Brain tumour stem cells

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Abstract | The dogma that the genesis of new cells is a negligible event in the adult mammalian brain has long influenced our perception and understanding of the origin and development of CNS tumours. The discovery that new neurons and glia are produced throughout life from neural stem cells provides new possibilities for the candidate cells of origin of CNS neoplasias. The emerging hypothesis is that alterations in the cellular and genetic mechanisms that control adult neurogenesis might contribute to brain tumorigenesis, thereby allowing the identification of new therapeutic strategies.

Abnormal ionic flux
Alterations in the transit of ions through specific channels (NMDA receptors) that is generated by the massive release of glutamate from damaged cells and which leads to excito-toxicity.

Reperfusion
CNS injury that is produced by tumour-induced transient ischaemia followed by blood re-oxygenation, which induces neural damage through the generation of reactive oxygen species.

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doi:10.1038/nrc1889

The central role of the brain in every aspect of bodily function, and the dramatic functional disturbances that arise with minimal disturbance to the neural cyto-architecture or circuitry, account for the severity of many brain tumours. These tumours are often lethal, as conventional anti-cancer treatments have limited or no efficacy.

There is now increasing awareness that a principal hurdle in understanding and successfully treating these cancers might be overcome by the identification of a defined cell that could function as a therapeutic target. Similar to haematopoietic and breast cancers, only a few atypical cells within the cancerous mass might be responsible for the growth and recurrence of some brain tumours. Evidence indicates that the real culprit is a peculiar, transformed CNS cell type that has the defining properties of somatic stem cells, as well as cancer-initiating ability — a brain tumour stem cell.

Here, we review the findings that demonstrate the presence and involvement of brain tumour stem cells in the initiation and propagation of brain tumours, particularly glioblastomas, medulloblastomas and ependymomas, for which the identification of specific tumour stem cells has recently been described. We provide a comparative analysis of the functional properties of brain tumour stem cells relative to normal neural stem cells and in the context of adult cell genesis. We also discuss the origin of brain tumour stem cells from normal adult neural stem cells or progenitor cells, and cover the possible involvement of normal neurogenetic regulatory mechanisms in the physiology of brain tumours and brain tumour stem cells. The theoretical and practical consequences of the idea that brain tumour stem cells are 'diseased' cells with deregulated self-renewal will be analysed, together with the conceptual and clinical implications.

Characteristics of malignant brain tumours

Different types of tumour of neuroepithelial origin have been identified and classified according to the cell types that predominate within the tumour mass (BOX 1). Whereas grade IV gliomas are the most frequent primary intra-parenchymal neoplasm in the elderly, medulloblastomas have the highest incidence in children.

The therapy of intracranial tumours presents many problems that result from the inherent vulnerability of the brain parenchyma. Abnormal ionic flux, excito-toxicity that is related to glutamate release from dead cells, reperfusion and compressive forces often cause irreversible damage to CNS tissues. In addition, the tendency of specific brain tumours, such as the astrocytic and embryonal subtypes, to extensively infiltrate the neighbouring brain structures and to relapse, often progressing toward malignancy, makes the prognosis of these cancers poor.

On the basis of World Health Organization classification, the most malignant form of glioma is grade IV, which is commonly known as glioblastoma multiforme. This highly aggressive tumour develops either *de novo* (primary glioblastoma multiforme) or as the result of the malignant progression from a low-grade glioma (secondary glioblastoma multiforme)[1]. In both cases, prognosis is poor and the median survival when radiotherapy and chemotherapy are combined is 14.6 months[2]. Most importantly, glioblastoma multiforme is characterized by a diffuse tissue-distribution pattern, with extensive dissemination of the tumour cells within the brain that hinders complete surgical resection[3]. Similarly, medulloblastoma, which is thought to arise from the malignant transformation of progenitors of the external granular layer of the cerebellum, is a highly malignant paediatric cancer with a poor prognosis, although there has been some progress recently. As opposed to glial tumours, medulloblastoma is primarily characterized by neuronal differentiation and is considered an embryonal tumour[4].

At a glance

- Adult somatic stem cells are a rare population of long-lived cells that have significant proliferative capacity, show extensive self-renewal and have a wide differentiation potential.
- Cells that have the cardinal properties of stem cells have been identified in restricted regions of the CNS, where they are arranged in specific lineage hierarchies.
- Similar to other adult stem cells, neural stem cells or their immediate progeny, which are called transiently dividing progenitors, can be considered a credible target for malignant transformation. This concept is supported by the finding that many of the molecular determinants that regulate normal neurogenesis seem also to be involved in tumorigenesis.
- Brain tumour stem cells have been identified and isolated from different types of brain tumour: in particular, glioblastoma multiforme and medulloblastoma.
- Brain tumour stem cells show all the features of stem cells, including the ability to generate new tumours that faithfully reproduce the phenotype of the human disease.
- The availability of brain tumour stem-cell lines provides a model system for the identification of specific antigenic and molecular markers that might target the tumour-initiating cell.
- The development of agents that selectively target and inhibit the tumour-initiating and propagation potential of brain tumour stem cells might reduce or eliminate primary tumour establishment, growth and recurrence.

