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Brain tumour stem cells: the undercurrents of human brain cancer and their relationship to neural stem cells

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Conceptual and technical advances in neural stem cell biology are being applied to the study of human brain tumours. These studies suggest that human brain tumours are organized as a hierarchy and are maintained by a small number of tumour cells that have stem cell properties. Most of the bulk population of human brain tumours comprise cells that have lost the ability to initiate and maintain tumour growth. Although the cell of origin for human brain tumours is uncertain, recent evidence points towards the brain's known proliferative zones. The identification of brain tumour stem cells has important implications for understanding brain tumour biology and these cells may be critical cellular targets for curative therapy.

Keywords: brain tumour; neural stem cell; cancer stem cell; hierarchy; self-renewal

1. INTRODUCTION

Brain tumours are a diverse group of neoplasms afflicting both children and adults and are among the human cancers with the poorest outcome (DeAngelis 2001). There are no known modifiable risk factors identified for brain tumour prevention. According to National Cancer Institute of Canada statistics (www.ncic.cancer.ca), 2500 new cases of brain tumours and 1650 deaths are expected to occur in adults in 2005, for a death to case ratio (0.67) only exceeded by cancer of the oesophagus, pancreas, lung and multiple myeloma. In adults, the commonest malignant brain tumour, the glioblastoma multiforme, has a 2-year survival rate of less than 10%. In children, brain tumours are the second commonest type of cancer (17% of all childhood cancer) and cause 25% of cancer deaths, just trailing leukaemia in both the categories. Survivors of brain tumours often have intellectual impairment related to chemotherapy and radiation therapy and even brain tumours classified as benign can be lethal due to their location in surgically inaccessible areas.

At the core of treatment failure is a poor understanding of the cellular and molecular mechanisms regulating tumour growth, for example which cells in the diverse tumour population initiate and maintain brain tumour growth? What are the molecular mechanisms involved? What is the normal cell in the brain that is transformed into a brain tumour?

Recently, by applying the principles of stem cell biology to the study of human brain tumours, new insight has been gained into understanding the cellular mechanisms that drive the growth of brain tumours.

A key finding is the demonstration that human brain tumours are organized as a cellular and functional hierarchy based on a subpopulation of brain tumour cells that have stem cell properties. Brain tumour stem cells have potent tumour-initiating and -maintaining ability, a property not shared by most of the cells in the tumour. Recent studies also suggest that brain tumours can be considered as caricatures of normal development, with relatively rare cancer stem cells aberrantly maturing into more differentiated cancer cells that lose the ability to proliferate. The discovery of brain tumour stem cells has important implications for brain tumour research and treatment and, together with earlier findings in human leukaemia and recent findings in human breast cancer, strongly implicates cancer stem cells as the undercurrent that fuels the growth of many human cancers. The implication is that if we can understand the mechanisms regulating the behaviour of cancer stem cells, we can develop new treatments that target the cellular source of cancer's growth. Much of the insight into brain tumour stem cells comes directly from neural stem cell (NSC) research.

2. APPLYING NEURAL STEM CELL BIOLOGY TO BRAIN CANCER

Most, if not all, of our normal somatic tissues are organized as a cellular hierarchy based on stem cells. So why could not human brain tumours, as aberrant organs, also be organized as such a hierarchy (see [figure 1](#))? The discovery of NSCs in the mammalian brain has laid the foundations for designing experiments to test whether this hierarchy also exists in human brain tumours.

NSCs are defined as rare cells in the brain that are capable of extensive self-renewal, proliferation and multilineage differentiation (Hall & Watt 1989; Potten & Loeffler 1990; Fuchs & Segre 2000;

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One contribution of 14 to a Theme Issue 'Stem cells and brain repair'.

