# The Neurobiology of Neurooncology

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The histological classification of brain tumors currently is based on the morphological appearance and protein expression patterns that reflect specific cell types within the central nervous system. Recent studies have suggested that the cells of origin for brain tumors may persist in the fully formed tumors, and that these "cancer stem cells" might represent the relevant cellular targets for anticancer therapy. In this regard, insights into the developmental neurobiology of brain tumors has significant impact on our understanding of the molecular and cellular pathogenesis of these devastating cancers, as well as the development of new strategies for treating brain tumors.

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Our knowledge of the cause of brain tumors has increased tremendously over the past 20 years and is leading to a deeper understanding of the molecular events essential for tumor formation. Simultaneously, we have gained insights into the developmental processes that cause the diversity of the normal cells that comprise the brain, and we are beginning to recognize the overlapping and common mechanisms regulating tumorigenesis and development in the nervous system. Understanding the interplay between tumorigenesis and development may have important implications for both neuroscience and neurooncology. Among these, one of the most important factors is determining the cell of origin for brain tumors. Identifying the cell from which a given tumor arises would allow us to compare tumor cells with their normal counterparts, so that key differences and vulnerabilities of tumor cells can be discovered. Furthermore, identifying the cell of origin would allow us to create more robust and relevant animal models with which to study brain tumor etiology, pathology, and treatment. Finally, recent studies suggest that the cell of origin, or a cell that resembles it, may persist in mature tumors, and this cell type may be critical for the continued growth and propagation of brain tumors.[1] Therefore, identifying the cell of origin for a particular central nervous system tumor may be critical for designing effective approaches to therapy. In this regard, certain subpopulations of cells in other cancers (e.g., melanoma) exhibit distinct sensitivities to chemotherapy as a result of differential expression of P-glycoprotein and adenosine triphosphate–binding cassette proteins.[2] Herein, we review what is known about the cell of origin for two major classes of brain tumors, medulloblastoma (MB) and astrocytoma, and discuss new approaches to addressing this important neurobiological issue.

## Histological Classification of Brain Tumors

Brain tumors currently are classified according to the World Health Organization (WHO) system, which derives from the pioneering work of Bailey and Cushing.[3] This classification system names tumors after the cell type that tumor cells resemble most in the developing embryo or adult. Based on these criteria, neuropathologists distinguish among glioma (astrocytoma), oligodendroglioma, and neuronal tumors.[4] Astrocytic tumors comprise a wide range of glial neoplasms, which are subdivided into four malignancy grades (WHO grades I-IV) based on the presence of specific criteria, such as nuclear atypia, mitotic activity, necrosis, and microvascular proliferation. Oligodendrogliomas are subdivided into those tumors composed of pure oligodendroglial tumor cells and those with a mixed oligodendroglial and astrocytic appearance. Neuronal tumors constitute a large proportion of brain tumors seen in children, and they include central neurocytoma, gangliioglioma, supratentorial primitive neuroectodermal tumors, and MB. MB, the most common pediatric brain tumor, is a malignant invasive neoplasm of the cerebellum composed of cells that exhibit primarily neuronal differentiation.

While the current WHO classification scheme is

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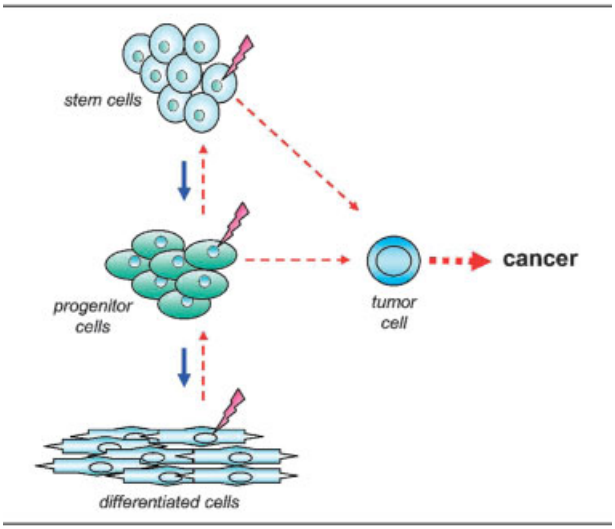


*Fig 1. Cancer-causing genetic changes in neural stem cells, progenitor cells, or differentiated cells cause brain tumor–associated neoplastic cells. Experimental evidence exists for the generation of brain tumors after the introduction of specific cancer-associated genetic changes (denoted by the* lightning bolts*) in neural stem cells, progenitor cells, and mature differentiated cell types. These brain tumor–associated mutations likely interfere with normal proliferation and differentiation.* Solid blue arrows *denote normal cellular differentiation in the developing nervous system, whereas the* dotted red arrows *denote the pathways to brain tumor formation.*

used widely, it has some notable limitations. First, many tumors consist of atypical-appearing cells that do not resemble any normal cell type in the brain. Second, central nervous system tumors are often morphologically diverse, and classification may rely on identification of a specific area within the tumor most characteristic of that particular tumor type. This may lead to inaccurate histological assignment based on a small region of tumor within an otherwise heterogeneous cellular mass. Third, tumor classification often depends on the use of immunocytological techniques to identify specific antigens or cell types. Because there are few antibodies that reliably or exclusively identify specific cell lineages, the presence or absence of a particular antigen suggests only a tumor type and does not necessarily allow definitive classification.

