVZ) NSCs[35,43] and $NG2^+$ progenitors[44–48] to mature astrocytes and neurons.[49,50] For example, conditional inactivation of *Tp53*, *Nf1* and *Pten* tumor suppressor genes in mouse SVZ NSCs can induce malignant astrocytomas.[35,43] In contrast, introducing platelet-derived growth factor into adult mouse white matter, tumor-initiating $NG2^+/GFAP^-$ progenitor cells recruits/activates other progenitors to promote malignant glioma.[47] Similarly, other studies have suggested that more differentiated glial cell types (astrocytes) might directly transform into glioma.[49] Even more surprising is the recent report that mature neurons can undergo dedifferentiation in response to glioma-associated genetic changes to create a progenitor state sufficient to initiate glioma formation.[50]

Genetically-engineered mouse (GEM) models have provided powerful instructive tools to study the cellular origins of brain tumors. While Parada and colleagues have demonstrated that malignant gliomas arise following biallelic *Nf1* and *Tp53* inactivation in NSCs within the lateral ventricle germinal zone,[35] the cells from which *Nf1* low-grade gliomas arise has proven more elusive. Using a series of Cre driver lines to conditionally inactivate the *Nf1* tumor suppressor gene in several potential progenitor cell types at various times during development, recent studies have revealed that *Nf1* mouse optic gliomas most likely arise from neuroglial progenitors within the germinal layer of the third ventricle (TVZ). In these experiments, $Nf1+/-$ mice in which the *Nf1* gene was inactivated in $NG2^+$ progenitors did not develop brain tumors, excluding this cell type as the cell of origin for optic glioma.[51] Similarly, $Nf1+/-$ mice in which the *Nf1* gene was inactivated in postnatal $GFAP^+$ astrocytes did not form gliomas:[52] Only $Nf1+/-$ mice in which *Nf1* gene inactivation occurred between 11.5 and 16.5 days of embryonic development in $GFAP^+$ neuroglial progenitors developed optic gliomas.[52] As both the lateral and TVZ contain neuroglial progenitors, it is possible that

both germinal zones could provide the cells of origin for *Nf1* optic glioma.

Subsequent transcriptomal analyses revealed that NSCs from the TVZ and those from the lateral ventricle SVZ represent two molecularly-distinct stem cell populations.[52] Moreover, the observation that TVZ NSCs, but not their lateral ventricle-SVZ counterparts, exhibit increased proliferation *in vitro* and increased glial differentiation *in vivo* following *Nf1* gene inactivation demonstrates that murine *Nf1* optic gliomas most likely arise from neuroglial progenitors residing within the TVZ. This conclusion is further supported by previous studies of human optic glioma, demonstrating that a discrete population of GFAP-positive radial glial cells extends bilaterally from the floor of the TVZ to the optic nerve.[53]

Unlike *Nf1* low-grade optic glioma, experimental mouse models for *f-BRAF*-associated sporadic PA are currently unavailable. Several studies have shown that ectopic expression of either constitutively-activated RAF-1 or $BRAF^{V600E}$ alone is not sufficient to induce gliomas in mice, suggesting that an activated *BRAF* mutation alone may not be an oncogenic driver.[54–56] However, *RAF-1* and $BRAF^{V600E}$ mutations are rare mutations in PA relative to the signature *f-BRAF* genetic alteration.[57] Using the most common *f-BRAF* genetic alteration ($KIAA1549^{ex16}$-$BRAF^{ex9}$), recent studies have revealed similar cell of origin effects.[34] While ectopic *f-BRAF* expression increases cerebellar mouse NSC proliferation, it has no effect on primary mouse astrocyte growth *in vitro*. In addition, mice transplanted with *f-BRAF*-expressing cerebellar NSCs develop low-grade glioma-like lesions within the cerebellum *in vivo*. While deregulated BRAF activity leads to increased proliferation in mouse NSCs, other studies have indicated that this effect is transient, and is followed by oncogene-induced senescence in human PA-associated glioma stem cells.[58,59] Collectively, these results underscore the importance of cellular context and developmental age as important determinants of low-grade glioma tumorigenesis.

BRAIN REGION

While malignant gliomas can arise in nearly any location within the central nervous system, glial cell tumors (astrocytomas or gliomas) are most frequently observed in the cerebellum, brainstem and optic pathway/hypothalamus in children.[3] An association between genetic mutation and tumor location is one of the earliest recognized features in low-grade gliomas. Somatic *BRAF* rearrangement is specific to sporadic PAs and more frequent in cerebellar, but rare in supratentorial hemispheric, PAs. In contrast, NF1-associated PAs are mainly seen in the optic pathway and brainstem, but uncommonly arise in the cerebellum or cortex.[22,57,60,61]

Although the molecular bases for these region-specific distributions have not been fully elucidated, one key determinant could be cell-intrinsic responses to specific glioma-associated genetic alterations. Previous studies from PAs and another glial neoplasm (ependymoma) have suggested that glial tumors of the same histological types often comprise clinically and molecularly-distinct subgroups retaining specific signatures that reflect their respective brain regions of origin.[38,40,62] In this regard, astrocytes from different brain regions (brainstem, optic nerve, cerebellum and neocortex) harbor unique gene expression signatures.[63] One of the differentially-expressed genes in mouse neocortical astrocytes relative to all other astrocyte populations was the *Nf1* tumor suppressor gene. Consistent with significantly lower levels of neurofibromin, loss of *Nf1* expression in cortical astrocytes had no effect on cell proliferation *in vitro*, whereas *Nf1* loss in optic nerve, cerebellar and brainstem astrocytes resulted in increased proliferation.[63] Similarly, mouse NSCs from the brainstem exhibit increased proliferation and gliogenesis following *Nf1* inactivation, whereas neocortical NSCs did not.[31] Unlike the situation with astrocytes, this differential response to *Nf1* loss was not the consequence of reduced neurofibromin expression, but rather resulted from differential expression of the mTOR component rictor.[31] In brainstem NSCs, which express fivefold more rictor relative to cortical NSCs, *Nf1* loss results in increased mTORC2-mediated Akt activation and p27 degradation, and leads to enhanced NSC proliferation and glial differentiation.