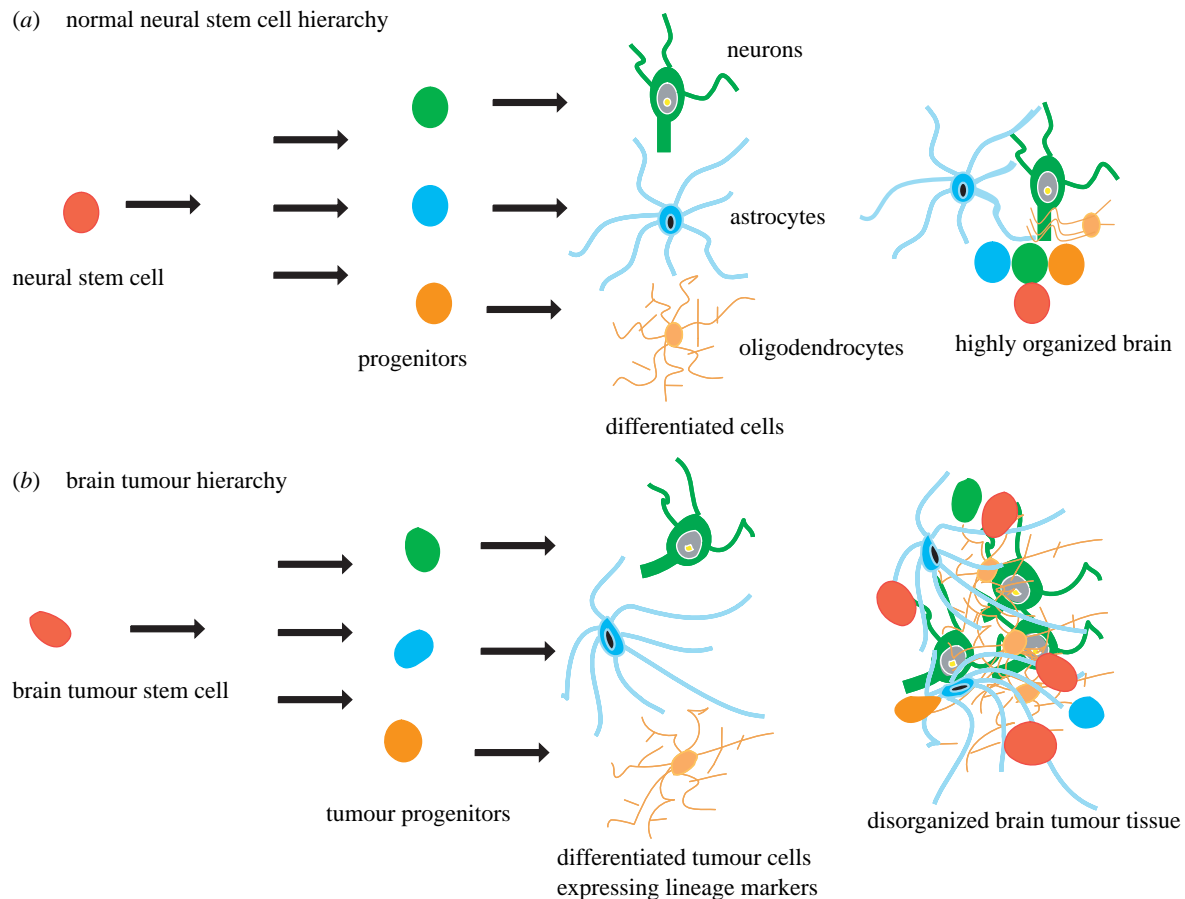


Figure 1. Stem cell hierarchy in (a) normal brain and (b) brain tumours. Rare stem cells in the brain are able to self-renew to maintain themselves and also generate progenitors that proliferate and then differentiate into mature cells that form the highly organized brain parenchyma. The hierarchy remains poorly defined in terms of markers that unequivocally define stem cells from progenitor cells. Brain tumours are also organized as a hierarchy based on cancer stem cells. Cancer stem cells are dysregulated in terms of self-renewal and proliferation and they aberrantly differentiate into cells that form the bulk of the disorganized cancer tissues.

Weissman 2000; Seaberg & van der Kooy 2003). NSCs can be isolated from the embryonic or adult mammalian brain of mice (Reynolds & Weiss 1992, 1996; Lois & Alvarez-Buylla 1993), primates (Gould *et al.* 1999) and humans (Eriksson *et al.* 1998; Flax *et al.* 1998; Fricker *et al.* 1999; Vescovi *et al.* 1999a,b; Roy *et al.* 2000a,b; Sanai *et al.* 2004). NSCs divide symmetrically for self-renewal and generate differentiated progeny (neurons, astrocytes, oligodendrocytes) through asymmetric divisions (figure 2; Gage 1998; Temple 2001). Postnatally, NSCs exist in the subventricular zones (SVZ) throughout the entire neuraxis persisting through adulthood into old age (Weiss *et al.* 1996; Tropepe *et al.* 1997; Gage 1998, 2000). Stem cells have also been demonstrated to exist in the adult hippocampus (Palmer *et al.* 1997), but there remains some controversy about whether the hippocampus contains a mixture of lineage-restricted progenitor cells with limited self-renewal ability as opposed to true self-renewing stem cells (Seaberg & van der Kooy 2002); these discrepancies may relate to differences in culture systems and assays used by different groups to characterize the cells. As we are now armed with a definitive knowledge that a small number of cells in the brain have stem cell potential, it suggests that these cells could be the origin of human brain tumours.

Although NSCs are now known to exist in very restricted zones of the brain, their precise purification has remained elusive. NSCs cannot yet be unambiguously identified with markers. Until recently, NSCs were mainly defined by the expression of nestin, a cytoplasmic intermediate filament protein (Hockfield & McKay 1985; Lendahl *et al.* 1990; Gates *et al.* 1995), but it is clear that nestin identifies neural progenitors as well as stem cells. *In vitro*, nestin expression is lost with differentiation of NSCs into neurons and glia and, *in vivo*, is retained postnatally only in proliferative zones, such as the cerebellar external granule layer (EGL; Hatten & Heintz 1995), and in the SVZ (Morshead *et al.* 1994). Recently, direct isolation of NSCs from human foetal brain using flow cytometry for the cell surface marker, CD133 (AC133, prominin1), was reported (Uchida *et al.* 2000). CD133 was originally shown to be a haemopoietic stem cell marker (Miraglia *et al.* 1997; Yin *et al.* 1997), but it is also expressed in the SVZ of developing mice and humans, being found on the apical membrane of cells lining the ventricle, with more restricted expression than nestin (Weigmann *et al.* 1997; Corbeil *et al.* 2000; Sawamoto *et al.* 2001). Isolated human foetal brain cells expressing CD133 have marked stem cell activity as determined in clonogenic neurosphere assays (Uchida *et al.* 2000). CD133+ human foetal brain cells transplanted into the

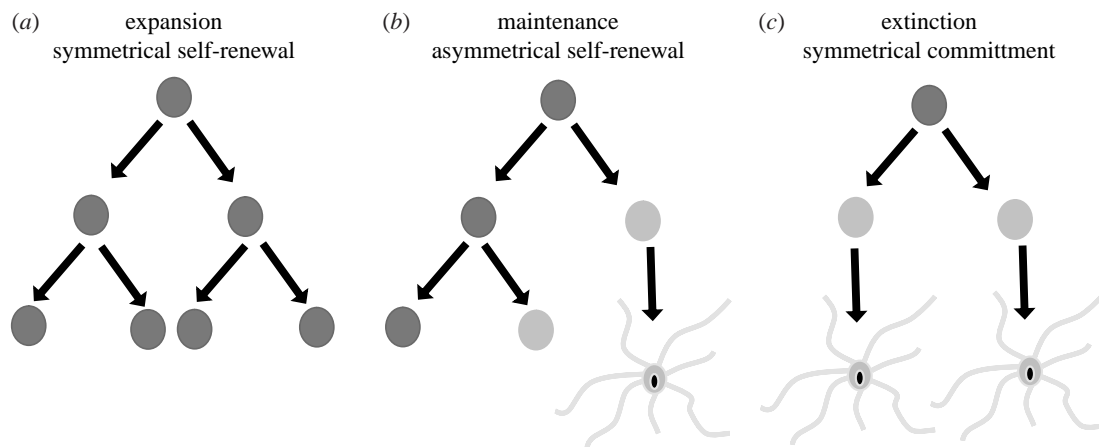


Figure 2. Stem cell division: symmetrical and asymmetrical. Stem cells make cell fate decisions with cell division, choosing to (a) replenish and expanding themselves symmetrically, or (b) maintaining themselves asymmetrically by generating one stem cell and one progenitor cell. These processes involve stem cell self-renewal. (c) Stem cells can theoretically divide symmetrically to generate two differentiated progeny, which would lead to stem cell extinction. Symmetry of cell division may be regulated by intrinsic or extrinsic factors. Currently, the molecular mechanisms regulation of cell fate decisions in brain tumour stem cells is unexplored, but could have important implications for understanding clonal expansion and maintenance of tumours.

mouse differentiate, persist and incorporate seamlessly into the brain (Tamaki *et al.* 2002).