Although the WHO classification scheme implies a cell of origin for many brain tumors, the cell of origin has not been unequivocally identified for any of them. It is hypothesized that MBs originate from neuronal precursors, whereas astrocytoma and oligodendroglioma arise from astrocytic or oligodendroglial precursors. In this fashion, brain tumors may form as a result of the acquisition of specific genetic changes in stem cells, progenitor cells, or differentiated cell types (Fig 1). These genetic changes deregulate cell growth and differentiation control pathways important for normal brain development and lead to increased cell growth as an initiating step in tumor formation. Cancer-causing genetic mutations in stem cells and progenitor cells likely result in the increased growth of immature cell types, whereas differentiated cell types (e.g., astrocytes) may acquire stem cell-like or progenitor cell–like properties as a result of specific genetic mutations. In the following sections, we discuss what is known and what remains to be learned about the origins of MB and astrocytoma.

## Cellular Origin of Medulloblastoma

Among central nervous system tumors, few have evoked more discussion and speculation regarding their cell of origin than those primarily composed of primitive neuroepithelial cells. The most representative tumor in this group, the cerebellar MB, was distinguished from other brain tumors in 1910 by James Homer Wright,[5] and later more specifically by Percival Bailey and Harvey Cushing.[6] In light of its predominant neuronal morphology, Wright[5] suggested that MBs derived from restricted neuronal precursors, or neuroblasts. In contrast, Bailey and Cushing[6] noted that these tumors often contained glial cells as well, and proposed that their cell of origin was a new embryonic neuroepithelial cell type ("medulloblast") capable of generating both glial and neuronal cells.

In addition to the debate about the neuronal/glial potential of the cell of origin, there has also been significant disagreement about the location of this progenitor cell within the developing cerebellum. It has long been recognized that the cerebellum contains two distinct germinal zones: the ventricular zone (VZ) that forms the innermost boundary of the cerebellum, and the external germinal layer (EGL) that lines the outside of the cerebellum (Fig 2).[7] In their original description of MB, Bailey and Cushing suggested that the medulloblast was located in the VZ, and that the tumor originated from this region. In contrast, many investigators have proposed that MBs arise from the EGL.[8,9] Because developmental studies have suggested that the VZ generates both neurons and glia, whereas the EGL contains primarily neuronally restricted granule cell precursors (GCPs), proponents of the EGL as the site of origin tend to favor the notion that these tumors arise from neuroblasts rather than multipotent progenitors.

Over the years, evidence has accumulated in support of both restricted neuronal progenitors and multipotent precursors as the cells of origin for MB. Most of this information has come from immunohistochemical staining and gene expression analyses of tumor tissue. For example, studies of human MB have shown that some tumors express markers associated with EGL-derived GCPs, such as $p75^{NTR}$, TrkC, Zic1, and Math1.[10–13] However, many MBs express markers of

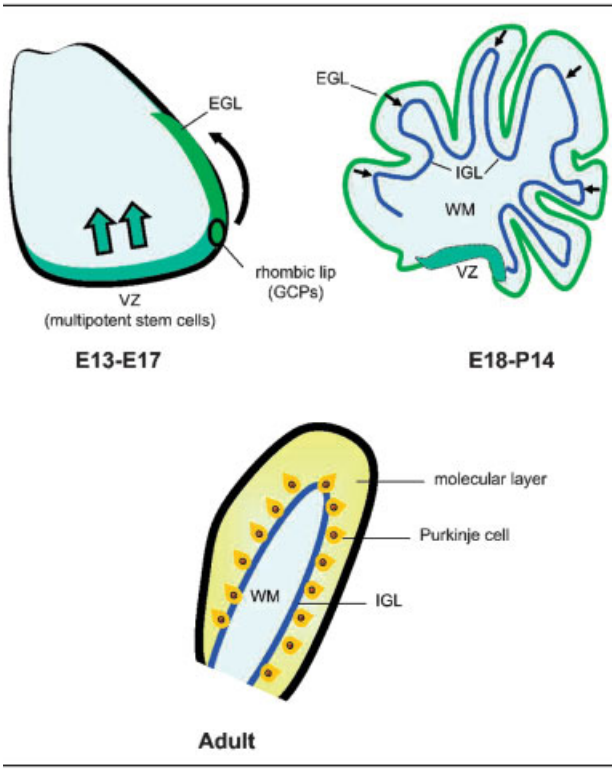


*Fig 2. Cellular origins of cerebellar neurons and glia. The developing cerebellum contains two distinct germinal zones: the ventricular zone (VZ), which contains multipotent stem cells that give rise to the majority of cerebellar neurons, and glia; and the external germinal layer (EGL), which contains committed granule cell precursors (GCPs) that only generate granule neurons. The VZ is most active during embryonic development (embryonic days 13–17 [E13-17] in mice), but may continue to generate cells postnatally. The EGL arises embryonically from a structure called the rhombic lip, but undergoes dramatic expansion during the late embryonic and early postnatal period (E18-P14). GCPs in the EGL proliferate for a brief period, then stop dividing and migrate inward to the internal granule layer (IGL). By adulthood, the surface of the cerebellum consists largely of granule cell axons and Purkinje cell dendrites (molecular layer), and most, if not all, proliferation has ceased. WM = white matter.*

VZ-derived progenitors, such as calbindin-D28K, parvalbumin, nestin, vimentin, and glial fibrillary acidic protein (GFAP).[14–17] Although some of these markers (e.g., nestin) can be found both in the EGL and in the VZ, most MBs express either EGL markers or VZ markers, but not both.

