THE MOLECULAR AND GENETIC BASIS OF NEUROLOGICAL TUMOURS

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There are no effective therapies for many tumours of the nervous system. This is, in part, a consequence of their location within relatively inaccessible tissues. It is also likely, however, that the unique characteristics of the cells that give rise to these tumours create a set of conditions that facilitate tumour development. Here, we consider recent advances in molecular genetics, the development of mouse models and developmental neurobiology as they relate to tumours of neuroectodermal origin. It is likely that these advances will provide insight into underlying mechanisms and provide a rational framework for the development of effective interventions.

NEURAL-CREST CELLS
Cells that originate in the dorsal lip of the neural tube that undergo epithelial-to-mesenchymal transition and migrate outwards to give rise to all cells of the peripheral, autonomic and enteric nervous systems. These cells also give rise to melanocytes and neuroendocrine cells.

SCHWANNOMA
A neural-crest-derived tumour that has morphological and molecular features of Schwann cells. These tumours are clonal in origin.

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In vertebrates, the embryonic neural tube (neuroectoderm) gives rise to the main cell types of the central nervous system (CNS), including neurons, astrocytes and oligodendrocytes. The NEURAL CREST derives from the dorsal lip of the neural tube, and its cells migrate extensively during embryonic development, giving rise to various tissues, including the peripheral nervous system (PNS). In both the CNS and PNS, the appearance of neurons (neurogenesis) developmentally precedes the appearance of glia (gliogenesis).

Neuroectodermal tumours (neurological tumours) include all neoplasms that have either a CNS- or PNS-derived cell of origin. The classification of neurological tumours is based on the predominant cell type(s), which is generally determined by morphological and immunohistochemical criteria (FIG. 1). After development ceases, neurons become post-mitotic and only a small compartment of stem cells remain, whereas glial cells retain the ability to proliferate throughout life. In this context, it is perhaps not surprising that most adult neurological tumours are of glial origin. These tumours are termed gliomas, and include tumours that are composed predominantly of astrocytes (astrocytomas), oligodendrocytes (oligodendrogliomas), mixtures of various glial cells (for example, oligoastrocytomas) and ependymal cells (ependymomas)[1–3]. In the case of the PNS, the neurofibroma and SCHWANNOMA are the two most common glial tumours[4] (FIG. 1).

Of all the various grading schemes, the World Health Organization (WHO) grading system is most widely used. The WHO grading system classifies gliomas into grades I–IV based on the degree of malignancy, as determined by histopathological criteria. In the CNS, grade I gliomas generally behave in a benign fashion and, in many cases, might even be circumscribed, whereas grade II–IV gliomas are malignant and diffusely infiltrate throughout the brain. Astrocytomas are the most common CNS neoplasms, accounting for more than 60% of all primary brain tumours. The most malignant form of infiltrating astrocytic neoplasm — glioblastoma multiforme (GBM) (WHO grade IV astrocytoma) — is one of the most aggressive human cancers, with a median survival of less than 1 year. Importantly, this statistic has not changed significantly over the past two decades[1–3].

In the PNS, neurofibromas and schwannomas are benign and belong to WHO grade I. Malignant PERIPHERAL-NERVE-SHEATH tumours (MPNSTs) are grade III–IV, and, like malignant astrocytomas, do not respond well to current therapies[4]. Neurofibroma is a complex tumour type of the PNS and can occur sporadically, but most commonly arises in individuals who are afflicted with neurofibromatosis type 1 (see below) and who have germ-line mutations in the tumour-suppressor *NF1* gene[5–7]. Moreover, most MPNSTs are derived from pre-existing neurofibromas. So, in this review, we focus on what recent lessons have been learned about the molecular biology and genetics of astrocytoma, as an example of CNS tumours, and compare these with those of neurofibroma and its malignant form, MPNST, as an example of PNS tumours.