Cultured NSCs have also been identified by the 'side population' phenotype using fluorescent activated cell sorting (cells which differentially exclude Hoechst 33342 DNA dye; Kim & Morshead 2003) as a relatively large cell that has low expression of CD24 and peanut agglutinin binding (Rietze *et al.* 2001) or as cells expressing the Sox2 transcription factor (Cai *et al.* 2002; D'Amour & Gage 2003; Graham *et al.* 2003; Brazel *et al.* 2005). *In vivo*, NSCs have also been identified as radial glial cells, expressing markers BLBP, GLAST and RC2 (Feng *et al.* 1994; Chanas-Sacre *et al.* 2000a,b; Malatesta *et al.* 2000; Hartfuss *et al.* 2001; Noctor *et al.* 2001; Parnavelas & Nadarajah 2001).

There is particularly strong evidence in mice and humans that the *in vivo* SVZ stem cell is a GFAP+ cell (therefore an astrocyte) identified as a morphological type B SVZ cell by electron microscopy (Doetsch *et al.* 1997, 1999; Morshead *et al.* 2003; Sanai *et al.* 2004). Although the A2B5 antigen and the PSA-NCAM cell surface protein have been used to isolate neuronal- and glial-restricted progenitors, respectively (Mujtaba *et al.* 1999), the hierarchy of stem, progenitor and differentiated cells remains poorly defined. In particular, GFAP marks a postnatal putative rare SVZ stem cell as well as a large number of differentiated astrocytes. However, GFAP may not identify mouse embryonic NSCs (Imura *et al.* 2003). Identification of more definitive markers within the normal NSC hierarchy should also be very informative for brain tumour research.

NSCs can be cultured in adherent (Johe *et al.* 1996; Kalyani *et al.* 1997) or non-adherent (Reynolds & Weiss 1992) conditions in the presence of serum-free media with EGF and FGF2. These cells are frequently studied in suspension as neurospheres owing to ease of clonogenic evaluation (figure 3). A neurosphere represents a clonal single cell-derived floating cluster of proliferating cells (Reynolds & Weiss 1992, 1996; Tropepe *et al.* 1999). A neurosphere contains thousands

of cells and is a mixture of stem and progenitor cells, but only 50–100 of these cells are stem cells. The number of stem cells in a neurosphere is determined in a clonogenic assay (limiting dilution analysis), which determines the ability of singly dissociated primary sphere cells to give rise to secondary spheres that are capable of differentiating down all three neural lineages (Tropepe *et al.* 1999). This assay also allows one to quantitate self-renewal, which is defined as distinct from proliferation: self-renewal involves a cell division that also involves a cell fate decision, resulting in at least one daughter cell that retains the full stem cell potential of the parent cell. A multipotent secondary sphere can only form from a stem cell. Undifferentiated neurospheres can be extensively passaged, but when plated onto an adherent substrate in serum, they differentiate into the three main neural lineages (neurons, astrocytes, oligodendrocytes).

3. CANCER STEM CELLS IN SOLID TUMOURS

The connection between normal stem cells and cancer (Reya *et al.* 2001; Pardal *et al.* 2003) is emerging in many tissues, particularly, blood (Lapidot *et al.* 1994; Bonnet & Dick 1997; Passegue *et al.* 2003), brain (Hemmati *et al.* 2003; Singh *et al.* 2003, 2004a,b; Galli *et al.* 2004; Yuan *et al.* 2004), mammary gland (Al-Hajj *et al.* 2003; Liu *et al.* 2003, 2004), gut (Gregorieff & Clevers 2005; Pinto & Clevers 2005; Radtke & Clevers 2005; Reya & Clevers 2005) and skin (Brown *et al.* 1998; Bolotin & Fuchs 2003; Perez-Losada & Balmain 2003; Tumber *et al.* 2004), and prospective isolation of cancer stem cells has been achieved in human leukaemia (Lapidot *et al.* 1994; Bonnet & Dick 1997), myeloma (Matsui *et al.* 2004), breast cancer (Al-Hajj *et al.* 2003) and brain tumours (Singh *et al.* 2004b). Human melanoma (Fang *et al.* 2005) and prostate cancer (Collins *et al.* 2005) also contain subpopulations of cells that demonstrate clonogenic ability *in vitro*, but these purified cells are yet to be demonstrated in a functional *in vivo* assay. Putative cancer stem cell populations have also been identified

in murine models of prostate cancer (Xin *et al.* 2005; Wang *et al.* 2006) and lung cancer (Kim *et al.* 2005).

Indeed, that cancer tissues resemble developing tissues is a long-standing observation, which has suggested a close connection between stem cells and cancer (McCulloch 1983; Pierce & Speers 1988; Huntly & Gilliland 2005). Till and McCulloch (1961) initially demonstrated that the bone marrow contained single cells that clonally gave rise to myeloerythroid colonies in the spleen (Till & McCulloch 1961; Becker *et al.* 1963). Clues that tumour growth may be driven by tumour stem cells were demonstrated by *in vitro* analysis of haemopoietic neoplasms and metastatic tumours, where only a minority of the cells plated formed clonogenic colonies (Bergsagel & Valeriote 1968; Hamburger & Salmon 1977), but, particularly, when the self-renewal of cells in the primary colony was tested in secondary replating assays (Buick *et al.* 1979). These studies suggested that the morphologically heterogeneous cancer tissues exist in a functional hierarchy with respect to proliferation and tumour initiation.