In an attempt to explain these discrepancies, a number of investigators suggested that different classes of MB may originate from distinct progenitors.[13,18] Although the WHO lists several histological subtypes of MB, the majority of tumors are described as either "desmoplastic" or "classic." Desmoplastic MBs account for 15 to 20% of all MBs, with a higher incidence in adult patients. These tumors are most often located in the cerebellar hemispheres, display extensive nodularity, and have a relatively favorable prognosis. In contrast, 75 to 80% of tumors are regarded as classic MBs. These are commonly located in the center of the cerebellum (the vermis), grow as relatively uniform sheets of cells with a high nuclear/cytoplasmic ratio, and have a tendency to invade adjacent brain and leptomeninges.[19]

Several studies have suggested that desmoplastic and classic MBs may have different origins. Desmoplastic tumors tend to express markers of the granule cell lineage (such as Math1 and $P75^{NTR}$), and have therefore been suggested to arise from GCPs in the EGL. Classic MBs more frequently express markers associated with non-granule neurons (e.g., calbindin) and, hence, have been suggested to originate from the VZ. Gene expression profiling also supports the concept of distinct origins for the different subtypes of MB. For example, both classic and desmoplastic MBs have separable genetic profiles, with desmoplastic MBs expressing genes associated with proliferating GCPs in the EGL.[20,21] In contrast, classic MBs express a distinct set of markers that are more heterogeneous and not clearly associated with any particular cerebellar cell type. These data suggest that desmoplastic MBs may derive from the EGL, whereas classic MBs arise from the VZ.

Although the dichotomy between EGL-derived desmoplastic tumors and VZ-derived classic MBs makes for an appealing model, some studies have cast some doubt on this view. First, some microarray analyses of human MB have failed to find a clear correlation between lineage markers and desmoplastic/classic histology.[22] In addition, a number of recent studies have suggested that both desmoplastic and classic MBs express high levels of stem-cell markers, including the VZ-associated glycoprotein CD133.[1,23] $CD133^+$ stemlike cells isolated from MBs can form proliferating clones characteristic of neural stem cells ("neurospheres"), can undergo self-renewal (ie, form secondary and tertiary neurospheres in culture), and under appropriate conditions, can be induced to differentiate into both neurons and glia. Finally, these $CD133^+$ cells can generate tumors after transplantation into immunocompromised mice. These data suggest that both histological subtypes of MB contain cells that resemble multipotent neural stem cells, but whether the tumors actually arise from stem cells remains unknown.

A number of genes and signaling pathways have been implicated in the genesis of MB, and these also shed some light on the cell of origin. The two most studied examples are the sonic hedgehog (SHH)-patched (PTCH) and the WNT signaling pathways (Fig 3). Patients with germline *PTCH* mutations (which activate the hedgehog pathway) acquire Gorlin's syndrome, a disease characterized by recurrent basal cell carcinomas of the skin, craniofacial abnor-

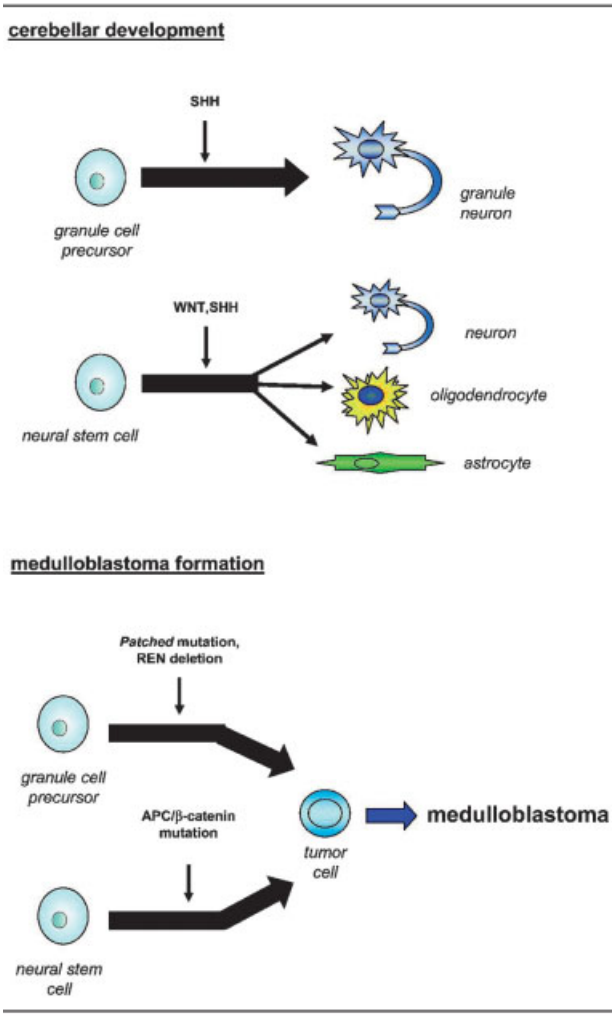


*Fig 3. Regulators of cerebellar development contribute to medulloblastoma formation. During normal cerebellar development, signaling pathways such as those induced by Sonic hedgehog (SHH) and WNT regulate growth and differentiation of granule cell precursors (GCPs) and neural stem cells (top). Mutations that activate these pathways, loss of PTCH, deletion of REN, or mutations in adenomatous polyposis coli (APC) or β-catenin, can cause excessive growth and failure of differentiation, and thereby predispose to medulloblastoma formation (bottom).*

malities, and an increased incidence of MB.[24,25] In addition, 20 to 30% of sporadic MBs harbor activating mutations in the SHH/PTCH pathway mutations, and mice engineered with *patched* mutations experience development of MB.[26–30] Because SHH signaling has been shown to control proliferation of GCPs,[31–33] it has been suggested that tumors arising from SHH pathway mutations are likely to arise from these cells. However, SHH/PTCH signaling may also influence multipotent neural stem cell growth.[34–37] Regardless of the cell of origin of SHH pathway tumors, there is hope that these tumors will be sensitive to small-molecule inhibitors of the hedgehog pathway. Such inhibitors have already been shown to dramatically improve the survival of tumor-bearing *Ptch* mutant mice.[38,39] Their effectiveness in treating human MB is not yet known, but is likely to be evaluated in the near future.