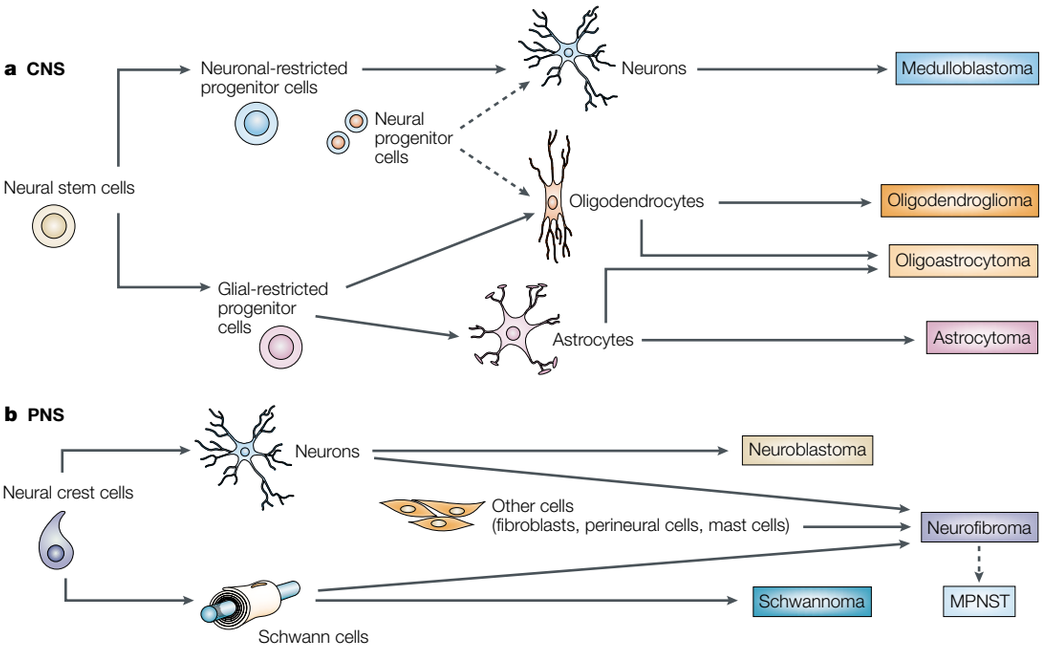


Figure 1 | **Developmental scheme of neuroectodermal (neurons and glia) cells and the classification of neurological tumours. a** | In the central nervous system (CNS), multipotent neural stem cells in the ventricular/subventricular zones of the embryonic neural tube give rise to three main cell types in the mature CNS — neurons, and oligodendrocytes and astrocytes. It is generally believed that neural stem cells first differentiate into two distinct progenitor cells — neuronal-restricted progenitor cells and glial-restricted progenitor cells, which further differentiate into neurons and glia, respectively. However, this view has been challenged by two recent studies, which indicate that oligodendrocytes might share the same progenitor (neural progenitor cells) with motor neurons, but not with astrocytes[136,137] (dotted line). The classification of neurological tumours is based on their predominant cell type(s). For example, astrocytoma is composed primarily of astrocytes, oligodendroglioma is composed primarily of oligodendrocytes, and oligoastrocytoma contains both astrocytic and oligodendroglial components. A few paediatric brain tumours, such as medulloblastoma, are derived from neuronal precursor cells. **b** | Neural-crest cells are neural-tube-derived multipotent stem cells, which give rise to various cell types, including neurons and glial cells (Schwann cells) in the peripheral nervous system (PNS). Neuroblastoma mainly occurs in children and derives from neuronal precursor cells of sympathetic neurons. Neurofibroma is a heterogeneous tumour that contains Schwann cells, fibroblasts, perineural cells, neuronal processes and mast cells. Schwannoma is a homogeneous tumour that is composed of Schwann cells. Most MPNSTs (malignant peripheral-nerve-sheath tumours) derive from neurofibromas.

Astrocytoma

Astrocytomas have the propensity to infiltrate throughout the brain. This feature is present even in low-grade tumours, making complete surgical resection impossible. Unfortunately, these tumours are largely resistant to radiation and chemotherapy. The most promising prospects for overcoming the unique features of astrocytoma are likely to come from recent studies into the genetics and molecular biology of these tumours. Particularly valuable insights have been gained by human genetic studies, developmental analysis of gliogenesis and efforts to model gliomas in mice.

Grade IV astrocytoma can be divided into two subtypes based on clinical characteristics: primary and secondary GBM. Primary GBM arises as a *de novo* process, in the absence of a pre-existing low-grade lesion, whereas secondary GBM develops progressively from low-grade astrocytoma, generally over a period of 5–10 years[1–3]. Genetic studies of GBMs, particularly secondary GBMs, indicate that there are distinct genetic pathways involved in the initiation and progression of these neoplasms (FIG. 2).

PERIPHERAL-NERVE SHEATH
A strong flexible cable that is composed of collagen fibres and a cellular tube (perineurum) that ensheaths, insulates and protects peripheral-nerve bundles.

Initiation pathways

p53. Certain genetic alterations are present in both low- and high-grade astrocytomas. These findings led to the hypothesis that these common mutations are involved in early phases of tumour formation. The p53 tumour suppressor — a transcription factor that regulates cell-cycle progression and apoptosis in response to many external insults (such as DNA damage and oncogenic mutations)[8] — fits this profile. Patients with the Li–Fraumeni syndrome — a familial cancer syndrome that is characterized by the presence of a germ-line mutation in the *TP53* gene (which encodes p53 in humans) — are predisposed to the development of various brain tumours, including astrocytomas[9,10]. Also, mutations in the *TP53* gene are found with equal frequency in all grades of sporadic astrocytoma (more than 60%)[11–16]. For example, one study reported that *TP53* mutations were already present in the first biopsy of 41 out of 46 patients[17]. In more prevalent human cancers, by contrast, *TP53* loss is associated with advanced stages[18]. The high incidence of *TP53* mutations, even in the low-grade astrocytomas, might point to the unique capacity of these tumours (even in the

Summary

- Tumours of neuroectodermal origin (neurological tumours) include all neoplasms of the central nervous system (CNS) and peripheral nervous system (PNS). The classification of neurological tumours is based on their predominant cell type(s) as they relate to normal cell types that are present in the CNS and PNS.
- Glial cells retain proliferative properties throughout life. So, most neurological tumours are of glial-lineage origin. In the CNS, gliomas include astrocytomas, oligodendrogliomas and oligoastrocytomas. In the PNS, neurofibromas and schwannomas are common tumours.
- Genetic pathways that are involved in the initiation and progression of astrocytomas have been identified. In secondary glioblastoma multiforme (GBM), loss of p53 and activation of the growth-factor–receptor-tyrosine-kinase signalling pathway initiates tumour formation, whereas disruption of the retinoblastoma (RB) pathway contributes to the progression of tumour development.
- Similar genetic pathways are disrupted in primary GBM, although through different mechanisms. The rapid growth nature of the primary GBM indicates that this type of malignancy might arise from the transformation of adult neural stem cells, which either are present in the brain or can be de-differentiated from astrocytes in response to oncogenic mutations.
- Dermal neurofibromas are thought to be derived from a component of mature Schwann cells, whereas plexiform neurofibromas are believed to arise from an embryonic Schwann-cell lineage. Most malignant peripheral-nerve-sheath tumours are derived from neurofibromas — particularly plexiform neurofibromas.
- Disruption of neurofibromatosis type 1 (NF1) in the Schwann-cell lineage initiates neurofibroma formation. In the setting of plexiform neurofibroma, the heterozygous state of tumour environment is important for tumour formation. The disruption of the p53 pathway is involved in the malignant progression of neurofibroma.

case of well-differentiated lesions) to infiltrate surrounding tissue — a trait that other tumour types do not show until advanced stages of neoplasia. The requisite early loss of *TP53* might reflect the need for astrocytoma cells to evade apoptosis in order to migrate and survive in a microenvironment that normally would not provide adequate trophic support.

Although these genetic studies indicate an association of *TP53* mutations with the initiation of astrocytoma, a causal link has not been established. Disruption of *Trp53* (which encodes p53 in mice) in the mouse germ line has provided more direct evidence regarding its role in astrocytoma initiation. Although *Trp53* homozygous ($p53^{-/-}$) and heterozygous ($p53^{+/-}$) mice do not develop astrocytomas[19,20], primary $p53^{-/-}$ astrocytes show increased growth and susceptibility to transformation[21,22]. These observations indicate that loss of *Trp53* alone is insufficient to initiate astrocytoma formation, and indicate that additional genetic or epigenetic events are required.