The definitive demonstration of cancer stem cells in human neoplasia was first made in 1994 in leukaemia (Lapidot *et al.* 1994). Injection of a small subpopulation of acute myelogenous leukaemia cells, identified by a cell surface phenotype also found in normal haemopoietic stem cells, caused a leukaemia in immunodeficient mice (Lapidot *et al.* 1994; Bonnet & Dick 1997). Importantly, the cellular heterogeneity of the resulting leukaemia matched that of the patient's leukaemia. The principle of cancer stem cells was then extended to solid tumours, first in breast cancer and then in brain tumours. In breast cancer, a rare fraction of CD44+ and CD24lo/- expressing human breast cancer cells uniquely caused tumours when small numbers of cells were engrafted into the mammary fat pads of non-obese diabetic severe combined immunodeficient (NOD/SCID) mice (Al-Hajj *et al.* 2003).

4. IDENTIFICATION OF BRAIN TUMOUR STEM CELLS

If NSCs can be isolated from the brain by culturing serum free in epidermal growth factor (EGF)/fibroblast growth factor (FGF), could this culture system also identify stem cells in brain tumours? Several groups studying human brain tumours identified small numbers of cells with clonogenic potential based on the neurosphere assay (Ignatova *et al.* 2002; Hemmati *et al.* 2003; Singh *et al.* 2003, 2004b; Galli *et al.* 2004; Yuan *et al.* 2004; Huhn *et al.* 2005). In culture, these brain tumour cells form self-renewing neurosphere-like colonies, and they have an ability to differentiate into one or more neural lineages. In neurosphere conditions, brain tumour cells express nestin and CD133. Brain tumours grown as spheres also express molecular markers associated with neural precursors, such as Sox2, Bmi1 (Hemmati *et al.* 2003), Notch, Emx2, Pax6 (Galli *et al.* 2004) and Jagged1 (Ignatova *et al.* 2002). Following differentiation, they express markers of mature neurons, astrocytes and oligodendrocytes. Often, multiple differentiated lineages are found in individual patient tumours, but there is a strong bias to differentiate into a cell type that is predominant in the

patient's tumour, so, for example, nestin+ astrocytoma cells grown as spheres in EGF and FGF differentiate into GFAP+ astrocytes after plating in serum. However, glioblastomas can differentiate into GFAP+ astrocytes and α -III-tubulin+ neurons (Ignatova *et al.* 2002; Galli *et al.* 2004; Singh *et al.* 2004b; Yuan *et al.* 2004), suggesting that they are derived from a cell that has multilineage differentiation capacity, a stem cell, as opposed to the traditional thinking that these tumours were derived from dedifferentiated normal astrocytes. Brain tumour cells grown in neurosphere conditions have now been very recently shown to more faithfully recapitulate the phenotype and genotype of primary patient tumours compared with serum-derived lines from the same patient tumours (Lee *et al.* 2006).

The sphere-forming population was then found to reside in the fraction of primary brain tumour cells that express the NSC/precursor cell surface marker CD133 (Uchida *et al.* 2000; Singh *et al.* 2003, 2004b). CD133+ cells represent a subpopulation of cells in brain tumours with a frequency of as low as 1% or less in low-grade tumours to as high as 30% in highly aggressive glioblastomas, with a relatively close correlation with clonogenic frequency based on a primary neurosphere-forming assay. However, within one pathological type of brain tumour, such as glioblastoma, the frequency of expression of this marker could be extremely variable, from 5 to 30% in different patients' tumours. It is uncertain at this time whether the CD133 fraction in a tumour has any prognostic significance.

CD133+ brain tumour cells, isolated by magnetic bead sorting, had proliferative and self-renewal ability *in vitro*, and they expressed neural precursor markers, such as nestin, but not differentiated markers, such as β -III-tubulin, GFAP and O4. These CD133+ brain tumour cells could be induced to differentiate to express mature neural cell lineage markers, highlighting, *in vitro*, how tumours resemble caricatures of normal development, with differentiated tumour cells deriving from undifferentiated tumour cells.

The definitive demonstration of a cancer stem cell requires an *in vivo* demonstration that the candidate-purified cancer stem cell is capable of re-initiating and maintaining growth of a tumour that resembles the patient's original tumour. Injection of 100–1000 uncultured malignant brain tumour cells, purified by magnetic bead sorting for CD133, could initiate formation of a serially transplantable tumour in the brains of NOD/SCID mice that was a phenocopy of the patient's original tumour (figure 4), whereas injection of 105 CD133- cells engrafted, but did not cause brain tumours (Singh *et al.* 2004b). Tumour xenografts diffusely infiltrated into the brain, which is a hallmark of malignant brain tumours. Purified populations of CD133+ cells injected into the brains of NOD/SCID mice induced tumours that were heterogeneous and had a minority of cells that expressed CD133, suggesting differentiation *in vivo*. Importantly, as well, sphere-forming glioblastoma cells have been shown to initiate tumours following transplantation into immunodeficient mice (Galli *et al.* 2004; Yuan *et al.* 2004).

More recently, human ependymomas, which are much less aggressive, but still malignant, brain

tumours, were also demonstrated to contain a subfraction of CD133+ cells that could initiate tumour formation in mice, a property not seen in the CD133-ependymoma cells (Taylor *et al.* 2005). Moreover, human ependymoma cells had gene expression profiles that resembled that of normal radial glial cells (putative NSCs) isolated from the same relative rostral-caudal position in the neuraxis as where the tumour was found. These latter results suggest that human ependymomas arise from regionally distinct populations of radial glial cells.

These findings demonstrate that brain tumours of different types are heterogeneous not only morphologically, but also functionally, for tumour-initiating ability. The brain tumour stem cells have distinct biological properties from the bulk of the tumour cells, establishing them as novel targets for treatment. Although cancer-initiating ability resides in the CD133 fraction, not every CD133+ cell initiates the formation of a sphere *in vitro*, demonstrating that not every CD133+ cell has stem cell properties *in vitro*. The 'true' cancer stem cell fraction will require further purification. The differentiation ability of tumour sphere cells or CD133+ cells suggests that some aspects of a normal developmental programme is maintained in these neoplastic cells and that differentiation therapy is a potential option for brain tumour treatment.