The WNT signaling pathway has likewise been implicated in a subset of MBs. Turcot's syndrome, which results from germline mutations in the adenomatous polyposis coli gene, have a high incidence of colon cancer and brain tumors, primarily MBs.[40] Although adenomatous polyposis coli mutations are relatively rare in sporadic MBs,[41,42] 5 to 15% of these tumors have been reported to contain mutations in β-catenin or Axin, each of which can result in WNT pathway activation.[41,43,44] Unlike the SHH pathway, the WNT pathway has not been implicated in growth or survival of GCPs. However, WNT signaling is known to be critical for the specification of the midbrain-hindbrain boundary from which the entire cerebellum develops,[45,46] and may therefore be important for the growth and survival of multipotent progenitors in the embryonic cerebellum. Alternatively, there may be other classes of progenitors in the developing cerebellum that depend on WNT signaling for proliferation or self-renewal. The cellular targets of transformation in WNT pathway–associated MBs remain an important area of investigation.

## Cellular Origins of Astrocytoma

As with MB, there has been a considerable amount of controversy and discussion regarding the cellular origin of astrocytoma. In 1846, Virchow[47] described the presence of glial cells in the brain and named them "neuroglia." He postulated that these cells may be causally related to the development of a number of brain tumors, and coined the term *glioma.*[47] Ramon y Cajal and Del Rio Hortega further defined this heterogeneous group of neuroglia, subdividing them into astrocytes and oligodendroglia. In 1926, Bailey and Cushing[3] proposed that astrocytic tumors are related to either the maturation of progenitor cells (bipolar spongioblasts) or astrocytes.

Using human tumor tissues, it has not been possible to conclusively demonstrate which cell type causes astrocytoma, and debate continues whether astrocytomas arise from differentiated astrocytes, astroglial progenitor cells, or neural stem cells. Immunohistochemical studies have shown that astrocytoma tumor cells express protein markers typically found in glial progenitor cells, including GFAP, nestin, brain lipid-binding protein, and OLIG-2.[48–52] However, identifying the cell of origin based on these lineage-specific markers is problematic. In this regard, although GFAP has long been regarded as a marker for differentiated astrocytes, recent studies have shown that GFAP expression begins in midembryogenesis and marks cells in the subven-

tricular zone with the ability to function as true neuroglial stem cells.[53,54] With the recognition that GFAP identifies a wide variety of cell types, ranging from stem cells to mature astrocytes, its use as a marker for differentiated astrocytes has been called into question. Efforts to identify additional differentiation markers for the astroglial lineage have been sought by studying glial cell differentiation in vitro; however, it is not clear that the "lineage-specific" markers expressed by differentiating astroglial cells grown in vitro reflect the different phases of astroglial cell maturation that occur in the intact animal in vivo.[55] Finally, similar to MBs, all histological grades of human astrocytoma contain $CD133^+$ stem cells, which, when explanted into naive mouse brains, result in the development of astrocytomas histologically identical to the original parental tumor.[1,56,57] As discussed earlier, these studies support the hypothesis that stem cells exist in human astrocytomas, and that these cells can regenerate astrocytomas in naive recipient mice, but do not prove that astrocytomas arise from these cells.

A number of specific genetic changes that influence astroglial cell differentiation from stem cells in vivo have been identified that have particular relevance to gliomagenesis (Fig 4). These include growth factors (epidermal growth factor [EGF] and platelet-derived growth factor [PDGF]), proteins of the interleukin family (interleukin 6 [IL-6] and leukemia inhibitory factor), and members of the SHH transcriptional control program (OLIG and GLI transcription factors). In this regard, PDGF is a potent mitogen for astroglial cell precursors and is a critical growth factor that specifies oligodendrocyte development.[58,59] EGF has been shown to promote astroglial cell differentiation from neural stem cells at the expense of neurons both in vitro and in vivo,[60–62] and mice lacking the EGF receptor exhibit abnormal astrocyte maturation.[63] Astrocyte differentiation is dependent on IL-6 and leukemia inhibitory factor receptor function,[64] such that leukemia inhibitory factor receptor–deficient mice exhibit a significant reduction in the number of $GFAP^+$ cells in the brain.[65] Lastly, SHH has been implicated in the maintenance of neural progenitor cells and in the differentiation of oligodendroglial and astroglial cells.[34,66,67]