Receptor tyrosine kinases and RAS. Normal cells require growth signals for survival and/or proliferation. Many growth signals are mediated by diffusible growth factors that are transmitted into the cell by a group of transmembrane proteins with intrinsic tyrosine kinase activity — receptor tyrosine kinases (RTKs). After binding to a growth factor, RTKs undergo receptor dimerization, autophosphorylation and recruitment of adaptor proteins (such as GRB2 and SHC) that interact with and activate various downstream effectors (FIG. 3). The small GTP-binding protein, RAS, is an important downstream effector of the growth-factor–RTK signalling pathway, and can activate at least three downstream cascades: RAF–MEK–MAPK (mitogen-activated protein kinase), phosphatidylinositol 3-kinase (PI3K)–AKT and CDC42–RAC–RHO[23] (FIG. 3). The growth-factor–RTK–RAS signalling cascade is one of the most frequently targeted genetic pathways in human cancers,

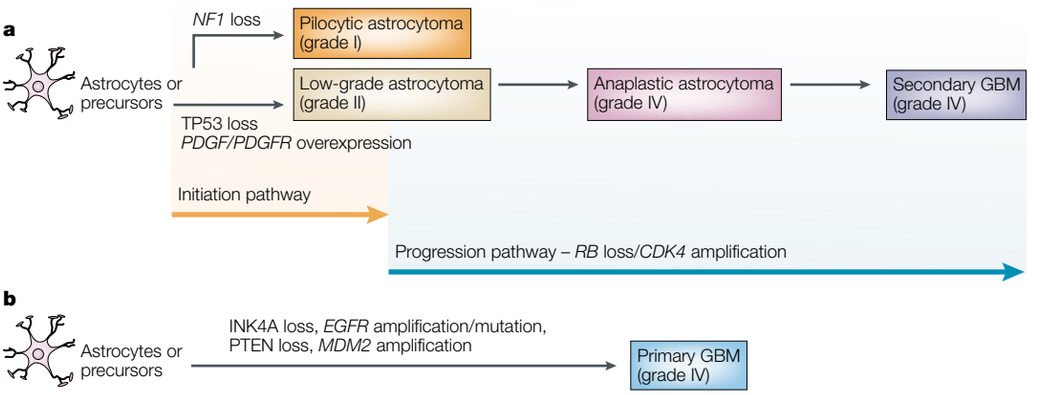


Figure 2 | **Genetic pathways involved in the development of primary and secondary astrocytoma. a** | Several genetic pathways are involved in the initiation versus progression of secondary glioblastoma multiforme (GBM). For example, loss of *TP53* and activation of the growth-factor–RTK (receptor tyrosine kinase)–RAS pathway (such as through overexpression of *PDGF*/*PDGFR* (platelet-derived growth factor/PDGF receptor) or loss of neurofibromatosis type 1 (*NF1*)) are involved in the initiation of pilocytic (grade I) or low-grade (grade II) astrocytoma. These can progress to anaplastic astrocytoma (grade IV) or secondary GBM (grade IV), which has been associated with disruption of the retinoblastoma (RB) pathway (through loss of *RB* or amplification/overexpression of *CDK4*). **b** | In primary GBM, the same genetic pathways are dismantled, although through different mechanisms. For example, disruption of the p53 pathway often occurs through loss of the gene that encodes ARF, or less frequently through amplification of *MDM2*. Disruption of the RB pathway occurs through loss of the gene that encodes INK4A. Amplification and/or mutation of the gene that encodes epidermal growth factor receptor (EGFR) is the most frequently detected genetic defect that is associated with primary GBM. Activity of the phosphatase PTEN is also frequently disrupted in this type of tumour.

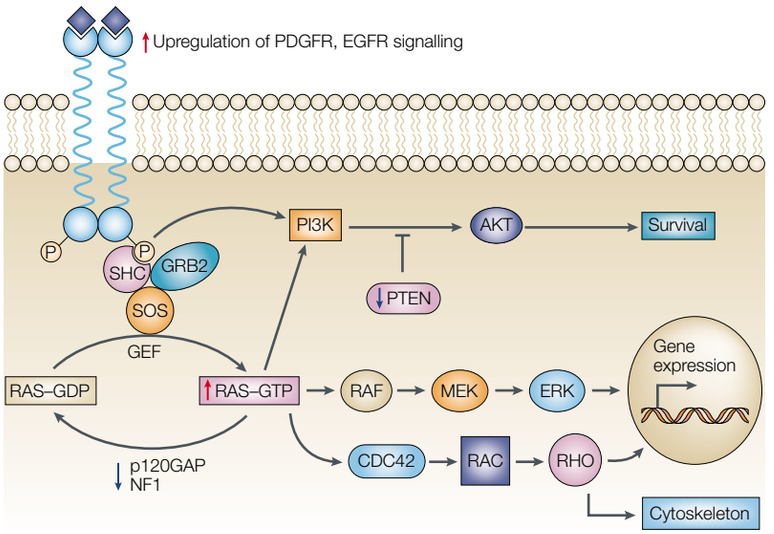


Figure 3 | **Signalling pathway mediated by growth-factor–RTK.** After ligand binding, receptor tyrosine kinases (RTKs), such as the platelet-derived growth factor receptor (PDGFR) or epidermal growth factor receptor (EGFR), dimerize, undergo autophosphorylation (P) and recruit adaptor proteins (such as GRB2 and SHC) that activate various downstream effectors. RAS is an important downstream effector of this signalling pathway and functions as a molecular switch by cycling between the active GTP-bound form and the inactive GDP-bound form. RAS activity is regulated positively by guanosine exchange factors (GEFs), such as SOS, and negatively by GTPase-activating proteins (GAPs), such as p120GAP, and the *NF1* gene product, neurofibromin. At least three downstream effectors can be activated by RAS. The RAF-mediated signalling cascade controls cell proliferation and differentiation in various cell types. The phosphotidylinositol-3 kinase (PI3K) pathway promotes cell survival. The CDC42-mediated signalling pathway regulates not only gene expression, but also cytoskeletal organization. Activation of these signalling pathways is frequently found in astrocytomas, and can also be caused by loss of activity of negative regulators such as NF1 or PTEN. Upward (red) arrows represent upregulated gene activity in tumours and downward arrows (blue) indicate downregulated/lost gene activity. ERK, extracellular signal-related kinase; MEK, mitogen-activated protein kinase kinase.

possibly because activating mutations render cancer cells independent of exogenous growth factors. Genetic analysis of astrocytomas indicates that RAS-mediated signalling is involved in the initiation of astrocytoma development. Elevated expression of growth factors and their cognate RTK receptors[24–26], including platelet-derived growth factor (PDGF) and platelet-derived growth-factor receptor (PDGFR)[27–30], are found in every grade of astrocytoma. Furthermore, PDGF and PDGFR are often co-expressed in the same tumour cells, indicating that astrocytoma cells establish an autocrine stimulatory loop[28,30]. These observations indicate that the PDGF/PDGFR-mediated signalling cascade could be involved in the initiation of astrocytoma development.