5. CELLS OF ORIGIN FOR BRAIN CANCER

Although there is accumulating evidence that tumours contain subpopulations of cells with potent cancer-initiating ability not shared by the bulk population, the cell of origin for the cancer stem cells of brain tumours has not yet been determined. The term 'cancer stem cell' does not mean that these tumour cells are derived from normal cells. It is not yet clear whether cancer-initiating events occur in NSCs, progenitors or differentiated cells (Passegue *et al.* 2003). However, NSCs are attractive candidates for the cells of origin of brain tumour stem cells, because these cells are long lived and theoretically have primed self-renewal ability, perhaps allowing an oncogene to more easily initiate uncontrolled proliferation. If we can understand the cell of origin for brain tumours, we will be better equipped to understand how the molecular alterations lead to cancer, and how we can target those alterations for treatment, or prevent them from occurring. The cell that is transformed may have important bearings on the behaviour of the neoplasm and therefore may also affect the patient prognosis.

Neuropathological observations of brain tumours have long suggested that they may be stem cell or progenitor cell derived. Brain tumours often comprise morphologically heterogeneous cells, with varying numbers of less-differentiated cells which can be identified by the neural precursor marker nestin (Dahlstrand *et al.* 1992; Tohyama *et al.* 1992) as well as cells expressing differentiated neural lineage markers (Burger *et al.* 1991), suggesting that the transformed cell has multipotentiality.

The potential for NSC transformation has been further considered based on the observations of brain

tumours occurring in the brain's putative stem cell or proliferative zones. Clinically, human brain tumours are also known to frequently arise deep in the brain near the SVZ. Indeed, the SVZ was suspected many years ago to contain embryonal rests that were thought to give rise to brain tumours, particularly those tumours occurring in the brain adjacent to the walls of the ventricular system (Globus & Kuhlenbeck 1944). This idea was then further considered after the identification of mitotically active cells in the subependymal regions of adult rodents and primates in the 1960s (Smart 1961; Altman 1963; Lewis 1968), and now especially since the brain SVZ has been defined as the normal NSC niche (Doetsch *et al.* 1997, 1999).

In the 1970s, periventricular tumours were demonstrated to occur in the SVZ region after intraventricular inoculation with avian sarcoma viruses, with a much higher rate of tumours occurring in neonatal rats versus adult rats (Copeland *et al.* 1975; Copeland & Bigner 1977; Vick *et al.* 1977). The incidence of brain tumours of a variety of types in mice inoculated with a pellet of carcinogen was much greater if the pellet was placed in the SVZ region versus the peripheral cortex (Hopewell & Wright 1969). In addition, there is an extensive older literature that demonstrates that a single dose of the alkylating agent ethylnitrosourea (ENU) to pregnant rats selectively induces periventricular brain tumours of a variety of phenotypes in the offspring (Druckrey *et al.* 1966; Koestner *et al.* 1971; Koestner 1990; Mennel *et al.* 2004). Animals are more vulnerable with prenatal exposure to ENU (when there is more neural precursor activity) and the preneoplastic lesions that occur are identified in the SVZ (Schiffer *et al.* 1978; Pilkington & Lantos 1979). In mice, ENU-induced brain tumorigenesis occurs only in the context of p53 homozygous deletion and, in these animals, preneoplastic/early neoplastic lesions are also identified in the SVZ (Oda *et al.* 1997; Leonard *et al.* 2001; Wilhelmsson *et al.* 2003). p53^{-/-} Mice have increased proliferative activity in the SVZ and more neurospheres can be isolated from this region, suggesting an expansion of the NSC pool, which may make it more susceptible to neoplastic transformation (Gil-Perotin *et al.* 2006; Meletis *et al.* 2006).

Furthermore, in a combined mutant mouse model deficient in p53 with *Lox-Cre* engineered conditional allele of NF1 in a GFAP+ (astrocyte) compartment, 100% of mice developed glioblastoma (Zhu *et al.* 2005). Interestingly, before full-blown tumours arise, preneoplastic/early neoplastic changes are seen in the SVZ, suggesting that cells targeted for transformation came from a GFAP+ stem cell in the SVZ stem cell compartment. The preneoplastic proliferative expansion of the granule cell precursors in the EGL of the cerebellum demonstrated in *Patched1* mutant mice also suggests that medulloblastomas that arise in these mice come from this cerebellar precursor compartment (Oliver *et al.* 2005). However, recently, cerebellar stem cells purified based on cell sorting for CD133/prominin1 have been demonstrated, suggesting that these cells are also possible cells of origin for medulloblastomas (Lee *et al.* 2005). Could medulloblastomas have two different cells of origin? It could be speculated that brain tumours of different