Similar to NF1, brain region heterogeneity also contributes to the patterning of sporadic *f-BRAF*-associated PA. While NSCs from the TVZ and cerebellum exhibit increased proliferation following *f-BRAF* expression, *f-BRAF*-expressing NSCs from the lateral ventricle-SVZ or neocortex do not.[34,52] This brain region-specific effect is not related to the ability of *f-BRAF* to activate MEK/mitogen-activated protein kinase signaling, but rather reflects the ability of f-BRAF to phosphorylate tuberin and activate mTOR in neocortex NSCs. Together, these findings suggest that unique populations of location-specific progenitor cells are the cells of origin for histologically-similar glial cell tumors, and may confer distinct molecular properties on gliomas arising in different brain regions.

LOCAL MICROENVIRONMENT

In order to recapitulate NF1-associated gliomagenesis in mice, it is necessary to inactivate both copies of the *Nf1* gene in astroglial progenitor cells. As conventional *Nf1* knockout ($Nf1-/-$) mice are embryonic lethal,[64,65] mice in which the *Nf1* gene was conditionally inactivated in specific cell populations were generated using Cre-LoxP technology.[66,67] Surprisingly, conditional knockout mice with complete loss of neurofibromin expression in $GFAP^+$ astroglial progenitor cells did not develop brain tumors, suggesting that other factors are required for tumorigenesis.

As children with NF1 start life with one functional and one non-functional *NF1* gene in every cell of their bodies ($NF1+/-$ humans), $Nf1+/-$ mice were employed to generate $Nf1+/-$ mice lacking *Nf1* expression in astroglial progenitor cells. Using two independent GFAP-Cre driver strains to inactivate the *Nf1* gene in neuroglial progenitors during embryogenesis, the majority of $Nf1+/-$ mice with astroglial *Nf1* loss ($Nf1+/-{}^{GFAP}$CKO mice) develop optic gliomas.[68] These unexpected findings suggested that *Nf1* optic gliomagenesis requires coupling of complete *Nf1* loss in neuroglial progenitors with reduced *Nf1* gene expression in non-neoplastic cells. Moreover, it argues that non-neoplastic $Nf1+/-$ stromal cells are required for *Nf1* optic glioma formation.

Previous studies have shown that endothelial cells, reactive astrocytes and microglia (macrophage-like immune system cells) are among the many non-cancerous cells capable of producing growth factors that increase preneoplastic and neoplastic cell growth.[35,69,70] Although the other stromal cell types are likely important for creating a permissive microenvironment for glioma formation and continued growth, subsequent studies focused on microglia, based on their abundance in human sporadic and NF1-associated PA.[71,72] In this respect, microglia comprise 30–50% of the total number of cells in these tumors, and have been implicated in the maintenance of brain homeostasis, including neuronal survival,[73] synaptic function[74,75] and neurotrophic factor production.[76] In addition, reactive gliosis in response to injury is partially dependent on microglia/macrophage-induced sonic hedgehog activation in astrocytes,[77] raising the possibility that microglia promote both reactive gliosis and endothelial cell proliferation.[78]

Consistent with this observation, increased numbers of microglia were found in $Nf1+/-{}^{GFAP}$CKO mouse optic nerves before obvious optic glioma formation.[71] Moreover, $Nf1+/-$ microglia, but not wild-type microglia, increase the proliferation of $Nf1-/-$ astrocytes in a paracrine fashion *in vitro*.[79] Additional evidence for a critical role for microglia in optic glioma

proliferation derives from pharmacologic and genetic microglia inhibition studies.[79–81] First, treatment of *Nf1*+/− ^{GFAP}CKO mice with minocycline to impair microglia function results in reduced optic glioma tumor proliferation. Second, *Nf1*+/− microglia (but not *Nf1*-deficient astrocytes) exhibit increased JNK activation, such that pharmacologic JNK inhibition of microglia in *Nf1*+/− ^{GFAP}CKO mice attenuates tumor cell proliferation. Third, genetically reducing microglia following gancyclovir-mediated death in *Nf1*+/− ^{GFAP}CKO mice expressing the thymidine kinase gene in monocytes (CD11b-TK mice) reduces optic glioma tumor proliferation.

To determine whether microglia are required for optic gliomagenesis, recent work has employed mice with impaired fractalkine receptor (CX3CR1) expression.[82] CX3CR1 is expressed in resident brain microglia and partly drives microglia infiltration and function in response to chemokines.[82,83] *Nf1*+/− ^{GFAP}CKO mice develop optic glioma by 2–3 months of age,[67,71] while mice in which one *Cx3cr1* allele was silenced (*Nf1*+/− ^{GFAP}CKO-CX3CR1 mice) have reduced numbers of optic nerve microglia at 6 weeks and 3 months of age and no evidence of glioma.[84] However, by 4 months of age, the numbers of microglia have normalized and optic gliomas are now evident, further underscoring the importance of these stromal cell types in both glioma formation and maintenance.

