Tumorigenesis in the Brain: Location, Location, Location

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Abstract

Emerging evidence from numerous laboratories supports the notion that brain tumors arise from cells with stem cell/neuroglial progenitor cell properties ("cancer stem cells"). Two recent studies suggest that histologically similar tumors from different brain regions are molecularly distinct because they arise from distinct populations of site-restricted progenitor cells. These new findings imply an interaction between the cell of origin, the tumor microenvironment, and specific cancer-causing genetic changes in the evolution of central nervous system tumors. [Cancer Res 2007;67(12):5579–82]

Introduction

Tumors of the central nervous system (CNS) account for less than 2% of all malignancies, yet brain tumors are the leading cause of cancer-related death in children and the fourth leading cause in adults (1). Glial cell tumors (gliomas and ependymomas) represent the most common CNS neoplasms in all age groups. Gliomas (astrocytomas) and ependymomas are composed of glial fibrillary acidic protein (GFAP)–immunoreactive neoplastic cells. Low-grade astrocytomas (e.g., pilocytic astrocytomas; PA) are more common in children and young adults and are typically located in the cerebellum, brainstem, and optic pathway. In contrast, malignant gliomas predominate in adults and usually arise supratentorially. Ependymomas are slow-growing tumors of children and young adults that originate from the wall of the cerebral ventricles or from the spinal canal, and are most commonly located in the posterior fossa and spinal cord.

It has long been recognized that histologically identical brain tumors could exhibit strikingly diverse clinical behaviors; however, the molecular basis for this variability is currently unknown. Some of these differences may be attributable to the effects of specific genetic changes or unique growth-regulatory cues present in the tumor microenvironment. However, it is also possible that these clinical differences reflect the inherent properties of the tumor cell of origin. With the advent of high-density molecular profiling, it is now possible to define the gene expression patterns of brain tumors relative to their putative cell of origin. Previous microarray-based studies have identified differentially expressed transcripts that distinguish low-grade gliomas from nonneoplastic tissue as well as histologically identical but molecularly distinct tumor subgroups. Interestingly, many of these differentially expressed genes encode proteins with important roles in brain development and neuroglial progenitor differentiation, including neural cell adhesion molecule and connexin-43 (2), growth-associated protein-43 (3), brain lipid–binding protein (4), doublecortin and semaphorin-3B (5), and archaete-scute complex-1 (6). The relationship between gliomagenesis and brain development has been further underscored by the finding that high-grade gliomas can be subclassified according to the expression of gene signatures (neural, proliferating, and mesenchymal-like) characteristic of distinct stages and patterns of differentiation (7). Collectively, these observations raise the possibility that molecularly distinct subgroups of gliomas arise from specific populations of progenitors.

This notion has recently been furthered by two groups employing gene expression microarray analyses to define molecular differences between histologically identical glial cell tumors arising in distinct CNS locations (8, 9). Both studies showed that the site-specific tumor gene expression signatures were similar to those seen in normal neuroglial precursor cell populations (e.g., astrocytes, radial glia, and neural stem cells) from the corresponding regions of the CNS. These findings raise the intriguing possibility that specific populations of progenitor cells within the nervous system give rise to histologically similar tumors with distinct molecular properties. Moreover, these observations underscore the relationship between neuro-oncology and developmental neurobiology and reinforce the need to view the process of CNS tumorigenesis in the context of normal brain development.

Regional Diversity in Normal Neural Tissues

The diversity of cell types in the mammalian brain is astounding. In the human brain, approximately 10^{12} neurons are supported by more than 10^{13} glial cells, and each of these two major cell types is composed of multiple distinguishable subtypes. It has been estimated that there are as many as 10,000 different forms of neurons; and numerous morphologic and molecular variants comprise the glial population. One of the most remarkable features of the CNS is the degree to which this cellular diversity is organized spatiality, resulting in anatomic regions with distinct histologic structures and functions. A variety of cell-autonomous and non–cell-autonomous mechanisms have been shown to shape and maintain regional specification in the developing and mature CNS, and regional differences in neural progenitor cells are evident at the inception of neurogenesis.