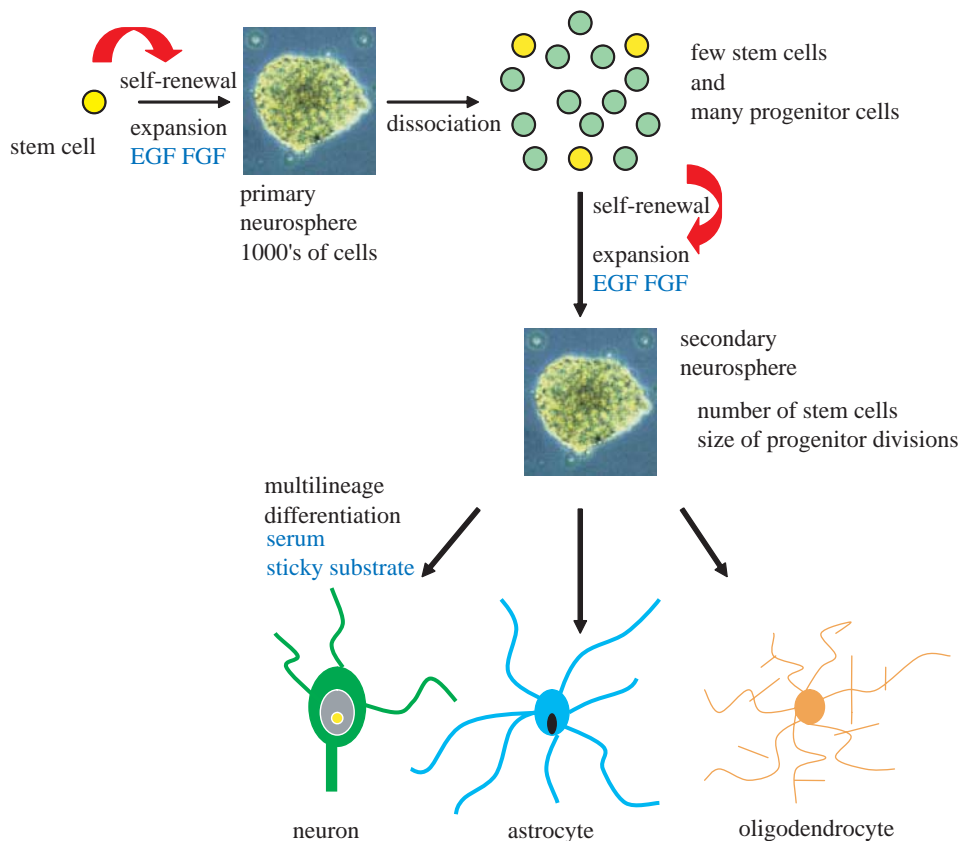


Figure 3. Neurosphere assay of NSCs *in vitro*. The neurosphere assay is used to study neural precursor cells in culture. Isolated from embryonic or adult brain, rare cells in serum-free conditions, in the presence of EGF and FGF, generate floating colonies of cells from single cells. Neurosphere cells express neural precursor markers, but not differentiated markers, but can be induced to differentiate into all three major neural lineages in the presence of serum and with cell adhesion. Neurosphere cells can be dissociated and replated in EGF and FGF to regenerate spheres. The replating efficiency of spheres is a measure of the number of stem cells, and the size of the sphere reflects progenitor proliferative activity. Human brain tumours can be similarly cultured in neurosphere conditions to understand the stem cell hierarchy of these neoplasms.

phenotypes, occurring in different locations and patients of different ages, may have different cells of origin. All the genetic models of brain tumours currently test for tumour initiation in young animals. Perhaps it is not surprising that, in these models, the more abundant neural precursor populations at young ages are most probably initiated into cancer, as opposed to differentiated brain cells. Currently, a mouse model has not been developed that tests for brain tumour initiation in adult animals.

Interestingly, in murine models of skin carcinogenesis, the tumour phenotype arising from H-Ras overexpression depended on the cell compartment in which the oncogene was expressed (Brown *et al.* 1998; Perez-Losada & Balmain 2003). Benign tumours resulted from H-Ras overexpression in suprabasal (differentiated) layers of the skin, but invasive carcinomas resulted if H-Ras was expressed in the hair follicle bulge region, the location of putative skin stem cells. Along these lines, perhaps there are different cells of origins for different types of brain tumours, more benign tumours arising from restricted progenitors or differentiated cells and aggressive malignant tumours from stem cells or early progenitors. Do deep aggressive brain tumours arise from SVZ stem cells? Do superficially less-aggressive tumours arise from regionally located but lineage-restricted progenitor cells? Or, does a transformed SVZ stem cell migrate away from

the ventricle into the cortex to cause a malignant tumour more peripherally? These questions currently remain unanswered.

Alternatively, the tumour phenotype may depend less on the cell type initiated and more on the molecular mechanism of tumour initiation. The oncogene would activate a signalling pathway that specifies differentiation down one lineage, but blockage down another. Regardless of cell of origin, the cancer stem cell hypothesis suggests that, once the tumour is initiated, the tumour is maintained by a subpopulation of cancer stem cells. The molecular events allow the original cell to acquire stem cell properties, particularly that of self-renewal. The cancer stem cells then initiate and maintain the tumour growth. Importantly, multilineage differentiation capacity should not be considered an essential property of a cancer stem cell. Although cancer stem cells probably will often show multilineage differentiation (as this is a hallmark of cancer tissues), differentiation will not occur in normal proportions or may be restricted to one lineage. A cancer stem cell regenerates the heterogeneous tumour mass matching that from which it was derived. The differentiation ability of the cancer stem cell may change with the accumulation of genetic changes with tumour progression.

Overexpression of human brain tumour-derived oncogenes has also been tested in different brain cell compartments *in vivo* in the neonatal mouse through

retroviral transduction. In these models, brain cells expressing neural precursor markers have been suggested to be more receptive to oncogenic transformation than differentiated brain cells (Holland *et al.* 1998, 2000). Expression of oncogenic EGFR (EGFRvIII), or combinations of K-Ras and Akt more potently induced glioblastoma in a nestin+ cell compartment compared with a GFAP+ compartment in the newborn mouse brain, with higher frequencies in the context of p16Ink4a/p19Arf cell cycle regulatory deficiency. Other studies manipulating cultured differentiated astrocytes versus cultured neurosphere precursors with oncogenes have suggested that a differentiated mouse neural cell is equally permissive to transformation if it has a key genetic alteration (p16Ink4a/p19Arf deficiency) (Bachoo *et al.* 2002). This alteration may facilitate dedifferentiation to a stem cell-like phenotype (Bachoo *et al.* 2002; Passegue *et al.* 2003).

The cell of origin question, as probed in these studies, is hampered by limited definition of the normal NSC hierarchy, especially by a paucity of promoters that can specify gene expression in distinct compartments of the stem cell hierarchy and by a paucity of cell surface markers that can distinguish stem cells from multipotent or lineage-restricted progenitors. Nestin, CD133 and Sox2 identify NSCs and progenitors, and no definite lineage-restricted progenitor cells have been identified. As well, GFAP marks a rare NSC population as well as an abundance of differentiated astrocytes, complicating interpretation of some of these studies.

6. DEVELOPMENTAL SIGNALLING IN BRAIN TUMOUR STEM CELL FUNCTION

A hypothesis that cancer growth is driven by cancer stem cells suggests that there should be mechanistic similarities between self-renewal of normal stem cells and that of cancer stem cells. Cancer could be considered a disease of unregulated self-renewal where mutations in normal stem cell self-renewal pathways give rise to abnormal proliferation and cancer growth.