The notion that neoplastic glial cells release soluble factors (stromagens) that recruit or activate microglia, which in turn produce additional tumor-promoting molecules (gliomagens) to increase the proliferation, survival and invasion of neoplastic cells has recently gained traction (Figure 4).[85–88] One of these potential gliomagens is the stroma-derived growth factor CXCL12, a ligand for the CXCR4 and CXCR7 chemokine receptors.[89] First, high levels of CXCL12 are found in young children and mice along the optic pathway, but not in adults.[90] Second, *Nf1*+/− microglia produce high levels of CXCL12 relative to their wild-type counterparts. Third, CXCL12 increases the survival of *Nf1*-deficient astrocytes, but causes apoptosis in wild-type astrocytes. As neurofibromin positively regulates cyclic adenosine monophosphate (cAMP) levels,[91,92] the lower intracellular cAMP levels in *Nf1*−/− astrocytes has been shown to enhance cell survival in response to CXCL12.[90] In this regard, defective cAMP generation in *Nf1*-deficient astroglial cells may influence gliomagenesis. Consistent with this hypothesis, treatment of *Nf1*+/− ^{GFAP}CKO mice with the phosphodiesterase-4 (PDE4) inhibitor, Rolipram, elevates cAMP levels by blocking cAMP degradation, and attenuates *Nf1* mouse optic glioma growth *in vivo*.[93] Conversely, lentiviral delivery of phosphodiesterase-4 into the forebrains of *Nf1*+/− ^{GFAP}CKO mice locally lowers cAMP levels, and results in ectopic glioma formation in some mice.[93] These findings argue that CXCL12 is one critical stromal determinant important for dictating NF1-associated gliomagenesis.

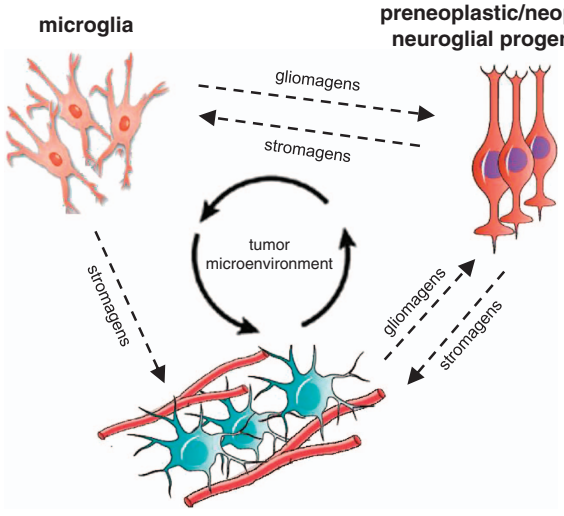

Figure 4. The glioma tumor microenvironment. Tumor initiation in preneoplastic/neoplastic cells results from complete loss of *Nf1* tumor suppressor gene expression. These *Nf1*-deficient neuroglial progenitors release factors (stromagens) that recruit or activate microglia, as well as other stromal cell types (endothelial cells and reactive astrocytes). Non-neoplastic stromal cells in turn elaborate gliomagens to create a supportive niche that facilitates the growth and expansion of preneoplastic/neoplastic neuroglial progenitors. The glioma tumor microenvironment is established by interactions between preneoplastic/neoplastic and non-neoplastic stromal cell populations, which together promote gliomagenesis and tumor progression.

Currently, little is known about the role of the tumor microenvironment in sporadic PAs. Although *f-BRAF* expression is sufficient to induce glioma-like lesions in the cerebellum of mice *in vivo*, it is possible that *f-BRAF* expression in the proper cell type (cerebellar NSCs) must be coupled with brain region-specific stromal constraints. For example, in MB, the expression of hepatocyte growth factor in cerebellar neural progenitor cells in the setting of enhanced sonic hedgehog signaling increases the frequency of MB formation.[94] With the recent development of a novel conditional *f-BRAF* mouse (Kaul A, Chen Y-H and Gutmann DH, manuscript in preparation), the influence of the local microenvironment is now being explored in mechanistic detail.

GENOMIC CONTRIBUTIONS TO GLIOMAGENESIS

Accumulating evidence from a number of studies has demonstrated that epigenetic changes may also influence tumorigenesis.[95,96] Reilly and colleagues[97] have employed *Nf1* GEM strains harboring heterozygous mutations in both the *Nf1* and *p53* genes (*NPcis* mice) to elucidate the role of genomic modifiers in gliomagenesis. In these studies, they have shown that gliomas form at a higher frequency on the inbred C57BL/6J (B6) genetic background relative to the 129S4/SvJae (129) background. Subsequent experiments demonstrated that B6 alleles on mouse chromosome 11 were found to confer this dominant susceptibility.[98] Subsequent studies have identified other modifier loci on mouse chromosomes 5 and 12.[99,100] Collectively, these findings establish that the existence of epigenetic or polymorphic differences that modify the susceptibility of mice to gliomagenesis, and raise the intriguing possibility that similar loci exist in children with NF1 that likewise predispose them to glioma development.