It should not be surprising that some of the cancer-associated changes important for astrocytoma formation involve the same genes important for astroglial cell differentiation during development (see Fig 4). In this regard, mutations in these genes would release the normal brakes on cell proliferation and the terminally differentiated state and facilitate the acquisition of a less differentiated and more proliferative cellular phenotype. Activating mutations in the EGF receptor are commonly seen in high-grade astrocytoma,[68] and mice engineered to express a mutationally activated EGF receptor in combination with other genetic changes develop astrocytoma.[69–71] Similarly, astrocytomas of many grades exhibit increased PDGF and PDGF receptor expression.[72] PDGF has been shown to dedifferentiate cultured astrocytes in vitro and result in oligodendroglioma formation in vivo.[73] Using a glioma-prone transgenic mouse model, IL-6 was found to be required for tumor formation.[74] Lastly, altered expression of members of the SHH pathway have been reported in astrocytomas,[75] and some of its downstream transcriptional targets have been implicated in astrocytoma formation.[52,76]

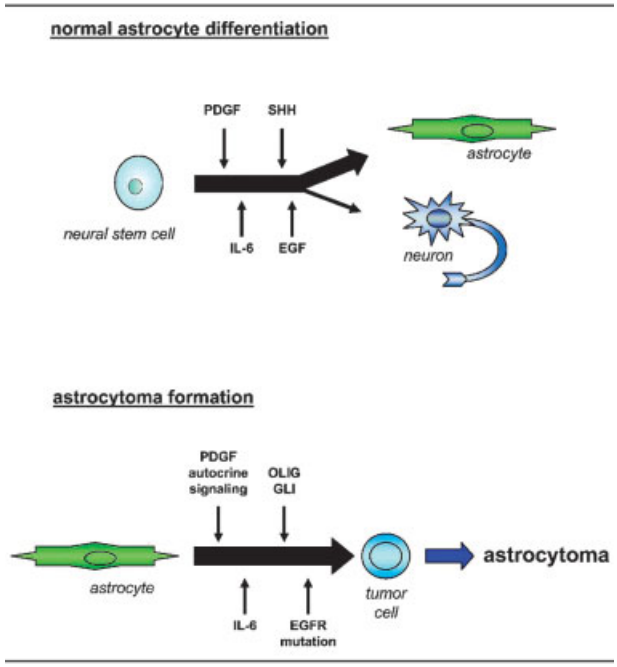


*Fig 4. Regulators of astrocyte differentiation play a critical role in astrocytoma formation. The same proteins involved in normal glial cell differentiation from glial progenitors are mutated or altered in astrocytoma. For example, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), as well as interleukin-6 (IL-6), are important for favoring glial differentiation from neural stem cells, whereas the sonic hedgehog (SHH) signaling pathway regulates the transcription factors of the GLI and OLIG family critical for astrocytic and oligodendrocytic lineage specification (top). In human glial neoplasms, mutation and deregulated expression of the EGF and PDGF receptors (EGFR and PDGFR, respectively), PDGF, members of the IL-6 family, and SHH pathway regulators are observed (bottom).*

A number of these regulators of brain and astrocytoma development are also potential targets for therapeutic drug design. Inhibition of EGF and PDGF receptors by specific small-molecule tyrosine kinase inhibitors are currently being investigated in clinical trials.[77,78] Similarly, monoclonal antibody therapy against EGF receptor is in preclinical evaluation for the treatment of glioma.[79] It is likely that additional tar-

geted therapies will derive from basic research aimed at defining the growth regulatory pathways important for glial cell growth and differentiation.

**Finding the Cell of Origin**

Despite intense scientific investigation, it is fair to say that the origins of human brain tumors remain unresolved. Moreover, several characteristics of human tumors may make it difficult to resolve this issue definitively. First, because human tumors can be studied only once they have already developed, the cell of origin can merely be inferred retrospectively from markers expressed in its progeny. In this regard, that a tumor cell expresses a marker of a particular lineage does not necessarily mean that the tumor arose from cells of that lineage. Second, because tumor cells undergo significant molecular changes as a result of transformation, they may express markers that are not expressed by their normal counterparts during development. Finally, the heterogeneity of human tumors and the discrepancy among the histopathological criteria used to classify tumors further complicates studies of the cell of origin for human brain tumors.

However, these limitations and obstacles do not mean that searching for the cell of origin for brain tumors is futile. A powerful alternative to studying the cell of origin in human tumors involves the use of genetically or virally engineered animal models. Mouse models based on a specific genetic mutation have a number of significant advantages. First, they are less genetically heterogeneous than human tumors, making it easier to draw conclusions about the cell of origin for any particular tumor. Second, using retroviral gene delivery or transgenic technology, genetic alterations can be introduced into specific subpopulations of normal cells, and the resulting animals can be used to prospectively test hypotheses about the cell of origin. Finally, mouse tumors can be studied at both early and late stages, so that progressive molecular and phenotypic changes in the cell of origin can be tracked as they happen, instead of being inferred from the end stages of the disease. A number of such studies have already been performed, and these have important implications for our understanding of the origins of brain tumors.

Studies in genetically engineered mice have provided important insights into the cellular origins of MB. In this regard, conditional knock-out methods (Cre-Lox technology) have been used to assess the role of the retinoblastoma (Rb) and p53 tumor suppressor genes in neoplastic transformation.[80] Mice expressing the Cre recombinase under the control of the GFAP promoter were mated with mice in which the Rb and p53 genes were flanked by LoxP sites, so that Rb or p53 would be deleted in $GFAP^+$ cells. Mice lacking both Rb and p53 expression in $GFAP^+$ cells experienced development of MBs. In this model, Rb and p53 inactivation occurred in astrocytes, but also in a population of neuronal precursors in the EGL. In a complementary approach, the RCAS-TVA viral transduction system has been used to target oncogenes to neural progenitors in the cerebellum.[81] Nestin-TVA transgenic mice, which express the avian retrovirus receptor (TVA) in $nestin^+$ cells, were infected as neonates with avian retroviruses encoding SHH. In this model, MB formation was observed in 9 to 15% of mice, suggesting that SHH-induced MB can arise from $nestin^+$ progenitors in the postnatal cerebellum.