Stem cells in the adult CNS

Cancer arises from a series of mutations that occurs in few or even single founder cells. These cells eventually acquire unlimited and uncontrolled proliferation potential[5]. Two hypothetical models can explain this phenomenon (BOX 2). The stochastic model predicts that all the cells in a tumour have a similar tumorigenic potential, which is activated asynchronously and at a low frequency in certain cells[6]. Conversely, the hierarchical model holds that only a rare subset of cells within the tumour have significant proliferation capacity and, particularly, the ability to generate new tumours, with the remainder of the tumour cells representing differentiating or terminally differentiated cells[6]. The latter hypothesis fits with the cancer-stem-cell theory and is now supported by a plethora of recent observations[7–9].

Recently, the concept of cancer stem cells has been extended to tumours of the brain, whose archetypically quiescent tissue has long been thought not to undergo significant cell turnover. This concept, known as the 'no new neuron' dogma, and the implied absence of brain stem cells in adulthood, was challenged in the early 1960s when the genesis of new, functional brain cells (neurogenesis) was described in the adult mammalian CNS[10–12]. However, the 'no new neuron' dogma withstood the challenge: although resting embryonic-like tissue remnants, which can persist within the CNS parenchyma, were also envisioned as the possible cellular source of brain tumours[13], tumour cells in the brain were hypothesized to derive mostly from the transformation of maturer neural cells such as astrocytes, oligodendrocytes or neuronal precursors.

However, in the early 1990s, following on from the pioneering work of Fernando Nottebohm's laboratory that showed the functional relevance of adult neurogenesis in songbirds[14], Reynolds and Weiss reported the isolation of a neural stem cell from the adult mouse brain[15]. A flurry of studies followed, which described persistent neurogenesis in the adult brains of rodents, tree shrews[16], monkeys[17] and humans[18] (reviewed in REF. 19). Therefore, neogenesis of mature cells persists throughout adult life within discrete brain regions, primarily in the dentate gyrus of the hippocampus and in the subventricular zone of the forebrain lateral ventricles (FIG. 1). This process is central to the generation and integration of new neurons into pre-existing neural circuitry, and is probably crucial for the maintenance of brain integrity, plasticity and optimal function[20].

The main implication of continued adult neurogenesis is the presence of undifferentiated, mitotically active stem and progenitor cells within discrete regions of the mature brain. Like those that are found in other renewing tissues, these populations might function as a source of cells for transformation, giving rise to tumour stem cells.

The overall structure of the cell neogenetic process in the adult brain, its basic cellular compartments and the fundamental organization of the lineage hierarchy within neurogenetic areas are comparable to those of other renewing tissues (FIG. 2). The stem-cell compartment contains highly undifferentiated cells that are endowed with an extensive proliferative potential, and is responsible for the whole process of tissue cell homeostasis throughout the life of the organism. Stem cells often produce the entire range of mature cell types that are found in the tissue of origin (multipotency). Most importantly, they have extensive self-renewal capacity — that is, the ability to maintain their number in adult tissues at a steady level throughout life. Stem cells can accomplish self-renewal by undergoing asymmetric divisions, by which a faithful

Box 1 | **WHO classification of brain tumours**

Astrocytic tumours
- Diffuse astrocytoma (grade II)
- Anaplastic astrocytoma (grade III)
- Glioblastoma (grade IV)

Oligodendroglial tumours
- Oligodendroglioma

Mixed gliomas
- Oligoastrocytoma

Ependymal tumours
- Ependymoma

Neuronal and mixed tumours
- Gangliocytoma

Neuronal/glial tumours
- Dysembryoplastic neuroepithelial tumour
- Ganglioglioma

Embryonal tumours
- Medulloepithelioma
- Ependymoblastoma
- Neuroblastoma

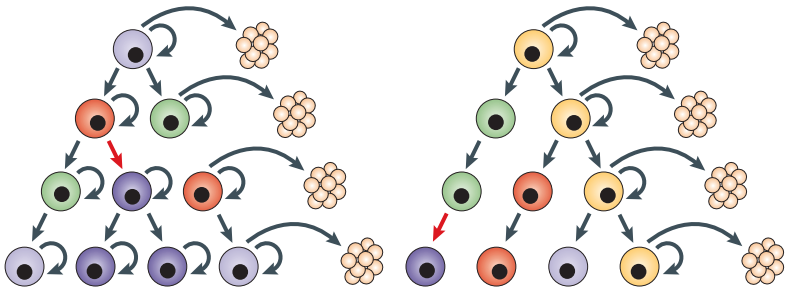
Primitive neuroectodermal tumours
- Medulloblastoma

Resting embryonic-like tissue
Remnants of cells that maintain the features of embryonic cells but are located in a semi-quiescent mature tissue.