Experiments in which Pdgf was expressed in mouse brain via a retroviral vector have shown that Pdgf signalling can induce astrocytoma formation[31]. In this study, 40% of mice that were infected with the vector developed brain tumours. These tumours also expressed Pdgfr, supporting a model of autocrine stimulation. Most tumours generated by this method, however, were either high-grade gliomas with the characteristics of GBMs or primitive neuroectodermal tumours (PNETs), which show consistent expression of nestin — a neural stem-cell marker. Recently, an avian viral system was established that allows delivery of Pdgf into specific cell types, both *in vitro* and *in vivo*. *In vitro*, overexpression of Pdgf stimulated proliferation of nestin-expressing progenitor cells and astrocytes, as determined by expression of the astrocyte marker *Gfap*. The overexpression of *Pdgf* was interpreted to reflect de-differentiation of astrocytes into glial progenitor cells, based on the criteria that they have similar morphological and molecular characteristics to O2A cells (BOX 1). *Pdgf* overexpression in neonatal mouse brains resulted in the development of oligodendrogliomas and oligoastrocytomas[32].

So far, astrocytoma mouse models that have been created by *Pdgf* overexpression fail to develop low-grade astrocytomas that reflect the human disease. Although this difference might be attributed to species differences in the cellular response to Pdgf, another explanation is that astrocytic lineage cells that would normally give rise to tumours might not express *Pdgfr* during the neonatal stage[33] — the stage at which these studies are performed[31,32]. So, co-expression of Pdgf and its receptor in mouse astrocytes might be required to induce astrocytoma formation. Alternatively, adult astrocytes might be the more susceptible target for Pdgf signalling as astrocytoma occurs mainly in adulthood.

Despite considerable recent progress, the precise role of PDGF/PDGFR signalling either in astrocyte development or in astrocytoma formation remains unclear. Although both *in vitro* and *in vivo* data indicate that PDGF/PDGFR signalling is a mitogenic signal for oligodendrocyte progenitor cells, this has not been shown for the astrocytic lineage. For example, no defects have been reported in astrocyte development of Pdgfa- or Pdgfb-null mice[34]. Low-grade infiltrating astrocytomas generally have low mitotic activity, despite their highly invasive nature, indicating that mitogenic factors are not central to the development of these tumours. So, if PDGF/PDGFR signalling functions in astrocyte development, it might be involved in functions other than proliferation. In *Drosophila*, a *PDGFR* homologue has been shown to regulate cell migration, lending support to this idea[35]. Further study on the role of PDGF in astrocyte migration should provide insight into the initiation of astrocytoma development.

Although *RAS* mutations are found in approximately 30% of human cancers, no such mutations have been identified in human astrocytomas[36]. Instead, in these tumours, RAS can be activated indirectly by upregulation of growth-factor–RTK pathways (for example, PDGF/PDGFR), loss of function of the *NF1* gene[37] or through other RAS-pathway-activating mechanisms (FIG. 3).

Neurofibromatosis type 1 is a familial cancer syndrome in which patients develop multiple benign and malignant tumours of the CNS and PNS. The *NF1* gene encodes the protein neurofibromin, which shares homology with the RAS GTPase-activating protein (GAP) family. RAS–GAPs catalyse the conversion of activated RAS–GTP to inactive RAS–GDP (FIG. 3),

Box 1 | **PDGF is a mitogen for the oligodendrocyte precursor cells**

Although O2A cells can differentiate into both oligodendrocytes and type-2 astrocytes *in vitro*, there is no clear evidence that O2A cells can produce astrocytes *in vivo*. Therefore, O2A cells are generally regarded as oligodendrocyte precursor cells (OPCs)[133,134]. In keeping with the role of platelet-derived growth factor (PDGF) in regulating OPC proliferation *in vitro*, mice lacking PDGFA — one of two PDGF ligands (PDGFA and PDGFB) — have reduced numbers of oligodendrocytes owing to impaired precursor proliferation[34]. Furthermore, the overexpression of PDGFA in post-mitotic neurons leads to overproduction of oligodendrocytes during development[135]. So, PDGF stimulates the proliferation of OPCs both *in vitro* and *in vivo*.

thereby negatively regulating cellular RAS activity. Patients with neurofibromatosis type 1 are predisposed to the development of astrocytoma, although most neurofibromatosis-type-1-associated astrocytomas are grade I benign pilocytic astrocytomas[5–7]. Loss of both *NF1* alleles has been shown in these tumours[38,39] (FIG. 2). Activation of RAS-mediated MAPK and PI3K cascades are found in both neurofibromatosis type 1 mutant-associated and sporadic astrocytomas[39]. Furthermore, in a mouse transgenic model, overexpression of oncogenic *Ras* in astrocytes leads to the development of astrocytomas[40], providing direct evidence that RAS-pathway activation is important for astrocytoma formation. In summary, these observations support the concept that growth-factor–RTK–RAS signalling cascades are involved in the initiation of astrocytoma development (FIG. 2).

Progression pathways

Genetic pathways that are specifically disrupted in high-grade but not low-grade astrocytoma are considered to be involved in tumour progression. To maintain tissue homeostasis, normal cells have several mechanisms to regulate cell-cycle progression and to prevent uncontrolled proliferation. One of these regulatory stages takes place at the G1/S-phase checkpoint (for a review, see REF. 41). The tumour suppressor retinoblastoma (RB) is a key regulator of the G1/S checkpoint (BOX 2). A hallmark of high-grade astrocytomas is high mitotic activity. It is, therefore, not surprising that the RB–CDK–CKI (cyclin-dependent kinase inhibitor) regulatory circuit is frequently disrupted in these tumours. Loss of INK4A (also known as p16; both INK4A and ARF — also known as p14 in humans and p19 in mice, and which uses an alternative reading frame — are encoded by the *CDKN2A* gene) is detected in 40–57% of GBMs[42–46]. CDK4 amplification is found in 12–14% of GBMs[43,46], and loss of *RB* is identified in 14–33% of GBMs[42,43,46]. Consistent with the preceding analysis, these genetic alterations are most often mutually exclusive, supporting the idea that mutation of any component of the INK4A–CDK4–RB pathway can have equivalent effects (BOX 2). In total, mutations in INK4A/CDK4/RB are detected in more than 80% of GBMs and in 50% of anaplastic astrocytomas. By contrast, such mutations are rare in low-grade astrocytomas. The remaining 20% of GBMS that lack detectable INK4A/CDK4/RB mutations might harbour mutations in other components of this pathway. Indeed, overexpression of CDK6 and cyclin D1 has been detected in a small number of GBMs[47,48]. In one study, underexpression of E2F1 and overexpression of cyclin E have been reported in some high-grade gliomas[49].