CONCLUSIONS

There are a number of clinical challenges to the management of patients with PA. Surgical resection is considered the treatment of choice in children, and usually results in excellent long-term survival rates.[101,102] Unfortunately, a significant number of these gliomas (for example, NF1-PA) involve the optic pathway or deep midline structures, and are not amenable to complete resection. For these tumors, nonsurgical strategies, including chemotherapy and radiation therapy, are usually implemented. However, radiotherapy is not recommended for the treatment of NF1-associated optic gliomas, as children with NF1 have a propensity to develop secondary central nervous system tumors following radiation,[103] as well as significant long-term neurocognitive and endocrine impairments, which may not be obvious for 5–10 years following the completion of treatment.[101,104]

Over the past decade, there has been an explosion in our understanding of the molecular basis for NF1-related gliomagenesis and the contributions of tumor microenvironment, cell of origin, and genetic modifiers to glioma formation.

Although *Nf1* inactivation in glial lineage cells is a necessary step in oncogenesis, it must occur in a specific susceptive cell type capable of expanding in response to loss of neurofibromin growth regulation. Moreover, glioma formation in children with NF1 is further restricted by developmental constraints, brain region-specific determinants, patient age, genomic background and the presence of a supportive local microenvironment (Figure 5). The fact that there exist a number of conditions that must be met in order for gliomagenesis to occur may partly explain why the majority of children with NF1 do not develop brain tumors. In addition, each of these factors not only influences tumorigenesis, but also glioma behavior (vision loss, progression) and perhaps response to therapy. With the availability of an emerging number of potential preclinical GEM glioma models, it now becomes possible to identify and evaluate potential therapeutic strategies that leverage these unique requirements and conditions.

Although less is known about the conditions that dictate *f-BRAF*-associated glioma formation and growth, it is highly likely that some of the same rules that govern NF1-PA biology are applicable to their sporadic counterparts. The potential for shared lessons is underscored by the finding that mTOR activation is a signature of both sporadic and NF1-associated PA. In this regard, future studies focused on understanding the interplay of all of these molecular and cellular determinants may facilitate the design of more efficient strategies to treat these low-grade brain tumors without negatively impacting on the developing brain. Moreover, it is equally plausible that further elucidation of these constraints and conditions will reveal predictive modifiers of glioma behavior. Recent clinical studies have shown that girls with NF1 younger than 2 years of age harboring gliomas involving the post-chiasmatic optic radiations are more likely to behave in a clinically-aggressive manner.[105] Future clinical studies coupled with basic science investigation and genomic analyses may one day yield a series of predictive biomarkers that allow clinicians to stratify children with low-grade gliomas into clinically-relevant groups for surveillance and potential targeted treatment.

