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Getting at the Root and Stem of Brain Tumors

Minireview

Trudy G. Oliver and Robert J. Wechsler-Reya*
Department of Pharmacology and Cancer Biology
Duke University Medical Center
Durham, North Carolina 27710

Brain tumors are among the most aggressive and intractable types of cancer. Recent studies indicate that brain tumor cells resemble neural stem cells in terms of phenotype, signaling, and behavior in vitro. In light of these similarities, it has been suggested that brain tumors arise from stem cells, that they co-opt stem cell strategies for self-renewal, and even that they contain "cancer stem cells" that are critical for tumor maintenance. We will examine these possibilities and discuss their implications for the understanding and treatment of brain tumors.

Due to their rapid growth and tendency to spread throughout the brain and spinal cord, brain tumors are extremely difficult to treat and often fatal. Of the 18,000 Americans diagnosed with primary malignant brain tumors each year, only one-third will survive beyond 5 years (American Cancer Society, 2004). Glioblastoma multiforme, the most common brain tumor in adults, has a mean survival time of 10–12 months. Medulloblastoma, the most common malignant brain tumor in children, has a more favorable prognosis: combinations of radiation, chemotherapy, and surgery can cure more than half of patients. However, children who survive medulloblastoma treatment often suffer severe cognitive and physical deficits and increased susceptibility to other cancers. To develop more effective therapies for these tragic diseases, we need a deeper understanding of their molecular and cellular basis. Recent studies suggest that this understanding may come from an appreciation of the relationship between brain tumors and neural stem cells (NSCs).

Genes and Signals Shared by Brain Tumors and Stem Cells

The fact that brain tumors frequently include a mixture of neuronal and glial cell types has often been cited as evidence that these tumors may contain multipotent progenitors. Consistent with this notion, many brain tumors contain cells that express markers of neural stem cells (Hemmati et al., 2003; Ignatova et al., 2002; Leung et al., 2004; Singh et al., 2003). The most common example of a shared antigen is the intermediate filament protein nestin, which is expressed in NSCs in the developing and adult CNS and has also been found in brain tumors and brain tumor-derived cell lines. Recent studies have reinforced this correlation by describing expression of other NSC markers—including CD133, *musashi-1*, *bmi-1*, and *sox-2*—in primary brain tumor cells. Although the phenotypic similarities between brain tumors and NSCs could be coincidental, the growing list of commonly expressed genes suggests a more intimate relationship between these cells.

In fact, the similarity between brain tumors and neural stem cells extends to the level of cell signaling as well. For example, the Sonic hedgehog (Shh) pathway plays a critical role in regulating growth of neural progenitors and has been implicated in the etiology of brain tumors. In the cerebellum, Shh is required for proliferation of granule cell precursors, and mutations in this pathway predispose to cerebellar tumors (medulloblastomas) in both mice and humans (reviewed in Wechsler-Reya, 2003). Recent studies suggest that Shh signaling may also regulate self-renewal of progenitors in other parts of the brain, including the subventricular zone, hippocampus, and olfactory bulb (Lai et al., 2003; Machold et al., 2003). These findings raise the possibility that Shh pathway activation may be involved in tumors besides medulloblastoma. Although a small percentage of glial tumors (gliomas) exhibit amplification of the Shh-responsive transcription factor *Gli1*, no other Shh pathway mutations have been found in these tumors. However, at least one report suggests that hedgehog pathway genes are expressed in gliomas and that growth of glioma cell lines is reduced by pharmacological inhibitors of the pathway (Dahmane et al., 2001). In light of recent evidence that ligand-dependent (and mutation-independent) hedgehog pathway activation contributes to tumorigenesis in other tissues (Berman et al., 2003), the role of Shh signaling in other brain tumors merits further study.

The Wnt pathway, which is critical for self-renewal of hematopoietic and epithelial stem cells (Reya et al., 2001), may also be involved in the growth of NSCs and brain tumors. Recent reports show that β-catenin, a central mediator of Wnt signaling, is important for NSC and progenitor cell proliferation throughout the CNS. Conditional deletion of *β-catenin* causes depletion of neuronal precursors in the ventricular zone, whereas overexpression of activated β-catenin results in massive expansion of the precursor pool and enlargement of the hindbrain, midbrain, and forebrain (Chenn and Walsh, 2002; Zechner et al., 2003). Activation of the Wnt pathway is also associated with brain tumors. In particular, a subset of medulloblastomas has been reported to harbor mutations in *β-catenin*, *Axin*, or *APC* (Wechsler-Reya, 2003). Since these tumors have a distinct phenotype and molecular profile from medulloblastomas associated with hedgehog pathway mutations, they may arise from a distinct set of progenitors. To date, there have been no reports of Wnt pathway mutations in brain tumors outside the cerebellum. However, given the importance of Wnt signaling in self-renewal of progenitors throughout the brain, it is worth considering whether dysregulation of this pathway occurs in other types of brain tumors as well.