Recent studies have now revealed a number of molecular mechanisms regulating stem cell self-renewal and proliferation. There is particular interest in the Notch (Hitoshi *et al.* 2002; Calvi *et al.* 2003; Chojnacki *et al.* 2003; Maillard *et al.* 2003; Radtke & Raj 2003), Sonic hedgehog (Shh; Rowitch *et al.* 1999; Dahmane & Ruiz-i-Altaba 1999; Wechsler-Reya & Scott 1999; Dahmane *et al.* 2001; Lai *et al.* 2003; Machold *et al.* 2003; Watkins *et al.* 2003; Palma & Ruiz i Altaba 2004) and Wntless (Chenn & Walsh 2002, 2003; Megason & McMahon 2002; Reya *et al.* 2003; Willert *et al.* 2003; Kuhnert *et al.* 2004) pathways as these have been shown to play a particularly important role in normal stem cell self-renewal as well as in cancer growth when these pathways are dysregulated. As well, homeobox transcription factors, such as HoxB4 (Sauvageau *et al.* 1995; Antonchuk *et al.* 2001), the PTEN phosphatase protein (Groszer *et al.* 2001) and the polycomb group of transcriptional repressors (Bmi-1; Lessard & Sauvageau 2003; Molofsky *et al.* 2003; Park *et al.* 2003; Raaphorst 2003), have been implicated in normal and cancer stem

cell self-renewal. In particular, Bmi1 seems to be a target of Shh signalling and is expressed in human medulloblastomas (Leung *et al.* 2004), and Bmi1 deficiency in adult mice results in reduction of neurosphere formation (Bruggeman *et al.* 2005; Molofsky *et al.* 2005).

A role for Notch in human brain tumours is emerging, with finding Notch receptor and ligand transcripts in cultures of gliomas (Ignatova *et al.* 2002) or protein expression in primary glial tumours (Purow *et al.* 2005), and also in medulloblastomas where Notch1 or 2 transcripts have been shown to be increased in patient specimens (Fan *et al.* 2004; Hallahan *et al.* 2004). Overexpression of Notch2-IC increases proliferation of medulloblastoma cell lines (Fan *et al.* 2004) and medulloblastoma cell line growth has been shown to be suppressed by γ -secretase inhibitor or a soluble Dll-1 treatments (Hallahan *et al.* 2004). RNA interference-mediated downregulation of Notch receptors and ligands inhibits glioma cell line proliferation and induces apoptosis. Interestingly, overexpression of an activated Shh co-receptor smoothed in the cerebellum causes activation of Notch pathway in the resulting tumours, suggesting crosstalk between these pathways (Hallahan *et al.* 2004).

The role for Shh signalling in human medulloblastomas has been clearly established (Ruiz i Altaba *et al.* 2002; Pasca di Magliano & Hebrok 2003), with the finding of mutations in multiple Shh pathway members, Ptc1 (Pietsch *et al.* 1997; Raffel *et al.* 1997; Wolter *et al.* 1997), Smo (Reifenberger *et al.* 1998; Lam 1999) and SuFu (Taylor *et al.* 2002), in these neoplasms, and through the occurrence of medulloblastomas in Ptc1+/- mice (Goodrich *et al.* 1997). Interestingly, human brain tumour cell lines with a variety of phenotypes (not only medulloblastoma) express all three Gli genes, the transcriptional effectors of Shh signalling; and brain tumour cell lines of different phenotypes are inhibited by the Shh pathway inhibitor cyclopamine (Dahmane *et al.* 2001; Ruiz i Altaba *et al.* 2002). Interestingly, Gli1 was initially found to be amplified in a glioblastoma line, although this phenomenon has not been seen in primary glioblastomas (Kinzler *et al.* 1987). Recent findings that the Shh pathway remains active in adult tissues (particularly in the brain; Lai *et al.* 2003; Machold *et al.* 2003) and plays a role in specific adult cancers (GI tract, pancreas, skin; Pasca di Magliano & Hebrok 2003) suggest that Shh could play a role in tumours of the brain other than childhood medulloblastomas.

As brain tumours seem to be organized as stem cell hierarchies, further studies of these pathways in the context of purified brain tumour stem cells will likely bring further understanding to brain tumours.

7. THE STEM CELL NICHE: IMPLICATIONS FOR BRAIN TUMOURS

An important emerging concept over the past several years in stem cell biology is the concept of the stem cell niche. This concept has been extensively discussed in a number of excellent reviews and has been best characterized in the gonads of *Drosophila* (Spradling *et al.* 2001; Alvarez-Buylla & Lim 2004; Fuchs *et al.*

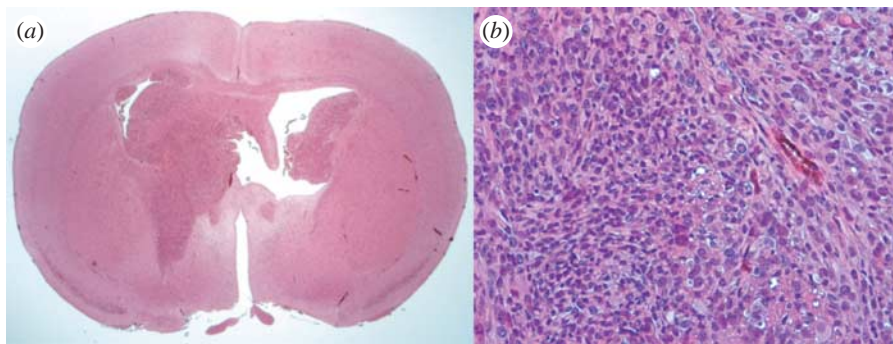


Figure 4. Intracranial xenograft of CD133+ human glioblastoma cells. (a) Low-power micrograph of a NOD-SCID mouse brain with a human glioblastoma tumour that arose following transplantation of CD133+ cells. (b) This tumour has typical histologic features of the patient's original glioblastoma.