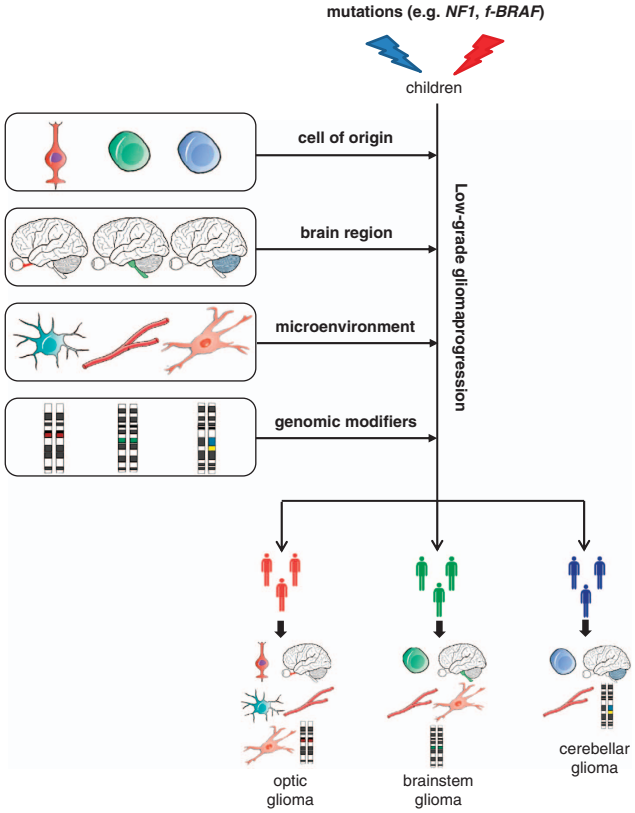

Figure 5. Pediatric gliomas are defined by numerous conditions. Pediatric glioma formation requires that cancer-causing genetic mutations occur in specific susceptible preneoplastic cells (glial cells or stem cells) within particular brain regions. The local micro-environment is highly influenced by the specific brain region, as well as genomic factors (patient sex, genomic modifiers). Moreover, the dynamic relationship between preneoplastic/neoplastic neuroglial and non-neoplastic stromal cells is also impacted by the patient age and genomic modifiers. Distinct subgroups of patients with low-grade glioma could reflect their unique clinicopathological features such as the cell of origin, brain region, stromal factors or genomic modifiers. Each of these subgroups of children may have different clinical outcomes and responses to conventional or targeted therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. *Neuro Oncol* 2012; **14**(Suppl 5): v1–v49.
2 Pfister S, Witt O. Pediatric gliomas. *Recent Results Cancer Res* 2009; **171**: 67–81.
3 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A *et al.* The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007; **114**: 97–109.
4 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**: 361–367.
5 Kluwe L, Hagel C, Tatagiba M, Thomas S, Stavrou D, Ostertag H *et al.* Loss of NF1 alleles distinguish sporadic from NF1-associated pilocytic astrocytomas. *J Neuropathol Exp Neurol* 2001; **60**: 917–920.
6 Lau N, Feldkamp MM, Roncari L, Loehr AH, Shannon P, Gutmann DH *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**: 759–767.
7 Listernick R, Ferner RE, Liu GT, Gutmann DH. Optic pathway gliomas in neurofibromatosis-1: controversies and recommendations. *Ann Neurol* 2007; **61**: 189–198.
8 Guillamo JS, Creange A, Kalifa C, Grill J, Rodriguez D, Doz F *et al.* Prognostic factors of CNS tumours in Neurofibromatosis 1 (NF1): a retrospective study of 104 patients. *Brain* 2003; **126**: 152–160.
9 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**: 896–902.
10 Jones DT, Kocialkowski S, Liu L, Pearson DM, Bäcklund LM, Ichimura K *et al.* Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res* 2008; **68**: 8673–8677.
11 Pfister S, Janzarik WG, Remke M, Ernst A, Werft W, Becker N *et al.* BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. *J Clin Invest* 2008; **118**: 1739–1749.
12 Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM *et al.* Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science* 1990; **249**: 181–186.
13 Andersen LB, Ballester R, Marchuk DA, Chang E, Gutmann DH, Saulino AM *et al.* A conserved alternative splice in the von Recklinghausen neurofibromatosis (NF1) gene produces two neurofibromin isoforms, both of which have GTPase-activating protein activity. *Mol Cell Biol* 1993; **13**: 487–495.
14 Costa RM, Yang T, Huynh DP, Pulst SM, Viskochil DH, Silva AJ *et al.* Learning deficits, but normal development and tumor predisposition, in mice lacking exon 23a of Nf1. *Nat Genet* 2001; **27**: 399–405.
15 Gutmann DH, Geist RT, Rose K, Wright DE. Expression of two new protein isoforms of the neurofibromatosis type 1 gene product, neurofibromin, in muscle tissues. *Dev Dyn* 1995; **202**: 302–311.