Other genes that may control self-renewal of both brain tumors and neural stem cells include the polycomb transcription factor Bmi-1 and the phosphatase PTEN. Bmi-1 regulates self-renewal of progenitors in the central and peripheral nervous systems (Molofsky et al., 2003), in part by repressing genes (such as p16Ink4a

*Correspondence: rw.reya@duke.edu

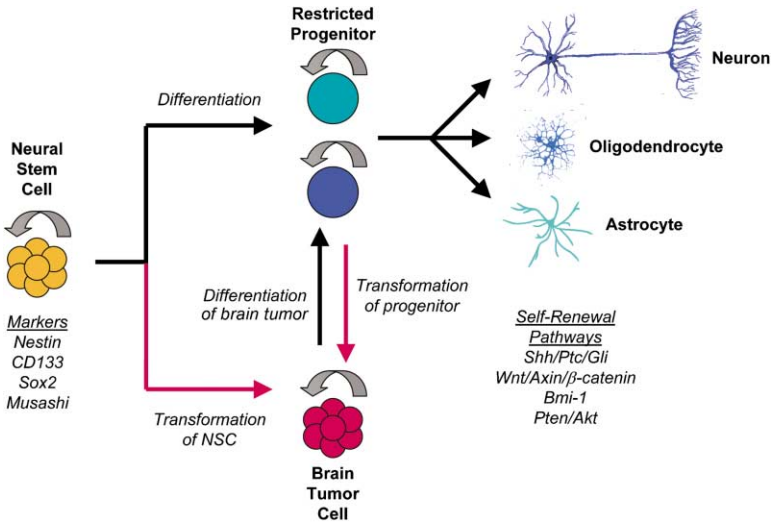

Figure 1. The Relationship between Brain Tumors and Neural Stem Cells

Brain tumors (red) share many characteristics with NSCs (yellow), including expression of intracellular and cell surface markers, activation of signaling pathways, and an ability to form self-renewing, multipotent neurospheres in vitro. One explanation for these similarities is that brain tumors result from transformation of NSCs, and there is evidence from transgenic mice to support this possibility. However, brain tumors may also arise from transformation of more restricted progenitors; in this case, the similarities in gene expression might represent acquisition of stem cell characteristics as a consequence of transformation. In either case, cells with stem cell-like properties are likely to be critical for growth of brain tumors, and targeting these cells may represent an important avenue for therapy.

and p19Arf) that promote cellular senescence and cell death. In addition, *bmi-1* has recently been shown to be a target of the Shh signaling pathway and to be required for self-renewal of neural progenitors in the cerebellum (Leung et al., 2004). Thus, elevated expression of *bmi-1* in medulloblastoma and other brain tumors (Hemmati et al., 2003; Leung et al., 2004) may reflect an increased capacity for self-renewal. *PTEN* is one of the most frequently mutated genes in primary glioblastoma (Li et al., 2003). Recent studies of mice in which *PTEN* has been conditionally deleted in nestin-expressing precursors suggest that it is a critical negative regulator of NSC proliferation and survival (Groszer et al., 2001). In addition, studies of $PTEN^{+/-}$ mice suggest that this gene may also be important for NSC migration (Li et al., 2003). Thus, dysregulation of PTEN may contribute to the aggressive growth and metastatic behavior associated with gliomas.

Self-Renewal and Multipotency of Brain Tumors and Stem Cells

The similarities in phenotype and signaling between brain tumor cells and stem cells may reflect similarities in their cellular behavior. Support for this notion comes from recent studies by Ignatova et al. (2002), Hemmati et al. (2003), and Singh et al. (2003). These investigators isolated cells from primary human brain tumors (including astrocytomas, glioblastomas, and medulloblastomas) and showed that, like NSCs, they form clonal neurospheres that can be repeatedly passaged in vitro. Upon withdrawal of growth factors, tumor-derived neurospheres can differentiate into both neurons and glia, consistent with the heterogeneous cell types found in the tumors from which they were derived. Although brain tumors have long been known to contain a mixture of neurons and glia, it has been unclear whether all of these cells are derived from the tumor or whether some represent normal cells that have become trapped within the tumor matrix. The observation that brain tumor-derived neurospheres can generate multiple cell types suggests that the heterogeneity within brain tumors may be an inherent characteristic of the tumor. Together, these studies suggest that brain tumor cells share with neural stem cells the capacity for self-renewal and multilineage differentiation.

In interpreting these studies, it is important to consider the possibility that prolonged culture of progenitors in high concentrations of growth factors may cause cells to adopt fates that they would not normally adopt in situ, or select for a subset of cells that does not reflect the behavior of the original population (Gabay et al., 2003). Thus, culture of brain tumor cells under neurosphere-generating conditions may dramatically alter their capacity for self-renewal and differentiation. Studying tumor cells that have not been subjected to extensive culture in growth factors may help shed light on their intrinsic properties. Notwithstanding these caveats, the demonstration of functional similarities between brain tumor cells and NSCs represents an important step beyond phenotypic characterization and brings us closer to understanding how these cells may be related to one another.