Similar to the approaches described for MB, mouse modeling studies have shown that specific oncogenic changes can also result in astrocytoma formation when introduced into either $GFAP^+$ or $nestin^+$ cells in vivo.[82] In addition, astrocytomas form in mice explanted with either neural stem cells or astrocytes engineered to harbor glioma-associated genetic changes.[70] However, there is a tendency for $nestin^+$ cells to be more sensitive than $GFAP^+$ cells to these glioma-associated genetic changes.[83] Similarly, more aggressive gliomas arise in genetically engineered neurofibromatosis-1 *(Nf1)* mutant mice when *Nf1* inactivation occurs in $GFAP^+$ cells at embryonic day 10 as opposed to embryonic day 14.[84,85] Collectively, these results suggest, but do not prove, that astrocytoma formation may be influenced by the timing of specific genetic changes related to gliomagenesis.

The use of animal models offers a powerful new approach to studying tumor origins, and in the long run, it may lead to definitive conclusions regarding the cell of origin. However, developing appropriate models depends on at least two critical types of information: (1) the identification of specific genes with deregulation that is sufficient to cause tumors in mice, and (2) the identification of cell type–specific promoters that can be used to drive expression of these genes in the appropriate cell types. The list of genes that have been shown to be mutated or misexpressed in human MB and astrocytoma is growing rapidly, but most have not yet been shown to cause tumors in mice. Similarly, a number of transgenes and mutations have been demonstrated to cause MB and astrocytoma in mice, but their contribution to the development of human brain tumors remains unknown. Further study of the key molecular switches that govern astrocyte and neuronal differentiation is likely to yield important insights into the specific genes and growth regulatory pathways deregulated in MB and astrocytoma.

Similarly, we have currently identified only a small number of cell-specific promoters for the in vivo interrogation of the cell of origin of MB and astrocytoma. For astrocytoma and MB, most genetically engineered mouse modeling studies have used the nestin or GFAP promoters, the interpretation of which is complicated by uncertainty regarding the identity of the cells in the

developing brain that express GFAP or nestin. In this regard, GFAP expression has been reported in both astrocytes and neural stem cells, whereas nestin expression has been observed in GCPs, neural stem cells, and radial glia. Future studies will be required to identify and use more specific genetic markers for astrocytes, neural stem cells, and GCPs that would enable the generation of additional genetically engineered mouse models to evaluate the contribution of these cell types to the origin of brain tumors. Progress in these areas will undoubtedly lead to new animal models for MB and astrocytoma and to a clearer definition of the cell of origin of these tumors.