16 Gutmann DH, Geist RT, Wright DE, Snider WD. Expression of the neurofibromatosis 1 (NF1) isoforms in developing and adult rat tissues. *Cell Growth Differ* 1995; **6**: 315–323.
17 Gutmann DH, Zhang Y, Hirbe A. Developmental regulation of a neuron-specific neurofibromatosis 1 isoform. *Ann Neurol* 1999; **46**: 777–782.
18 Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. *Nature* 1992; **356**: 713–715.
19 Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P *et al.* Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. *Nat Genet* 1996; **12**: 144–148.
20 Xu GF, Lin B, Tanaka K, Dunn D, Wood D, Gesteland R *et al.* The catalytic domain of the neurofibromatosis type 1 gene product stimulates ras GTPase and complements ira mutants of *S. cerevisiae*. *Cell* 1990; **63**: 835–841.
21 Martin GA, Viskochil D, Bollag G, McCabe PC, Crosier WJ, Haubruck H *et al.* The GAP-related domain of the neurofibromatosis type 1 gene product interacts with ras p21. *Cell* 1990; **63**: 843–849.
22 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**: 878–887.
23 Sievert AJ, Jackson EM, Gai X, Hakonarson H, Judkins AR, Resnick AC *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**: 449–458.
24 Eisenhardt AE, Olbrich H, Roring M, Janzarik W, Anh TN, Cin H *et al.* Functional characterization of a BRAF insertion mutant associated with pilocytic astrocytoma. *Int J Cancer* 2011; **129**: 2297–2303.
25 Janzarik WG, Kratz CP, Loges NT, Olbrich H, Klein C, Schäfer T *et al.* Further evidence for a somatic KRAS mutation in a pilocytic astrocytoma. *Neuropediatrics* 2007; **38**: 61–63.
26 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**: 2119–2123.
27 Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C *et al.* Analysis of BRAF V600E mutation in 1320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. *Acta Neuropathol* 2011; **121**: 397–405.
28 Sharma MK, Zehnbauer BA, Watson MA, Gutmann DH. RAS pathway activation and an oncogenic RAS mutation in sporadic pilocytic astrocytoma. *Neurology* 2005; **65**: 1335–1336.
29 Cin H, Meyer C, Herr R, Janzarik WG, Lambert S, Jones DT *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**: 763–774.
30 Dasgupta B, Yi Y, Chen DY, Weber JD, Gutmann DH. Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in neurofibromatosis 1-associated human and mouse brain tumors. *Cancer Res* 2005; **65**: 2755–2760.
31 Lee dY, Yeh TH, Emnett RJ, White CR, Gutmann DH. Neurofibromatosis-1 regulates neuroglial progenitor proliferation and glial differentiation in a brain region-specific manner. *Genes Dev* 2010; **24**: 2317–2329.
32 Hegedus B, Banerjee D, Yeh TH, Rothermich S, Perry A, Rubin JB *et al.* Preclinical cancer therapy in a mouse model of neurofibromatosis-1 optic glioma. *Cancer Res* 2008; **68**: 1520–1528.
33 Banerjee S, Crouse NR, Emnett RJ, Gianino SM, Gutmann DH. Neurofibromatosis-1 regulates mTOR-mediated astrocyte growth and glioma formation in a TSC/Rheb-independent manner. *Proc Natl Acad Sci USA* 2011; **108**: 15996–16001.
34 Kaul A, Chen YH, Emnett RJ, Dahiya S, Gutmann DH. Pediatric glioma-associated KIAA1549:BRAF expression regulates neuroglial cell growth in a cell type-specific and mTOR-dependent manner. *Genes Dev* 2012; **26**: 2561–2566.
35 Alcantara LS, Chen J, Kwon CH, Jackson EL, Li Y, Burns DK *et al.* Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer Cell* 2009; **15**: 45–56.
36 Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C *et al.* Subtypes of medulloblastoma have distinct developmental origins. *Nature* 2010; **468**: 1095–1099.
37 Kalamarides M, Stemmer-Rachamimov AO, Niwa-Kawakita M, Chareyre F, Taranchon E, Han ZY *et al.* Identification of a progenitor cell of origin capable of generating diverse meningioma histological subtypes. *Oncogene* 2011; **30**: 2333–2344.
38 Taylor MD, Poppleton H, Fuller C, Su X, Liu Y, Jensen P *et al.* Radial glia cells are candidate stem cells of ependymoma. *Cancer Cell* 2005; **8**: 323–335.
39 Merkle FT, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc Natl Acad Sci USA* 2004; **101**: 17528–17532.
40 Johnson RA, Wright KD, Poppleton H, Mohankumar KM, Finkelstein D, Pounds SB *et al.* Cross-species genomics matches driver mutations and cell compartments to model ependymoma. *Nature* 2010; **466**: 632–636.
41 Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC *et al.* Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* 2012; **123**: 465–472.
42 Schuller U, Heine VM, Mao J, Kho AT, Dillon AK, Han YG *et al.* Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell* 2008; **14**: 123–134.
43 Wang Y, Yang J, Zheng H, Tomasek GJ, Zhang P, McKeever PE *et al.* Expression of mutant p53 proteins implicates a lineage relationship between neural stem cells and malignant astrocytic glioma in a murine model. *Cancer Cell* 2009; **15**: 514–526.
44 Assanah M, Lochhead R, Ogden A, Bruce J, Goldman J, Canoll P. Glial progenitors in adult white matter are driven to form malignant gliomas by platelet-derived growth factor-expressing retroviruses. *J Neurosci* 2006; **26**: 6781–6790.
45 Lindberg N, Kastemar M, Olofsson T, Smits A, Uhrbom L. Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. *Oncogene* 2009; **28**: 2266–2275.
46 Liu C, Sage JC, Miller MR, Verhaak RG, Hippenmeyer S, Vogel H *et al.* Mosaic analysis with double markers reveals tumor cell of origin in glioma. *Cell* 2011; **146**: 209–221.
47 Masui K, Suzuki SO, Torisu R, Goldman JE, Canoll P, Iwaki T. Glial progenitors in the brainstem give rise to malignant gliomas by platelet-derived growth factor stimulation. *Glia* 2010; **58**: 1050–1065.
48 Sugiarto S, Persson AI, Munoz EG, Waldhuber M, Lamagna C, Andor N *et al.* Asymmetry-defective oligodendrocyte progenitors are glioma precursors. *Cancer Cell* 2011; **20**: 328–340.
49 Bachoo RM, Maher EA, Ligon KL, Sharpless NE, Chan SS, You MJ *et al.* Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer Cell* 2002; **1**: 269–277.
50 Friedmann-Morvinski D, Bushong EA, Ke E, Soda Y, Marumoto T, Singer O *et al.* Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science* 2012; **338**: 1080–1084.
51 Solga A, Gianino SM, Gutmann DH. NG2-cells are not the cell of origin for murine neurofibromatosis-1 (*Nf1*) optic glioma. *Oncogene*. (e-pub ahead of print 14 January 2013; doi:10.1038/onc.2012.580).
52 Lee dY, Gianino SM, Gutmann DH. Innate neural stem cell heterogeneity determines the patterning of glioma formation in children. *Cancer Cell* 2012; **22**: 131–138.
53 Tchoghandjian A, Fernandez C, Colin C, El Ayachi I, Voutsinos-Porche B, Fina F *et al.* Pilocytic astrocytoma of the optic pathway: a tumour deriving from radial glia cells with a specific gene signature. *Brain* 2009; **132**: 1523–1535.
54 Gronych J, Korshunov A, Bageritz J, Milde T, Jugold M, Hambardzumyan D *et al.* An activated mutant BRAF kinase domain is sufficient to induce pilocytic astrocytoma in mice. *J Clin Invest* 2011; **121**: 1344–1348.
55 Lyustikman Y, Momota H, Pao W, Holland EC. Constitutive activation of Raf-1 induces glioma formation in mice. *Neoplasia* 2008; **10**: 501–510.
56 Robinson JP, VanBrocklin MW, Guilbeault AR, Signorelli DL, Brandner S, Holmen SL. Activated BRAF induces gliomas in mice when combined with Ink4a/Arf loss or Akt activation. *Oncogene* 2010; **29**: 335–344.
57 Jacob K, Albrecht S, Sollier C, Faury D, Sader E, Montpetit A *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**: 722–733.
58 Jacob K, Quang-Khuong DA, Jones DT, Witt H, Lambert S, Albrecht S *et al.* Genetic aberrations leading to MAPK pathway activation mediate oncogene-induced senescence in sporadic pilocytic astrocytomas. *Clin Cancer Res* 2011; **17**: 4650–4660.
59 Raabe EH, Lim KS, Kim JM, Meeker A, Mao XG, Nikkhah G *et al.* BRAF activation induces transformation and then senescence in human neural stem cells: a pilocytic astrocytoma model. *Clin Cancer Res* 2011; **17**: 3590–3599.
60 Hasselblatt M, Riesmeier B, Lechtape B, Brentrup A, Stummer W, Albert FK *et al.* BRAF-KIAA1549 fusion transcripts are less frequent in pilocytic astrocytomas diagnosed in adults. *Neuropathol Appl Neurobiol* 2011; **37**: 803–806.
61 Hawkins C, Walker E, Mohamed N, Zhang C, Jacob K, Shirinian M *et al.* BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res* 2011; **17**: 4790–4798.