Do Brain Tumors Arise from Neural Stem Cells?

One explanation for the resemblance between brain tumors and stem cells is that brain tumors result from transformation of NSCs (see Figure 1). But does the fact that brain tumor cells look and act like NSCs mean that they actually arise from them? Or can they result from acquisition of stem cell characteristics by more differentiated progenitors? There is evidence for both possibilities.

Support for the notion that brain tumors can arise from transformation of neural stem cells comes from mouse models in which expression of oncogenes or tumor suppressors is perturbed in an NSC-specific manner. For example, Holland and colleagues have generated mice that express the avian retrovirus receptor (TVA) in nestin+ cells, and then used avian retroviruses to deliver oncogenes into neural stem cells (reviewed in Holland, 2001). Using this system, they have shown that transduction of constitutively active epidermal growth factor receptor, oncogenic Ras and Akt, or platelet-derived growth factor into forebrain nestin+ progenitors results in tumors that resemble human gliomas. Similarly, intracerebellar injection of retroviruses carrying *shh* and c-*myc* can cause medulloblastoma in nestin-TVA mice (Rao et al., 2003). To the extent that transgene expression in these mice is restricted to neural stem cells (nestin expression has also been observed in more

committed progenitors), these studies suggest that NSCs can serve as targets of transformation in brain tumors.

But there is also evidence that brain tumors can result from transformation of more differentiated cells. Shh pathway-associated medulloblastomas in both mice and humans have a phenotype and gene expression profile that strongly resembles that of committed granule cell precursors (Wechsler-Reya, 2003). Moreover, in studies using the TVA system, gliomas can be generated by retroviral transduction of genes into GFAP+ glial progenitors as well as nestin+ NSCs (Holland, 2001). Expression of oncogenic Ras or SV40 T antigen under the control of a *GFAP* promoter also results in gliomas in a large percentage of animals (Ding et al., 2001; Xiao et al., 2002). Finally, transgenic mice expressing v-erbB driven by an *S100*-β promoter (which is active in glial precursors) develop oligodendrogliomas (Weiss et al., 2003). These studies strongly suggest that restricted progenitors may be targets for transformation by oncogenes. However, since GFAP and S100β may also be expressed in neural stem cells (Goldman, 2003; Weiss et al., 2003), it will clearly be necessary to identify more definitive markers of stem cells and progenitors to determine the cell of origin of brain tumors.

Do Brain Tumors Contain "Cancer Stem Cells"?

The presence of NSC-like cells in brain tumors has also been used to suggest that these tumors contain "cancer stem cells" that are critical for their propagation (Hemmati et al., 2003; Singh et al., 2003). While this is an intriguing possibility, it is critical to stress the distinction between tissue stem cells (such as NSCs) and cancer stem cells (Reya et al., 2001). Tissue stem cells exhibit extensive self-renewal and have the capacity to differentiate into multiple cell types in the normal tissue from which they are derived (e.g., neurons, astrocytes, and oligodendrocytes in the CNS). Cancer stem cells may also exhibit extensive self-renewal and multipotency. But most importantly, cancer stem cells are defined by their capacity to maintain the long-term growth of a tumor in vivo. This capacity for tumor propagation is most compellingly demonstrated by transplanting putative cancer stem cells into a new host and showing that they can recapitulate the growth of the tumor. Such experiments have been carried out with human leukemia and breast cancer cells and have shown that a very small fraction of the original tumor is uniquely capable of recapitulating the tumor following transplant (Al-Hajj et al., 2003; Bonnet and Dick, 1997).

In the case of brain tumors, Singh et al. show that a subset of brain tumor cells (those expressing CD133) have the exclusive ability to form multipotent neurospheres in vitro. While isolation of this population is an important step, the ability of these cells to generate and maintain tumors in vivo remains to be tested. If CD133+ cells (or any other population within these tumors) represent true cancer stem cells, it would have significant implications for the understanding and treatment of brain tumors. First, it would suggest that despite the heterogeneity inherent in many brain tumors (reflected, for example, by the term glioblastoma *multiforme*), a single cell type may be responsible for tumor growth and maintenance. Identification of this cell type might provide important markers that can be used for tumor detection and diagnosis. Finally, targeting of this cell may be necessary (and perhaps even sufficient) to disrupt the growth of the tumor.

The studies described above suggest that brain tumors and neural stem cells resemble one another at a cellular and molecular level. Whether brain tumors actually arise from NSCs in most cases remains unclear, and a definitive answer may have to await the development of better markers for neural stem cells and progenitors. Nevertheless the fact that these cells express similar genes and show activation of similar signaling pathways suggests that they use similar strategies to achieve self-renewal and to generate heterogeneous populations of cells within a tissue. Understanding stem cell signaling pathways and developing ways to manipulate them will be critical in our effort to develop new treatments for tumors of the nervous system.

Selected Reading

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