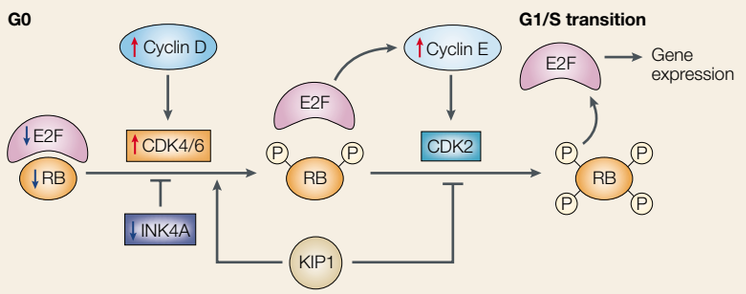
These retrospective studies of human tumours indicate that the RB-mediated regulatory circuit of cell-cycle progression is important for the progression of astrocytoma development, but not in the initiation process. Data from studies in mouse models support this idea. Neither Ink4a-homozygous nor Ink4a-heterozygous mice develop astrocytomas, although they are cancer prone[50–52]. Similarly, neither astrocytic inactivation of Rb[53] or overexpression of Cdk4 (REF. 54) in mouse brains fails to induce astrocytomas in mice. However, a recent study reported that inactivation of Rb proteins by expression of a truncated SV40 T antigen leads to the development of high-grade astrocytomas[55], indicating redundancy of Rb-family proteins (Rb, p107 and p130) in tumour suppression of mouse astrocytes.

Primary GBM

Although primary and secondary GBMs have similar histopathological characteristics and clinical outcomes,

Box 2 | **RB regulation**

In quiescent cells, RB proteins are hypophosphorylated (P) and sequester E2F transcription factors. In response to proliferative signals, RB is partially phosphorylated by cyclin-dependent kinases 4 and 6 (CDK4/6), and completely phosphorylated by CDK2. Hyperphosphorylated RB proteins release E2F transcription factors, which activate the expression of a set of genes that are required for G1/S transition and the initiation of DNA synthesis. CDK activity is positively regulated by cyclins. D-type cyclins form a complex and activate CDK4 and CDK6, whereas E-type cyclins bind and activate CDK2. Cyclin-dependent kinase inhibitors (CKIs) provide negative regulation of this pathway. The INK4 family, including INK4A (also known as p16), INK4B (also known as p15), INK4C (also known as p18) and INK4D (also known as p19) specifically inhibit activation of the cyclin D and CDK4/6 complex. WAF1 (also known as p21), KIP1 (also known as p27) and KIP2 (also known as p57) are more general cyclin/CKIs. Upward arrows (red) represent upregulated gene activity in tumours and downward arrows (blue) indicate downregulated/lost gene activity.



the kinetics of tumour development in these two subtypes are dramatically different[1–3]. Primary GBMs arise rapidly (<3 months) without clinical or histological evidence of pre-existing low-grade lesions, which makes it difficult to distinguish between genetic alterations that contribute to the initiation of primary GBMs and those that are associated with the progression of primary GBMs. However, mutational analysis indicates that the same genetic pathways are dismantled in both primary and secondary GBMs — namely p53, growth-factor–RTK–RAS and RB-mediated pathways (FIG. 2). But what underlies the distinction between the mechanisms that lead to rapid- versus slow-progressing glial tumours? Certain genetic alterations that have been predominately identified in primary GBMs provide clues concerning this distinction.

INK4A/ARF. Mutations in the gene that encodes INK4A — most of which are homozygous deletions — are common in primary GBMs (~40%) and rare in secondary GBMs (4%)[56]. *TP53* mutations, by contrast, are frequently detected in secondary GBMs (>60%), but are less common in primary GBMs (~10%)[57]. Furthermore, in these tumours, mutations in *TP53* and the gene that encodes INK4A are mutually exclusive[58], although INK4A is involved in the RB-mediated cell-cycle regulatory pathway. This conundrum is probably reconciled by the identification of a second transcript, ARF, from the *CDKN2A* locus[59]. ARF stabilizes p53 proteins by antagonizing MDM2 (amplification and overexpression of *MDM2* are detected in primary GBMs that lack *TP53* mutations[60]), which targets p53 for ubiquitin-mediated degradation. So, in secondary GBMs, *TP53* is directly mutated, whereas in primary GBMs, the p53 pathway is altered, resulting either from loss of ARF or from upregulation of *MDM2*. Homozygous deletion of the *CDKN2A* locus ablates both INK4A and ARF function, simultaneously dismantling both RB and p53 pathways. This might explain why primary GBMs manifest so rapidly. In mice, simultaneous disruption of both *Nf1* and *Trp53* genes results in the development of high-grade astrocytomas in certain genetic backgrounds, whereas stepwise loss of Nf1 and p53 function does not[61]. These mouse studies support the concept that simultaneous loss of two key growth-regulatory pathways in a cell might present more favourable conditions for the development of cancer.

EGF/EGFR. Amplification of the gene that encodes epidermal growth-factor receptor (EGFR) is found in ~40% of primary GBMs, but is rare in secondary GBMs[57,62]. The specific role of the EGFR signalling pathway in primary GBMs is consistent with the observation that *EGFR* amplification is associated with mutation in the gene that encodes INK4A and is mutually exclusive with the *TP53* mutation[63,64]. Moreover, most tumours with *EGFR* amplifications (~77%) have additional genetic alterations[65], most of which are intragenic rearrangements that lead to a truncated and constitutively active EGFR. Overexpression of this truncated EGFR confers a growth advantage and tumorigenic properties in glioma cell lines[66]. *In vivo*, expression of this *Egfr* mutant in neonatal mouse brains failed to induce tumour formation. Overexpression of this mutant form of *Egfr* in the Ink4a–Arf mutant background does, however, lead to the development of glioma-like lesions[54]. These data support a model in which the cooperation of EGFR activation and INK4A–ARF deficiency contributes to tumour formation.