62 Sharma MK, Mansur DB, Reifenberger G, Perry A, Leonard JR, Aldape KD *et al.* Distinct genetic signatures among pilocytic astrocytomas relate to their brain region origin. *Cancer Res* 2007; **67**: 890–900.
63 Yeh TH, Lee dY, Gianino SM, Gutmann DH. Microarray analyses reveal regional astrocyte heterogeneity with implications for neurofibromatosis type 1 (NF1)-regulated glial proliferation. *Glia* 2009; **57**: 1239–1249.
64 Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW *et al.* Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. *Genes Dev* 1994; **8**: 1019–1029.
65 Jacks T, Shih TS, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. Tumour predisposition in mice heterozygous for a targeted mutation in Nf1. *Nat Genet* 1994; **7**: 353–361.
66 Bajenaru ML, Zhu Y, Hedrick NM, Donahoe J, Parada LF, Gutmann DH. Astrocyte-specific inactivation of the neurofibromatosis 1 gene (NF1) is insufficient for astrocytoma formation. *Mol Cell Biol* 2002; **22**: 5100–5113.
67 Zhu Y, Harada T, Liu L, Lush ME, Guignard F, Harada C *et al.* Inactivation of NF1 in CNS causes increased glial progenitor proliferation and optic glioma formation. *Development* 2005; **132**: 5577–5588.
68 Bajenaru ML, Hernandez MR, Perry A, Zhu Y, Parada LF, Garbow JR *et al.* Optic nerve glioma in mice requires astrocyte Nf1 gene inactivation and Nf1 brain heterozygosity. *Cancer Res* 2003; **63**: 8573–8577.
69 Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B *et al.* A perivascular niche for brain tumor stem cells. *Cancer Cell* 2007; **11**: 69–82.
70 Hanisch UK. Microglia as a source and target of cytokines. *Glia* 2002; **40**: 140–155.
71 Bajenaru ML, Garbow JR, Perry A, Hernandez MR, Gutmann DH. Natural history of neurofibromatosis 1-associated optic nerve glioma in mice. *Ann Neurol* 2005; **57**: 119–127.
72 Watters JJ, Schartner JM, Badie B. Microglia function in brain tumors. *J Neurosci Res* 2005; **81**: 447–455.
73 Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M. Microglia promote the death of developing Purkinje cells. *Neuron* 2004; **41**: 535–547.
74 Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K *et al.* BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005; **438**: 1017–1021.
75 Roumier A, Bechade C, Poncer JC, Smalla KH, Tomasello E, Vivier E *et al.* Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. *J Neurosci* 2004; **24**: 11421–11428.
76 Elkabes S, DiCicco-Bloom EM, Black IB. Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J Neurosci* 1996; **16**: 2508–2521.
77 Amankulor NM, Hambardzumyan D, Pyonteck SM, Becher OJ, Joyce JA, Holland EC. Sonic hedgehog pathway activation is induced by acute brain injury and regulated by injury-related inflammation. *J Neurosci* 2009; **29**: 10299–10308.
78 Lin EY, Pollard JW. Tumor-associated macrophages press the angiogenic switch in breast cancer. *Cancer Res* 2007; **67**: 5064–5066.
79 Daginakatte GC, Gutmann DH. Neurofibromatosis-1 (Nf1) heterozygous brain microglia elaborate paracrine factors that promote Nf1-deficient astrocyte and glioma growth. *Hum Mol Genet* 2007; **16**: 1098–1112.
80 Simmons GW, Pong WW, Emnett RJ, White CR, Gianino SM, Rodriguez FJ *et al.* Neurofibromatosis-1 heterozygosity increases microglia in a spatially and temporally restricted pattern relevant to mouse optic glioma formation and growth. *J Neuropathol Exp Neurol* 2011; **70**: 51–62.
81 Daginakatte GC, Gianino SM, Zhao NW, Parsadanian AS, Gutmann DH. Increased c-Jun-NH2-kinase signaling in neurofibromatosis-1 heterozygous microglia drives microglia activation and promotes optic glioma proliferation. *Cancer Res* 2008; **68**: 10358–10366.
82 Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, Sher A *et al.* Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol* 2000; **20**: 4106–4114.
83 Hoshiko M, Arnoux I, Avignone E, Yamamoto N, Audinat E. Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J Neurosci* 2012; **32**: 15106–15111.
84 Pong WW, Higer SB, Gianino SM, Emnett RJ, Gutmann DH. Reduced microglial CX3CR1 expression delays neurofibromatosis-1 glioma formation. *Ann Neurol* 2013; **73**: 303–308.
85 Kostianovsky AM, Maier LM, Anderson RC, Bruce JN, Anderson DE. Astrocytic regulation of human monocytic/microglial activation. *J Immunol* 2008; **181**: 5425–5432.