Progenitor cell populations capable of generating glia and neurons include both radial glia (RG) and neural stem cells (NSC). RG arise from neuroepithelial cells throughout the CNS at the start of neurogenesis, and represent the predominant neuronal progenitor (10). These cells generally display a radial morphology and mixed primitive cell/glial immunophenotype, but the fate and a function of RG varies markedly from region to region within the CNS. In this regard, studies in which the fate of specific RG populations are traced genetically *in vivo* (11), *ex vivo* studies of RG differentiation (12), and live time-lapse video microscopy studies (13) collectively suggest that dorsal and ventral telencephalic RG

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doi:10.1158/0008-5472.CAN-07-0760

are mainly neurogenic and gliagenic, respectively. These fate differences may represent an inherent property of RG because many RG generate only one single cell type exclusively. For example, >80% of RG cultured from embryonic day (E) 10 mouse cortex are neurogenic (14), and *in vivo* clonal analyses of E9 mouse brain show that >80% of RG are fate-restricted to generate either neurons or glial cells (15). RG are not only heterogeneous within the cerebral cortex, but also display distinct molecular and cellular characteristics between different CNS regions (16). Similarly, NSCs from different brain regions also have different proliferative capacities and varying abilities to generate astrocytes, oligodendrocytes, and neurons that may reflect distinct patterns of gene expression (17–19). In this regard, NSC neurosphere cultures from fetal human rostral brain locations proliferate faster than those from caudal regions, whereas forebrain neurospheres generate more astrocytes and spinal cord neurospheres produce greater numbers of oligodendroglial cells. Thus, both RG and NSCs are heterogeneous populations of precursor cells that likely play major roles in region-specific functional patterning within the CNS.

Regional Diversity in Brain Tumors

Two recent studies identified molecular differences between histologically identical glial cell tumors arising in distinct CNS locations, similar to their nonneoplastic counterparts (8, 9). Taylor and colleagues (8) analyzed a series of 32 ependymomas from the cerebral hemispheres, posterior fossa, and spinal cord using a combination of gene expression profiling and array comparative genomic hybridization methods. They found that subsets of ependymoma exhibit distinct patterns of gene expression and regions of chromosome gain and loss that correlate closely with the anatomic location of the tumor. Intriguingly, these distinct gene expression signatures were also seen in RG in the corresponding regions of the embryonic mouse CNS; and cancer stem cells (CSC) isolated from human ependymomas were shown to possess RG-like properties.

In an analysis of 41 PAs, Sharma and colleagues (9) identified a set of 36 genes that distinguished PAs arising in the posterior fossa from those located in the cerebral hemispheres or hypothalamus/optic nerves (supratentorial location). This expression signature also included genes that are involved in normal brain region–specific development (e.g., LHX2 and NR2E1). Interestingly, a subset of the PA gene expression pattern that distinguished tumors by brain location was shared with normal primary $GFAP^+$ cells (glia) and NSCs from these distinct brain regions. Moreover, some of these region-specific genes were common to both PAs and ependymomas. Together, these findings raise the intriguing possibility that specific populations of site-restricted progenitor cells within the CNS are the cells of origin of histologically similar glial cell tumors with distinct molecular properties. Thus, regional differences in neuroglial precursor cells may dictate a significant component of CNS tumor gene expression and biology.

Regionally Specified Precursor Cells as a Source of Brain CSCs

The CSC hypothesis is predicated on the idea that not all cancer cells have equal proliferative potential, and that cells with the greatest ability to proliferate and form new tumors have phenotypic and functional properties similar to NSCs. Over the past few years, multiple investigators have shown that CSCs isolated from human glial cell tumors (gliomas and ependymomas) undergo self-renewal and multilineage cell differentiation, similar to normal neural stem and precursor cells (20). Glial tumor CSCs express antigens shared with NSCs and progenitors, including nestin, CD133, and brain lipid–binding protein (or fatty acid–binding protein-7). Moreover, similar to normal NSCs, brain CSCs reside in perivascular niches that maintain the stem-like properties of these cancer cells (21). CD133+ glial CSCs form tumors in the brains of mice that are histologically identical to the original tumor, suggesting that these CSCs retain the capacity to generate all cell types found in the parent tumor and can fully recapitulate the neoplastic phenotype *in vivo*. The discovery by Taylor and colleagues (8) and Sharma and colleagues (9) that glial tumors share intrinsic, lineage-specific molecular signatures that reflect the brain region in which their nonmalignant predecessors originated, supports the notion that glial tumor CSCs arise directly from transformed NSCs or precursor cells.