PTEN. Loss of the long arm of chromosome 10 is the most common genetic alteration that is associated with GBMs[67,68]. Several genetic loci that are associated with these tumours have been identified in this region. Among them, loss of *PTEN* — phosphatase and tensin homologue on chromosome 10 (REF. 69), which is also known as MMAC (mutated in multiple advanced cancers)[70] or TEP1 (transforming growth-factor-β-regulated and epithelial-cell-enriched phosphatase 1)[71] — is found in more than 30% of primary GBMs, but is rare in secondary GBMs (4%)[72]. The PTEN protein can function as both a protein and lipid phosphatase, and its activity seems to be essential for tumour suppression as many mutations are found in its phosphatase domain[73–75]. The observation that some mutant forms of the PTEN protein still retain protein phosphatase activity indicates that the ability to dephosphorylate lipid might be more important for tumour suppression[76].

The identification of phosphatidylinositol (3,4,5)-triphosphate (PIP3, a PI3K product) as a PTEN substrate[75] indicates that PTEN could function as a negative regulator of a well-known growth-control signalling pathway — the PI3K–AKT pathway[77] (FIG. 3). This is confirmed by the observation that enhanced AKT activity has been detected in PTEN-deficient tumours and cell lines from both humans and mice[78–80]. Furthermore, overexpression of constitutive *Akt*, as well as oncogenic *Ras*, in mouse neural stem cells leads to the development of GBMs[80], supporting the idea that the PI3K–AKT pathway is pivotal in the aetiology of GBMs.

Neural stem cells: cell of origin of primary GBM?

In vitro, EGF, but not PDGF, can stimulate the proliferation of neural stem cells that have the ability to undergo self-renewal and generate all three CNS cell types: neurons, oligodendrocytes and astrocytes[81,82]. Specific inactivation of Pten in mouse neural stem cells causes increased proliferation, at least in part, by shortening the cell cycle of neural stem cells[83]. The unique roles of EGF signalling and PTEN in neural stem-cell proliferation raise the intriguing possibility that primary GBMs might derive from neural stem cells. Furthermore, adult neural stem cells have been identified in various species, including humans and rodents[84–87]. Intriguingly, adult neural stem cells express the astrocytic marker — GFAP[88,89] — indicating that a close link might exist between the astrocytic lineage and adult neural stem cells. This idea is further supported by the observation that

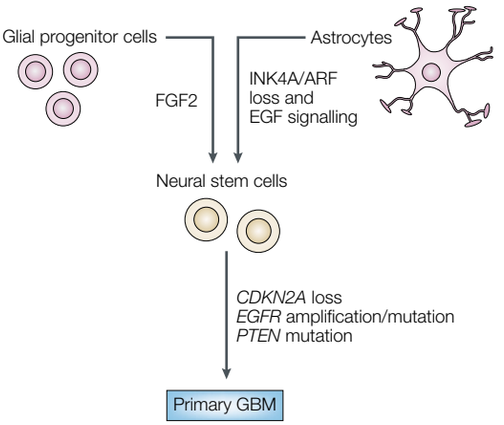


Figure 4 | **A model for the development of primary GBM.** Primary glioblastoma multiforme (GBM) can arise from transformation of adult neural stem cells, which are either present in some areas of the brain (for example, the subventricular zones) or de-differentiated from more mature cells. For example, glial progenitor cells, such as O2A cells, can be reprogrammed to develop into neural stem cells in response to exogenous fibroblast growth factor 2 (FGF2). Astrocytes can be de-differentiated into neural stem cells in response to loss of INK4A/ARF and epidermal growth factor (EGF) signalling. After this, loss of *CDKN2A*, amplification of the gene that encodes the EGF receptor (EGFR) or *PTEN* mutations can lead to primary GBM.

radial glial cells — traditionally considered to be astrocyte precursors — show characteristics of neural stem cells both *in vivo* and *in vitro*[90–93]. In mice, it has been shown that neural stem cells are more susceptible to transformation than are differentiated astrocytes[54,80]. The above observations are consistent with the notion that the rapid growth of primary GBMs might be a consequence of transformation of neural stem cells. This is not consistent, however, with the fact that primary GBMs arise mainly in patients who are older than 55 years of age[1,2], as neural stem-cell activity reduces with age[85].

These seemingly contradictory observations are likely to be reconciled by several recent provocative studies. One study reported that oligodendrocyte precursor cells can be reprogrammed to become neural stem cells in response to certain exogenous growth factors[94]. Moreover, a recent study showed that loss of both Ink4a and Arf, but not of Ink4a, Arf or p53 alone, results in de-differentiation of neonatal mouse astrocytes into neural stem cells in response to Egf signalling. After transplantation into brains, Ink4a/Arf$^{-/-}$ astrocytes, as well as Ink4a/Arf$^{-/-}$ neural stem cells, can lead to the development of high-grade astrocytomas in response to Egf signalling[95]. Taken together, these observations support a model in which primary GBMs can arise from neural stem cells, which either exist in the adult brain or can be de-differentiated from more differentiated cell types (such as astrocytes) in response to oncogenic mutations (for example, deficiency of the gene that encodes INK4A/ARF) (FIG. 4).

SCHWANN CELL
A glial-cell component of the peripheral nervous system that is derived from the neural crest and composed of myelinating and non-myelinating cells.

PERINEURAL CELL
A mesenchyme-derived cell that is recruited by forming peripheral nerves to undergo mesenchymal-to-epithelial transformation. These cells form the perineurum and secrete matrix that forms the perineural sheath.

Tumours in the PNS

More than 80% of MPNSTs are high-grade malignant tumours, which correspond to WHO grade III–IV[4]. Like GBMs in the CNS, MPNSTs are resistant to conventional therapies, and their deep location in the body and locally invasive growth prevent complete surgical resection. The 5-year survival rate for patients with MPNSTs ranges from 34–52%. Most MPNSTs (>60%) arise from pre-existing benign neurofibromas, particularly from plexiform neurofibromas in the setting of neurofibromatosis type 1. Less frequently, MPNSTs can arise *de novo* from peripheral nerves without histological evidence of low-grade lesions. In rare conditions, MPNSTs might also arise from pre-existing schwannomas. Human genetic studies, particularly from neurofibromatosis type 1 patients, have shed insight into the genetic pathways that are responsible for the initiation and progression of this highly aggressive malignant tumour.