86 Markovic DS, Vinnakota K, Chirasani S, Synowitz M, Raguet H, Stock K *et al.* Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion. *Proc Natl Acad Sci USA* 2009; **106**: 12530–12535.
87 Platten M, Kretz A, Naumann U, Aulwurm S, Egashira K, Isenmann S *et al.* Monocyte chemoattractant protein-1 increases microglial infiltration and aggressiveness of gliomas. *Ann Neurol* 2003; **54**: 388–392.
88 Wesolowska A, Kwiatkowska A, Slomnicki L, Dembinski M, Master A, Sliwa M *et al.* Microglia-derived TGF-beta as an important regulator of glioblastoma invasion—an inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. *Oncogene* 2008; **27**: 918–930.
89 Calatozzolo C, Canazza A, Pollo B, Di Pierro E, Ciusani E, Maderna E *et al.* Expression of the new CXCL12 receptor, CXCR7, in gliomas. *Cancer Biol Ther* 2011; **11**: 242–253.
90 Warrington NM, Woerner BM, Daginakatte GC, Dasgupta B, Perry A, Gutmann DH *et al.* Spatiotemporal differences in CXCL12 expression and cyclic AMP underlie the unique pattern of optic glioma growth in neurofibromatosis type 1. *Cancer Res* 2007; **67**: 8588–8595.
91 Dasgupta B, Dugan LL, Gutmann DH. The neurofibromatosis 1 gene product neurofibromin regulates pituitary adenylate cyclase-activating polypeptide-mediated signaling in astrocytes. *J Neurosci* 2003; **23**: 8949–8954.
92 Tong J, Hannan F, Zhu Y, Bernards A, Zhong Y. Neurofibromin regulates G protein-stimulated adenylyl cyclase activity. *Nat Neurosci* 2002; **5**: 95–96.
93 Warrington NM, Gianino SM, Jackson E, Goldhoff P, Garbow JR, Piwnica-Worms D *et al.* Cyclic AMP suppression is sufficient to induce gliomagenesis in a mouse model of neurofibromatosis-1. *Cancer Res* 2010; **70**: 5717–5727.
94 Binning MJ, Niazi T, Pedone CA, Lal B, Eberhart CG, Kim KJ *et al.* Hepatocyte growth factor and sonic Hedgehog expression in cerebellar neural progenitor cells costimulate medulloblastoma initiation and growth. *Cancer Res* 2008; **68**: 7838–7845.
95 Fanelli M, Caprodossi S, Ricci-Vitiani L, Porcellini A, Tomassoni-Ardori F, Amatori S *et al.* Loss of pericentromeric DNA methylation pattern in human glioblastoma is associated with altered DNA methyltransferases expression and involves the stem cell compartment. *Oncogene* 2008; **27**: 358–365.
96 Widschwendter M, Fiegl H, Egle D, Mueller-Holzner E, Spizzo G, Marth C *et al.* Epigenetic stem cell signature in cancer. *Nat Genet* 2007; **39**: 157–158.
97 Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. *Nat Genet* 2000; **26**: 109–113.
98 Reilly KM, Tuskan RG, Christy E, Loisel DA, Ledger J, Bronson RT *et al.* Susceptibility to astrocytoma in mice mutant for Nf1 and Trp53 is linked to chromosome 11 and subject to epigenetic effects. *Proc Natl Acad Sci USA* 2004; **101**: 13008–13013.
99 Amlin-Van Schaick J, Kim S, Broman KW, Reilly KM. Scram1 is a modifier of spinal cord resistance for astrocytoma on mouse Chr 5. *Mamm Genome* 2012; **23**: 277–285.
100 Amlin-Van Schaick JC, Kim S, DiFabio C, Lee MH, Broman KW, Reilly KM. Arlm1 is a male-specific modifier of astrocytoma resistance on mouse Chr 12. *Neuro Oncol* 2012; **14**: 160–174.
101 Armstrong GT, Conklin HM, Huang S, Srivastava D, Sanford R, Ellison DW *et al.* Survival and long-term health and cognitive outcomes after low-grade glioma. *Neuro Oncol* 2011; **13**: 223–234.
102 Wisoff JH, Sanford RA, Heier LA, Sposto R, Burger PC, Yates AJ *et al.* Primary neurosurgery for pediatric low-grade gliomas: a prospective multi-institutional study from the Children's Oncology Group. *Neurosurgery* 2011; **68**: 1548–1554, discussion 54-5.
103 Sharif S, Ferner R, Birch JM, Gillespie JE, Gattamaneni HR, Baser ME *et al.* Second primary tumors in neurofibromatosis 1 patients treated for optic glioma: substantial risks after radiotherapy. *J Clin Oncol* 2006; **24**: 2570–2575.
104 Shalitin S, Gal M, Goshen Y, Cohen I, Yaniv I, Phillip M. Endocrine outcome in long-term survivors of childhood brain tumors. *Horm Res Paediatr* 2011; **76**: 113–122.
105 Fisher MJ, Loguidice M, Gutmann DH, Listernick R, Ferner RE, Ullrich NJ *et al.* Visual outcomes in children with neurofibromatosis type 1-associated optic pathway glioma following chemotherapy: a multicenter retrospective analysis. *Neuro Oncol* 2012; **14**: 790–797.