It remains possible that some brain CSCs arise from differentiated cells, like glia, which acquire stem cell properties as a result of mutation. Retroviral transduction of postnatal day 5 *Ink4a/Arf*$^{-/-}$ mouse astrocytes with activated *Egfr* has been shown to induce a transformed phenotype, suggesting that certain mutations may "de-differentiate" mature glial cells (22). However, because RG persist into the first week of life in the mouse and give rise to adult stem cells, stem or progenitor populations may have been the actual target of transformation in these experiments. Furthermore, if mature cells in the CNS are a major source of CSCs, then one might expect to see a more diverse spectrum of brain tumor types in the adult population than is actually observed. Instead, we suggest that the predominance of atypical teratoid/rhabdoid, primitive neuroectodermal, choroid plexus, PAs, and ependymomas among children reflects the large pool of neural precursor cells that exist during early brain development: it is this progenitor cell population that serves as the cellular target of transforming mutations which lead to these unusual tumor types in childhood.

Implications for CNS Tumorigenesis

If glial tumors arise from region-specific progenitor cells, then hard-wired genetic programs in these cells might render them uniquely susceptible to distinct "cancer-causing" genetic mutations (Fig. 1). Implicit in this model are the notions that NSC populations from different CNS regions are not identical and that the effect of specific genetic changes may not be equivalent in all stem cell/progenitor cell populations. Such an effect might account for the different patterns of genetic alterations seen in glial tumors arising in different regions of the CNS. For example, cortical ependymomas have been shown to delete the *INK4A/ARF* tumor suppressor locus and activate NOTCH signaling—genetic changes that promote the self-renewal of normal cortical NSCs (8, 23, 24). Similarly, the differential sensitivity of neural stem and progenitor cells to inactivation of the neurofibromatosis-1 (*NF1*) gene could potentially explain why children with germline *NF1* mutations preferentially develop tumors in the optic pathway, whereas histologically identical PAs arise in the cerebellum in children without NF1 (25). Finally, the transformation of region-specific progenitor cells by combinations of specific genetic changes (e.g., epidermal growth factor receptor amplification and *PTEN* loss) might also involve unique signals from the local microenvironment (e.g., SHH and CXCL12) that contribute to the activation of specific stem cell programs that vary according to brain region (26). Current studies in our laboratories are focused on understanding the consequences of specific genetic mutations in region-specific progenitors on CNS