Dermal and plexiform neurofibromas

Although neurofibromas can arise sporadically, the development of multiple neurofibromas is the hallmark of neurofibromatosis type 1 disease[5–7]. Neurofibromas have two characteristic features: they invariably arise within peripheral nerves, and they are heterogeneous tumours that contain every cell type that is present in normal peripheral nerves. These include SCHWANN CELLS, neuronal processes, PERINEURAL CELLS, fibroblasts and infiltrating mast cells. Two main types of neurofibroma occur in the setting of neurofibromatosis type 1. The most common type is the dermal neurofibroma, which arises in association with small, peripheral nerve twigs within the dermis, usually after the time of puberty. Although potentially disfiguring, dermal neurofibromas spontaneously cease growth and rarely progress to malignancy. The second type is the plexiform neurofibroma, which is thought to be embryonic in origin, and which arises in spinal or cranial nerves in a diffuse infiltrative pattern that causes expansion of nerve trunks and their branches. Due to their size and location, these tumours often physically impede normal neurological function. In addition, a significant proportion of plexiform neurofibromas (5%) undergo malignant transformation. The unique susceptibility to malignant transformation of plexiform neurofibromas indicates that this tumour type might contain a unique 'susceptible cell type'.

The clinical behaviours of these two neurofibromas indicate that plexiform neurofibromas are probably derived from embryonic Schwann-cell lineage, whereas dermal neurofibromas can arise from more mature Schwann-cell lineage. During development, neural-crest cells migrate into peripheral nerves, and a subset of cells commit to the Schwann-cell lineage. In rodents, neural-crest cells first differentiate into Schwann-cell precursors, which require neuregulin-1 (NRG1) for survival *in vitro* and *in vivo*[96]. After birth, immature Schwann cells develop into two types of mature Schwann cells: myelinating and non-myelinating. However, recently, a population of multipotent neural

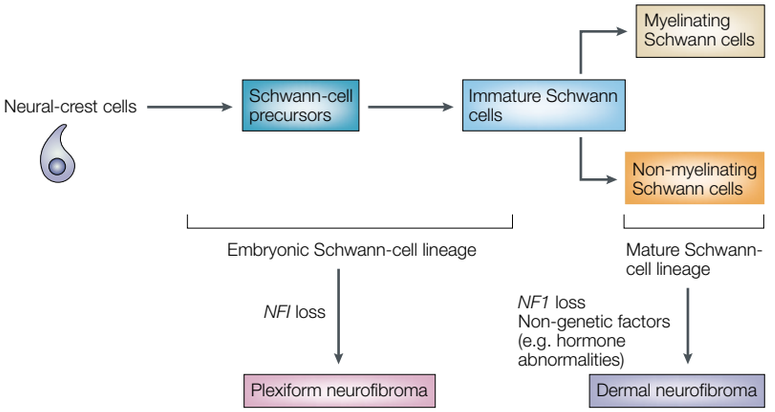


Figure 5 | **Development of Schwann cells and neurofibromas.** In rodents, a subset of migrating neural-crest cells first differentiate into Schwann-cell precursors, which give rise to immature Schwann cells, marked by expression of the Schwann-cell marker S100. After birth, immature Schwann cells differentiate into two types of mature Schwann cells: myelinating and non-myelinating. For simplification, we group neural-crest cells, Schwann-cell precursors and immature Schwann cells into the 'embryonic Schwann-cell lineage'. Plexiform neurofibromas might arise from transformation of embryonic Schwann-cell lineage. In mature Schwann cells, disruption of NF1 and other, non-genetic factors, such as hormone abnormalities, can lead to dermal neurofibromas.

stem cells has been identified from the E14–17 rat peripheral nerve, which can give rise to several cell types, including neurons, Schwann cells and muscle cells[97]. These observations raise the intriguing possibility that stochastic mutation of *NF1* could affect neural-crest-derivative components of peripheral nerve at different developmental stages — in turn, leading to different biological consequences. Whether plexiform neurofibromas are derived from early stages of Schwann-cell differentiation, and dermal neurofibromas are originated from more differentiated Schwann cells, remains to be determined (FIG. 5).

Initiation pathway and *NF1*

Loss of both alleles of the tumour suppressor *NF1* gene has been found in several malignant tumours, including MPNSTs[98], myeloid leukaemias[99] and PHEOCHROMOCYTOMAS[100]. However, the inactivation of both *NF1* alleles in benign neurofibromas has been questionable until recent years. So far, loss of heterozygosity (LOH) has been detected in only a minority of dermal neurofibromas, ranging from 13 to 36%[101–103]. This might, in part, be attributed to the fact that the *NF1* gene spans 350 kb of the human genomic sequence, making it difficult to detect subtle mutations with conventional screening techniques. Also, as *NF1* negatively regulates RAS-mediated signalling (FIG. 3), it is possible that other components in the pathway might be genetically altered in those neurofibromas that lack detected *NF1* mutations. Although activated *RAS* mutations have not been detected in neurofibromas, acquired expression of *EGFR* has been detected in neurofibromas and MPNSTs, but not in normal Schwann cells[104].

Studies from mouse models were initially not particularly informative of the role of *Nf1* in neurofibroma formation. *Nf1*-homozygous mice ($Nf1^{-/-}$) die due to abnormal cardiac development during embryonic development, and *Nf1*-heterozygous mice ($Nf1^{+/-}$) do not develop classic neurofibromatosis type 1 hallmarks, such as neurofibromas and hyperpigmented skin[105,106]. Lack of neurofibroma formation in $Nf1^{+/-}$ mice indicates that loss of both *Nf1* alleles might be essential for neurofibroma formation. To increase the mutation rate in mouse cells, chimeric mice harbouring both $Nf1^{-/-}$ and $Nf1^{+/+}$ cells were generated. Interestingly, these chimeric mice developed plexiform neurofibromas that were exclusively derived from the $Nf1^{-/-}$ cells[107]. However, no dermal neurofibromas were reported in these chimeric mice. These studies provide compelling evidence that loss of both *Nf1* alleles is an obligate step for plexiform neurofibroma formation. Current mouse models fail to develop dermal neurofibromas. In human patients, these tumours arise after puberty. Together, these results indicate that other factors (genetic or non-genetic) might be crucial in this type of tumour formation (FIG. 5).

Schwann-cell origin and neurofibromas

Neurofibromas are heterogeneous tumours in which not all of the cells necessarily undergo LOH. So, the presence of these 'normal cells' might mask the LOH of *NF1*-deficient cells within the tumours. One study reported the presence of both *NF1*-positive and *NF1*-negative cells in plexiform neurofibromas, lending support for this idea[108]. It is assumed that a genetic bottleneck exists that initiates the formation of these rare heterogeneous multicellular tumours. A significant goal has therefore been to identify the cell that first undergoes LOH, and thereby initiates tumour formation. Retrospective analysis of human tumour samples has indicated a prevalence of LOH in Schwann-cell populations, leading to the hypothesis that these cells represent the source of LOH and tumour formation[109–111]. However, these studies suffer from the fact that analysis has been limited to samples that do not represent early events in tumour formation.