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## References

1. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. Nature 2004;432:396–401.
2. Frank NY, Margaryan A, Huang Y, et al. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. Cancer Res 2005;65:4320–4333.
3. Bailey P, Cushing H. A classification of the tumors of the glioma group on a histogenic basis with a correlation study of prognosis. Philadelphia: Lippincott, 1926.
4. Kleihues P, Louis DN, Scheithauer BW, et al. The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol 2002;61:215–226.
5. Wright J. Neurocytoma and neuroblastoma, a kind of tumor not generally recognized. J Exp Med 1910:556–561.
6. Bailey P, Cushing H. Medulloblastoma cerebelli: a common type of midcerebellar glioma of childhood. Arch Neurol Psychiatry 1925;14:192–223.
7. Altman J, Bayer SA. Development of the cerebellar system: in relation to its evolution, structure and functions. Boca Raton, FL: CRC Press, 1997.
8. Katsetos CD, Del Valle L, Legido A, et al. On the neuronal/neuroblastic nature of medulloblastomas: a tribute to Pio del Rio Hortega and Moises Polak. Acta Neuropathol (Berl) 2003;105:1–13.
9. Kadin ME, Rubinstein LJ, Nelson JS. Neonatal cerebellar medulloblastoma originating from the fetal external granular layer. J Neuropathol Exp Neurol 1970;29:583–600.
10. Pomeroy SL, Sutton ME, Goumnerova LC, Segal RA. Neurotrophins in cerebellar granule cell development and medulloblastoma. J Neurooncol 1997;35:347–352.
11. Buhren J, Christoph AH, Buslei R, et al. Expression of the neurotrophin receptor p75NTR in medulloblastomas is correlated with distinct histological and clinical features: evidence for a medulloblastoma subtype derived from the external granule cell layer. J Neuropathol Exp Neurol 2000;59:229–240.
12. Yokota N, Aruga J, Takai S, et al. Predominant expression of human zic in cerebellar granule cell lineage and medulloblastoma. Cancer Res 1996;56:377–383.
13. Salsano E, Pollo B, Eoli M, et al. Expression of MATH1, a marker of cerebellar granule cell progenitors, identifies different medulloblastoma sub-types. Neurosci Lett 2004;370:180–185.
14. Pelc K, Vincent S, Ruchoux MM, et al. Calbindin-d(28k): a marker of recurrence for medulloblastomas. Cancer 2002;95:410–419.
15. Valtz NL, Hayes TE, Norregaard T, et al. An embryonic origin for medulloblastoma. New Biol 1991;3:364–371.
16. Maria BL, Wong D, Kalnins VI. Dibutyryl cyclic AMP induces vimentin and GFAP expression in cultured medulloblastoma cells. Can J Neurol Sci 1990;17:15–20.
17. Herpers MJ, Budka H. Primitive neuroectodermal tumors including the medulloblastoma: glial differentiation signaled by immunoreactivity for GFAP is restricted to the pure desmoplastic medulloblastoma ("arachnoidal sarcoma of the cerebellum"). Clin Neuropathol 1985;4:12–18.
18. Katsetos CD, Burger PC. Medulloblastoma. Semin Diagn Pathol 1994;11:85–97.
19. Ellison D. Classifying the medulloblastoma: insights from morphology and molecular genetics. Neuropathol Appl Neurobiol 2002;28:257–282.
20. Pomeroy SL, Tamayo P, Gaasenbeek M, et al. Prediction of central nervous system embryonal tumour outcome based on gene expression. Nature 2002;415:436–442.
21. Kho AT, Zhao Q, Cai Z, et al. Conserved mechanisms across development and tumorigenesis revealed by a mouse development perspective of human cancers. Genes Dev 2004;18:629–640.
22. Lee Y, Miller HL, Jensen P, et al. A molecular fingerprint for medulloblastoma. Cancer Res 2003;63:5428–5437.
23. Hemmati HD, Nakano I, Lazareff JA, et al. Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci U S A 2003;100:15178–15183.
24. Gorlin RJ. Nevoid basal-cell carcinoma syndrome. Medicine (Baltimore) 1987;66:98–113.
25. Levanat S, Gorlin RJ, Fallet S, et al. A two-hit model for developmental defects in Gorlin syndrome. Nat Genet 1996;12:85–87.
26. Pietsch T, Waha A, Koch A, et al. Medulloblastomas of the desmoplastic variant carry mutations of the human homologue of Drosophila patched. Cancer Res 1997;57:2085–2088.
27. Raffel C, Jenkins RB, Frederick L, et al. Sporadic medulloblastomas contain PTCH mutations. Cancer Res 1997;57:842–845.
28. Taylor MD, Liu L, Raffel C, et al. Mutations in SUFU predispose to medulloblastoma. Nat Genet 2002;31:306–310.
29. Goodrich LV, Milenkovic L, Higgins KM, Scott MP. Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 1997;277:1109–1113.
30. Oliver TG, Read TA, Kessler JD, et al. Loss of patched and disruption of granule cell development in a pre-neoplastic stage of medulloblastoma. Development 2005;132:2425–2439.
31. Wechsler-Reya RJ, Scott MP. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron 1999;22:103–114.
32. Wallace VA. Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. Curr Biol 1999;9:445–448.
33. Dahmane N, Ruiz i Altaba A. Sonic hedgehog regulates the growth and patterning of the cerebellum. Development 1999;126:3089–3100.
34. Machold R, Hayashi S, Rutlin M, et al. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 2003;39:937–950.
35. Lai K, Kaspar BK, Gage FH, Schaffer DV. Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. Nat Neurosci 2003;6:21–27.