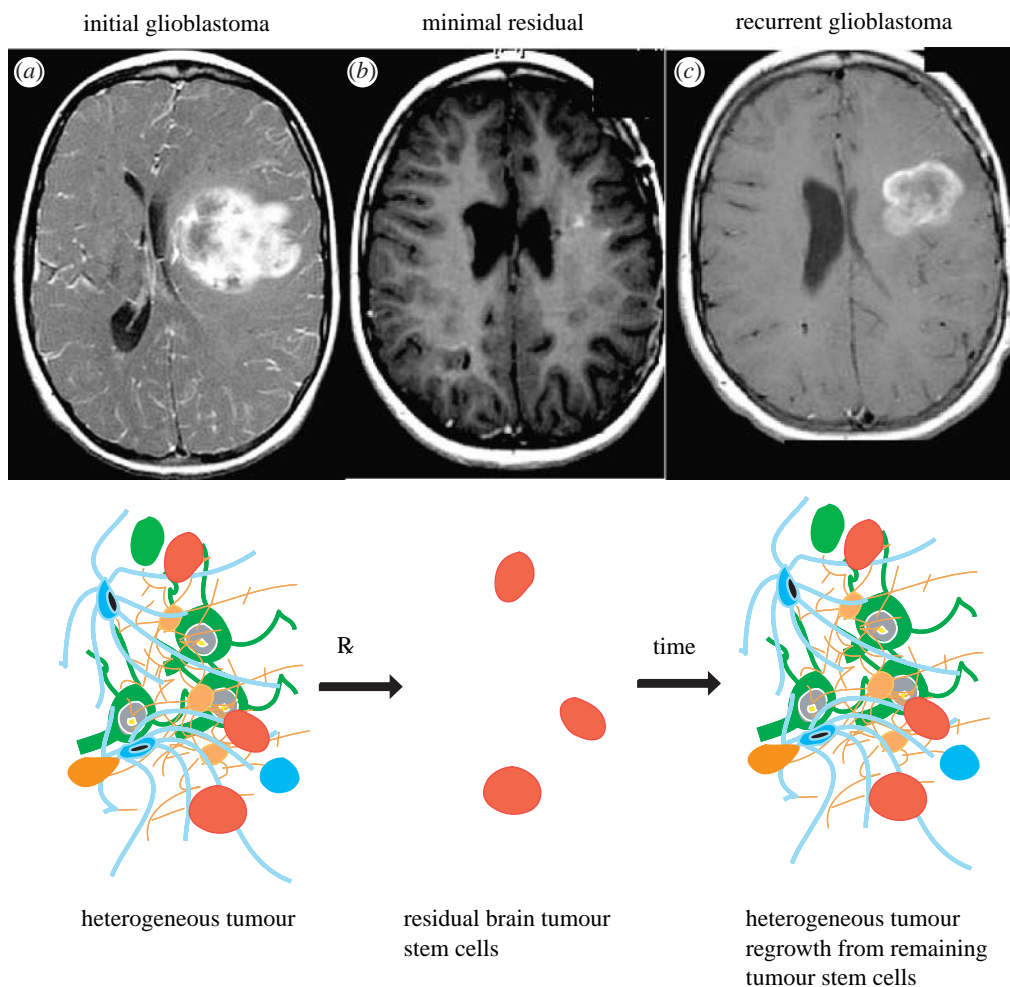


Figure 5. Cancer stem cells cause tumour regrowth after apparently successful therapy. The cancer stem cell hypothesis predicts that cancer stem cells are refractory to conventional therapies and will regrow the tumour. Top, MRIs of a left-hemisphere glioblastoma is shown at (a) initial patient presentation, (b) following multimodality treatment and then (c) at recurrence. It is hypothesized that treatment eliminated bulk tumour but spared cancer stem cells (below in red). These cancer stem cells regrew the heterogeneous tumour leading to clinical recurrence.

2004). In mammalian systems, the niche has been best characterized in the haemopoietic system (Calvi *et al.* 2003) and the skin (Tumbar *et al.* 2004). The stem cell niche is defined as the local microenvironment of stem cells that functions to indefinitely maintain stem cell self-renewal and multipotency. The niche can consist of extracellular matrix components or adjacent tissue non-stem cells. Within a given tissue, there may be several different types of niches for stem cells and the niche may be different at different developmental stages. For the

postnatal nervous system, the niche of neural precursors is beginning to be defined in the hippocampal region and the forebrain SVZ, and differentiated neural cells or blood vessel cells have been implicated as niche elements. Further, in-depth understanding of molecular mechanisms between niche elements and NSCs should be forthcoming soon.

In brain tumours, a concept of niche as a supportive environment for the self-renewal of brain tumour stem cells may also be extremely important for

understanding brain tumour growth. One important consideration and uncertainty is that we really do not know how the niche functions and whether it supports stem cell self-renewal or constrains self-renewal. In addition, so far there is little, if any, published data that define niche elements within tumours and, presently, we are left with many interesting questions. Does the tumour vasculature provide signals to brain tumour stem cells that enhance their self-renewal? Does the bulk population of tumour cells support brain tumour stem cell self-renewal and differentiation? Do brain tumour stem cells become independent of a niche leading to uncontrolled proliferation, or does the niche expand with tumour leading to further tumour enlargement? Answers to these questions should provide some fascinating insight into tumour stem cell behaviour.

8. SUMMARY

Recent studies demonstrate a close link between developmental biology, stem cells and cancer. Not every cancer cell is functionally equal. Brain tumours are heterogeneous in part because they exist as a stem cell hierarchy, relatively fewer stem cells driving the growth of the tumour, through their own self-renewal and the generation of the bulk tumour population. The brain tumour stem cells express neural precursor markers, suggesting that they may be derived from normal neural precursors and are capable of differentiation, although aberrant. The fact that any differentiation is possible suggests that brain tumour cells retain some aspects of a functional developmental programme, demanding further research into understanding the role of developmental signalling pathways in brain tumours.

Although there is evidence that is pointing more clearly towards the origin of brain tumours from brain proliferative compartments, we still do not know the cell of origin for brain tumours, and these may vary from one tumour type to another or may be different in tumours occurring at different patient ages. One could argue that once the tumour exists, its cell of origin is not relevant, what is relevant is the cancer stem cell and the directing therapy to this cell to effect a cure. The cancer stem cell hypothesis suggests that the cancer stem cell must be eliminated to cure cancer, but it is likely that different components of the tumour hierarchy will need to be targeted. This hypothesis suggests that our current therapies spare cancer stem cells leading to tumour regrowth and clinical recurrence (figure 5). One key factor for treatment may be the cell cycle status of the stem cells, as most currently available treatments target cells that are rapidly cycling. Although normal stem cells, and leukaemic stem cells, have been shown to be quiescent, the cell cycle status of solid cancer stem cells has not yet been well characterized. If the brain tumour stem cell is relatively quiescent, these cells will probably require distinct therapy from tumour progenitors that are rapidly proliferating.

These are early days for characterizing stem cells in solid tumours, and these cells must be more highly purified. The next challenge is to understand what

regulates tumour stem cell behaviour and to determine the genes expressed in cancer stem cells to identify novel targets. Hopefully, these studies will lead to badly needed breakthroughs for brain tumours in children and adults.

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