Mouse models afford an alternative strategy to address early events in tumour formation. Almost all cell types that are present in neurofibromas derived from $Nf1^{-/-}$ mice have some degree of abnormality in culture. For example, $Nf1^{-/-}$ peripheral neurons show the ability to survive in the absence of neurotrophins[112]. Furthermore, $Nf1^{-/-}$ Schwann cells show a growth advantage and can readily be transformed[113,114]. Interestingly, studies in mast cells provide compelling evidence that loss of one copy of *Nf1* (haploinsufficiency) leads to hyperactivation of Ras proteins in mast cells, which results in increased proliferation of $Nf1^{+/-}$ mast cells, as compared with its wild-type counterparts in response to stem-cell factor[115]. This hyperproliferative effect seems to be mediated by both the PI3K and RAS–MEK–MAPK signalling pathways[116].

Taken together, these data indicate that any or all the lineages of *NF1*-deficient cells within neurofibromas could contribute to tumour formation in one way or another. These studies do not identify, however, the cell type(s) that must undergo LOH at the *NF1* locus to generate neurofibromas. Recently, Schwann-cell-specific ablation of *Nf1* in mice was

PHEOCHROMOCYTOMA
A neuroendocrine tumour that is formed in neural-crest-derived adrenal chromaffin cells.

shown to give rise to multiple plexiform neurofibromas, with similar immunohistological, ultrastructural and morphological characteristics to human counterparts. So, direct evidence is now available indicating that loss of *NF1* in Schwann cells is the genetic bottleneck for neurofibroma formation[117]. Interestingly, tumour formation in this model seems to depend on the heterozygous state of surrounding cells. Only $NF1^{+/-}$ mast cells can extensively infiltrate into nerves before tumour formation, indicating that $NF1^{+/-}$ mast cells might be actively involved in tumour development.

Progression pathway

The molecular mechanisms that are responsible for malignant progression of neurofibromas are largely unknown. So far, only two genetic alterations have been identified as progression pathways. *TP53* mutations have been identified exclusively in MPNSTs but not in benign neurofibromas, indicating that, unlike in astrocytomas, the p53-mediated pathway is involved in the progression of tumour development[118,119]. Consistent with the role of p53 in the progression of MPNSTs, $Trp53^{-/-}$ and $Trp53^{+/-}$ mice develop neither neurofibromas nor MPNSTs[19,20]. However, mice that harbour both *Nf1* and *Trp53* mutations develop MPNSTs[107,120]. Taken together, both human genetic studies and mouse models support the notion that loss of *NF1/Nf1* initiates tumour formation, whereas malignant progression requires additional genetic lesions, such as in *TP53/Trp53*.

Genetic alterations in the gene that encodes INK4A are also frequently identified in MPNSTs but not in neurofibromas[121,122], indicating that additional loss of genes in this locus might also contribute to malignant progression of neurofibromas. ARF stabilizes p53 by antagonizing MDM2-mediated degradation[59]. So, loss of ARF function in the progression of MPNSTs is consistent with the idea that p53-mediated pathways are important for the progression of MPNSTs. A high percentage of homozygous deletions (50–60%) that disrupt both ARF and INK4A has been identified in MPNSTs[121,122]. This indicates that loss of INK4A might also contribute to tumour progression. In human tumours, loss of INK4A is exclusively found in MPNSTs, but not in neurofibromas. In addition, genetic alterations of *RB* and amplifications in *CDK4* have been detected in the MPNSTs that lack mutations in the gene that encodes INK4A[123], indicating that the RB-mediated G1/S-checkpoint pathway might be crucial in tumour progression. Furthermore, loss of expression of another component of the RB pathway — KIP1 — has been identified in most MPNSTs (91%) compared with only 6% of neurofibromas[124]. These studies rely on a small series of tumour samples. Further analysis of human and mouse tumours will be required to establish whether the RB-mediated pathway is pivotal in the progression of MPNSTs.

Future directions

Despite the recent progress in understanding the molecular and genetic mechanisms that underlie tumorigenesis in both the CNS and PNS, effective treatment for these malignancies remains elusive. The rapid advances in genomic technology, coupled with the exploitation of relevant mouse models, might allow the tumour type to be defined and classified based on specific gene-expression profiles. Progress is still needed in several areas. First, in keeping with the rapid advances in genomic technology, efforts should be made to continue to incorporate molecular genetic information into clinical classification and grading schemes[125,126]. This new 'molecular grading' system, combined with the traditional histopathological approaches, should allow more accurate and reproducible diagnoses. Improved imaging techniques will probably provide clinicians with opportunities to make ever-earlier diagnoses of these neoplasms.

Although important genetic pathways that are involved in the initiation of secondary GBMs have been identified, it remains unknown whether alterations of these pathways are sufficient to induce tumour formation. To this end, accurate mouse models that, through similar genetic alterations, can reproduce clinical symptoms in humans will be essential. Not only might relevant mouse models provide precise evidence regarding the causal role of particular genetic pathway(s) for tumorigenesis, but they will also provide a platform for further characterization of molecular mechanisms, as well as preclinical models for therapeutic drug screening.

We need to understand the correlation of genetic alterations and tumour phenotype to better understand how these genetic alterations (for example, *TP53* and *GF–RTK–RAS*) cause astrocytoma cells to become highly invasive and render malignant neurological tumours resistant to conventional therapies. Downstream effectors of these genetic pathways also need to be identified, which will provide additional therapeutic targets. Several molecularly targeted strategies have recently been developed. Virally mediated gene transfer has been used to specifically kill *TP53*-deficient cells[127,128]. Small compounds that block tyrosine kinase activity, such as antagonists of PDGFR and EGFR, are undergoing clinical trials[129]. Inhibitors for components of the RAS–MEK–MAPK pathway are also at various stages of clinical trials[130]. In addition, strategies to target the deficiency of the RB-mediated pathway have been used[131,132].

The highly invasive nature of astrocytoma cells might reflect a unique feature of astrocytes during development — their ability to undergo extensive migration. Compared with neuronal or oligodendroglial development, astrocytic development remains poorly understood, and is a crucial area for investigation. As discussed above, genetic mutations in neural stem cells might give rise to a markedly different outcome, compared with those occurring in more differentiated cells. So, a more complete understanding of the biology of stem cells should provide important insights into the evolution of cancer cells. Taken together, the integration of the multiple disciplines, including molecular biology, genetics, neuro-oncology and developmental neurobiology, will be a crucial feature of our quest to cure for these devastating diseases.

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