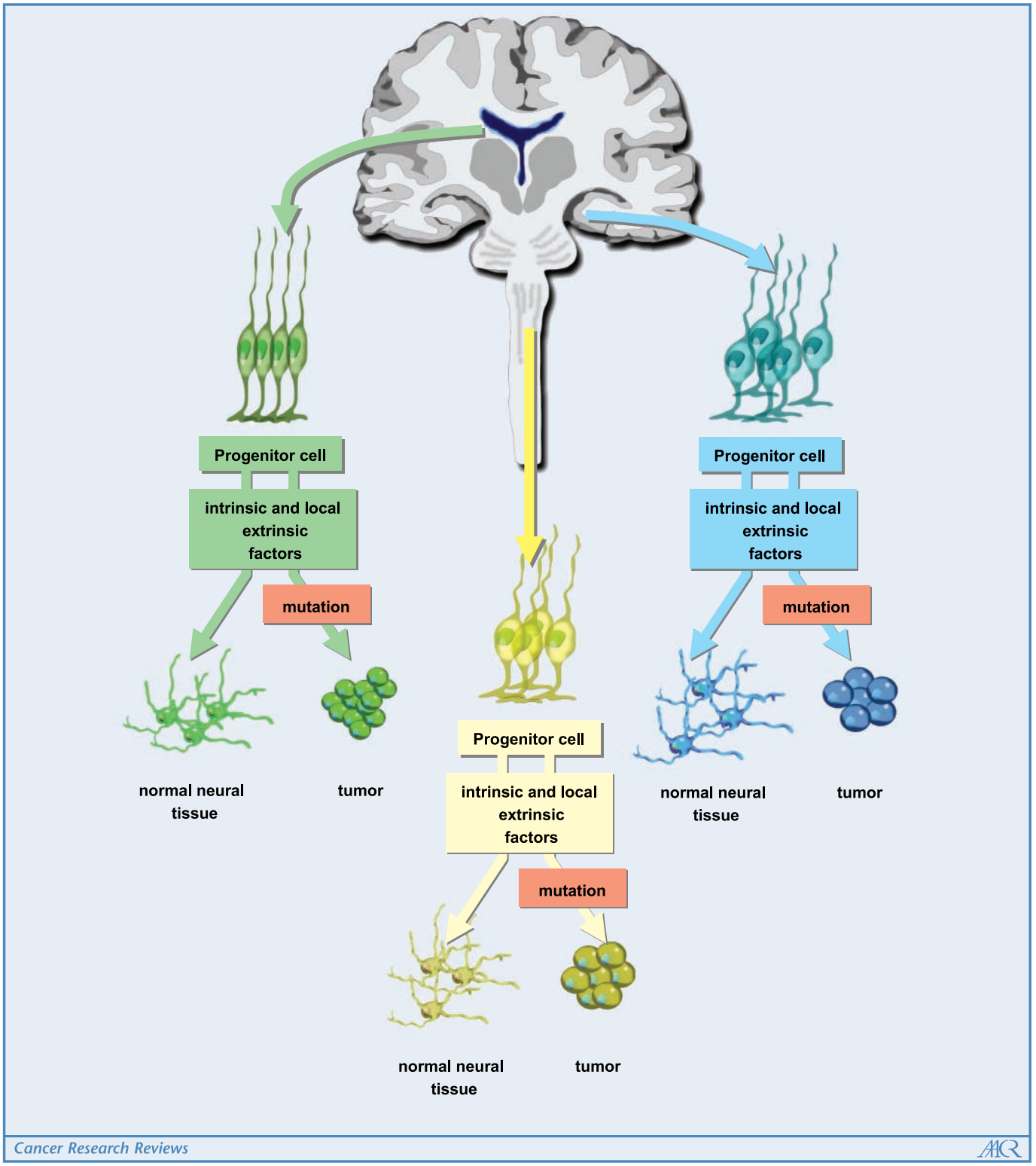

Figure 1. Model of how regional heterogeneity of neural progenitor cells in the CNS may contribute to brain tumor diversity. Neural progenitor/stem cells in different regions of the CNS display different molecular and cellular characteristics, represented in the figure by different colors. Molecular differences include site-specific gene expression signatures whereas cellular differences include distinct proliferative and differentiation potential. Progenitor/stem cells undergo differentiation to form appropriate site-specific normal neural tissue under the control of progenitor/stem cell-intrinsic and local microenvironmental (extrinsic) cues. Molecular differences among progenitor/stem cells in the different regions of the CNS render them uniquely susceptible to different cancer-causing mutations. Accumulation of these mutations, possibly in association with local cues, results in the generation of glial cell tumors with distinct biological properties that reflect the tumor cell of origin (site-specific progenitor/stem cell).

tumorigenesis. As we begin to unravel the significance of these molecular profiles and their effects on progenitor cell biology, it is possible that future targeted therapies will not only have to consider the specific cancer-causing change (e.g., epidermal growth factor receptor mutation), but also the developmental and molecular subgroup of the cells that gave rise to the tumor (cell of origin).

Acknowledgments

Received 2/23/2007; revised 4/25/2007; accepted 5/3/2007.

Grant support: NS058433 and 1-UO1-CA84314 (D.H. Gutmann). R.J. Gilbertson holds the Sydney Schlobohm Leadership Chair of Research from the Brain Tumor Society and is supported by NIH grants CA096832 and CA081457, and the American Lebanese Syrian Associated Charities.

We apologize to those authors whose work we could not cite owing to space limitations.

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Cancer Res 2007;67:5579-5582.