36. Palma V, Ruiz i Altaba A. Hedgehog-GLI signaling regulates the behavior of cells with stem cell properties in the developing neocortex. Development 2004;131:337–345.
37. Ahn S, Joyner AL. In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. Nature 2005;437: 894–897.
38. Romer J, Curran T. Targeting medulloblastoma: small-molecule inhibitors of the Sonic Hedgehog pathway as potential cancer therapeutics. Cancer Res 2005;65:4975–4978.
39. Sanchez P, Ruiz i Altaba A. In vivo inhibition of endogenous brain tumors through systemic interference of Hedgehog signaling in mice. Mech Dev 2005;122:223–230.
40. Turcot J, Despres JP, St Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon: report of two cases. Dis Colon Rectum 1959;2:465–468.
41. Koch A, Waha A, Tonn JC, et al. Somatic mutations of WNT/wingless signaling pathway components in primitive neuroectodermal tumors. Int J Cancer 2001;93:445–449.
42. Huang H, Mahler-Araujo BM, Sankila A, et al. APC mutations in sporadic medulloblastomas. Am J Pathol 2000;156:433–437.
43. Zurawel RH, Chiappa SA, Allen C, Raffel C. Sporadic medulloblastomas contain oncogenic beta-catenin mutations. Cancer Res 1998;58:896–899.
44. Dahmen RP, Koch A, Denkhaus D, et al. Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas. Cancer Res 2001;61:7039–7043.
45. McMahon AP, Bradley A. The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. Cell 1990;62:1073–1085.
46. Thomas KR, Musci TS, Neumann PE, Capecchi MR. Swaying is a mutant allele of the proto-oncogene Wnt-1. Cell 1991;67: 969–976.
47. Virchow R. Zur Entwicklungsgeschichte des Krebses. Virchows Arch 1847;1:94.
48. Lu QR, Park JK, Noll E, et al. Oligodendrocyte lineage genes (OLIG) as molecular markers for human glial brain tumors. Proc Natl Acad Sci U S A 2001;98:10851–10856.
49. Dahlstrand J, Collins VP, Lendahl U. Expression of the class VI intermediate filament nestin in human central nervous system tumors. Cancer Res 1992;52:5334–5341.
50. Tohyama T, Lee VM, Rorke LB, et al. Nestin expression in embryonic human neuroepithelium and in human neuroepithelial tumor cells. Lab Invest 1992;66:303–313.
51. Godbout R, Bisgrove DA, Shkolny D, Day RS 3rd. Correlation of B-FABP and GFAP expression in malignant glioma. Oncogene 1998;16:1955–1962.
52. Ligon KL, Alberta JA, Kho AT, et al. The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. J Neuropathol Exp Neurol 2004;63:499–509.
53. Doetsch F, Caille I, Lim DA, et al. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97:703–716.
54. Quinones-Hinojosa A, Sanai N, Soriano-Navarro M, et al. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. J Comp Neurol 2006;494:415–434.
55. Liu Y, Rao MS. Glial progenitors in the CNS and possible lineage relationships among them. Biol Cell 2004;96:279–290.
56. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 2004;64:7011–7021.
57. Yuan X, Curtin J, Xiong Y, et al. Isolation of cancer stem cells from adult glioblastoma multiforme. Oncogene 2004;23: 9392–9400.
58. Richardson WD, Pringle N, Mosley MJ, et al. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 1988;53:309–319.
59. Raff MC, Lillien LE, Richardson WD, et al. Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. Nature 1988;333:562–565.
60. Jori FP, Galderisi U, Piegari E, et al. EGF-responsive rat neural stem cells: molecular follow-up of neuron and astrocyte differentiation in vitro. J Cell Physiol 2003;195:220–233.
61. Doetsch F, Petreanu L, Caille I, et al. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuron 2002;36:1021–1034.
62. Kuhn HG, Winkler J, Kempermann G, et al. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. J Neurosci 1997;17:5820–5829.
63. Kornblum HI, Hussain R, Wiesen J, et al. Abnormal astrocyte development and neuronal death in mice lacking the epidermal growth factor receptor. J Neurosci Res 1998;53:697–717.
64. Taga T, Fukuda S. Role of IL-6 in the neural stem cell differentiation. Clin Rev Allergy Immunol 2005;28:249–256.
65. Koblar SA, Turnley AM, Classon BJ, et al. Neural precursor differentiation into astrocytes requires signaling through the leukemia inhibitory factor receptor. Proc Natl Acad Sci U S A 1998;95:3178–3181.
66. Marshall CA, Novitch BG, Goldman JE. Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. J Neurosci 2005;25:7289–7298.
67. Rowitch DH, S-Jacques B, Lee SM, et al. Sonic hedgehog regulates proliferation and inhibits differentiation of CNS precursor cells. J Neurosci 1999;19:8954–8965.
68. Friedman HS, Bigner DD. Glioblastoma multiforme and the epidermal growth factor receptor. N Engl J Med 2005;353: 1997–1999.
69. Ding H, Shannon P, Lau N, et al. Oligodendrogliomas result from the expression of an activated mutant epidermal growth factor receptor in a RAS transgenic mouse astrocytoma model. Cancer Res 2003;63:1106–1113.
70. Bachoo RM, Maher EA, Ligon KL, 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.
71. Holland EC, Hively WP, DePinho RA, Varmus HE. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. Genes Dev 1998;12:3675–3685.
72. Board R, Jayson GC. Platelet-derived growth factor receptor (PDGFR): a target for anticancer therapeutics. Drug Resist Updat 2005;8:75–83.
73. Dai C, Celestino JC, Okada Y, et al. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. Genes Dev 2001;15:1913–1925.
74. Weissenberger J, Loeffler S, Kappeler A, et al. IL-6 is required for glioma development in a mouse model. Oncogene 2004;23: 3308–3316.
75. Katayam M, Yoshida K, Ishimori H, et al. Patched and smoothened mRNA expression in human astrocytic tumors inversely correlates with histological malignancy. J Neurooncol 2002;59:107–115.
76. Kinzler KW, Ruppert JM, Bigner SH, Vogelstein B. The GLI gene is a member of the Kruppel family of zinc finger proteins. Nature 1988;332:371–374.
77. Reardon DA, Egorin MJ, Quinn JA, et al. Phase II study of imatinib mesylate plus hydroxyurea in adults with recurrent glioblastoma multiforme. J Clin Oncol 2005;23:9359–9368.
78. Reardon DA, Quinn JA, Vredenburgh JJ, et al. Phase 1 trial of gefitinib plus sirolimus in adults with recurrent malignant glioma. Clin Cancer Res 2006;12:860–868.

79. Perera RM, Narita Y, Furnari FB, et al. Treatment of human tumor xenografts with monoclonal antibody 806 in combination with a prototypical epidermal growth factor receptor-specific antibody generates enhanced antitumor activity. Clin Cancer Res 2005;11:6390–6399.
80. Marino S, Vooijs M, van Der Gulden H, et al. Induction of medulloblastomas in p53-null mutant mice by somatic inactivation of Rb in the external granular layer cells of the cerebellum. Genes Dev 2000;14:994–1004.
81. Fults D, Pedone C, Dai C, Holland EC. MYC expression promotes the proliferation of neural progenitor cells in culture and in vivo. Neoplasia 2002;4:32–39.
82. Holland EC. Progenitor cells and glioma formation. Curr Opin Neurol 2001;14:683–688.
83. Holland EC, Celestino J, Dai C, et al. Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat Genet 2000;25:55–57.
84. Bajenaru ML, Hernandez MR, Perry A, et al. Optic nerve glioma in mice requires astrocyte Nf1 gene inactivation and Nf1 brain heterozygosity. Cancer Res 2003;63:8573–8577.
85. Zhu Y, Harada T, Liu L, et al. Inactivation of NF1 in CNS causes increased glial progenitor proliferation and optic glioma formation. Development 2005;132:5577–5588.