Box 2 | **Tumour initiation and development**

Two alternative models have been put forward to explain how tumours initiate and develop. The stochastic model (**a**) proposes that tumour cells are heterogeneous, but that virtually all of them can function as a tumour-founding cell, although this might happen only rarely. Conversely, the hierarchical model (**b**) implies that only a small subpopulation of tumour stem cells can proliferate extensively and sustain the growth and progression of a neoplastic clone. Figure reproduced with permission from REF. 6 © (2001) Macmillan Publishers Ltd.

a Stochastic model **b** Hierarchical model



copy of the mother cell, together with a maturer progenitor, is generated. The same result is achieved when, within a given stem-cell pool, equivalent numbers of symmetrical cell cycles take place, yielding either two stem cells or two maturer progenitors[21]. Extensive self-renewal is often used to distinguish somatic stem cells from their immediate descendants, which constitute the transiently dividing progenitor compartment and can only reproduce themselves in a limited fashion. These descendants eventually give rise to the terminally differentiated elements of the mature cell compartment. Disruption of the regulatory mechanisms that control self-renewal are probably involved in the genesis of cancer-initiating stem-like cells[6,22].

Neurogenesis in the mature CNS

The largest neurogenetic region in the adult mammalian brain is the subventricular zone, which is located between the lateral ventricle and the parenchyma of the striatum (FIG. 1a). Despite previous controversies about their actual identity and nature, a subset of the subventricular zone cells that express the astroglial marker glial fibrillary acidic protein (GFAP) have been identified as the putative adult neural stem cells in this region[23]. These cells can reconstruct the entire neurogenetic structure when all of the other mitotically active cells have been ablated. Relatively quiescent, with a proposed cell-cycle time of 28 days, these astrocyte-like adult neural stem cells are called type B cells and represent a small subset of the total astrocyte population in the subventricular zone[23]. Notably, they can be distinguished from mature astrocytes, particularly those of the mature brain parenchyma, as these do not seem to possess stem-cell properties. *In vivo*, adult neural stem cells generate transiently dividing progenitor cells that are characterized by a cell-cycle time of about 12 hours[24]. These progenitors, which are called type C cells, retain multipotency and give rise to maturer transiently dividing progenitors that are restricted to neurons and are called type A cells. These type A cells migrate in bundles through the rostral extension of the subventricular zone into the olfactory bulb, where they integrate as new interneurons in the cortical layers (FIG. 1a).

Interestingly, the subventricular zone seems to be present in the human adult brain[25–27]. However, in humans, newly generated cells do not migrate in bundles towards the olfactory bulb, but instead leave the periventricular region as single cells, the destination of which remains to be identified[25,27] (FIG. 1c).

There is a similar hierarchical neurogenetic system in the subgranular zone of rodents[28,29] (FIG. 1b) and humans[18,30] (FIG. 1d). The subgranular zone is located between the hippocampal granular layer and the hilus, and contains astrocytes that are tightly associated with blood vessels to form foci of proliferating cells. Similar to their subventricular-zone equivalents, subgranular-zone astrocytes operate as neural stem cells and give rise to transiently amplifying precursors, which are called type D cells, from which new neuronal precursors are generated that migrate a short distance to functionally integrate into the granule-cell layer (reviewed in REF. 20).

The persistence of germinal regions and the presence of stem cells and transiently dividing progenitor compartments in the adult CNS has important conceptual and practical implications, and reinforces the idea that mature neural cells are not the only possible source of tumour cells in the adult mammalian brain.

Identifying neural stem cells and tumour stem cells

A valuable feature of adult neural stem cells is that they are readily and extensively expandable when placed in culture and stimulated with the appropriate growth factors, such as epidermal growth factor (EGF)[15,23,31] and fibroblast growth factor 2 (FGF2)[32,33]. This culture condition allows adult neural stem cells to be isolated, and their functional characteristics and developmental potential to be investigated. This was demonstrated by Reynolds and Weiss in 1992 who isolated a small population of cells (<0.1% of total cells) from the adult striatum that could proliferate and generate multipotent clones of cells — neurospheres — *in vitro*[15].

This neurosphere approach represents a serum-free, selective culture system in which most differentiating or differentiated cells rapidly die, whereas neural stem cells respond to mitogens, divide and form neurospheres that can be dissociated and re-plated to generate secondary spheres. This process can be repeated by serial subculturing, which results in an exponential increase in the total number of cells and neurospheres that are generated[34] (FIG. 3). It is worth emphasizing that the neural stem-cell content in this system is variable and depends on the stage of the culture. The neural stem-cell titre is actually extremely high (although it never reaches absolute purity) soon after the dissociation and re-plating (which selects against cells other than neural stem cells), but tends to decline progressively until the next subculturing step is carried out. This is due to the production of maturer precursors that occurs spontaneously during cell proliferation and neurosphere formation. Notably, on mitogen removal, the progeny of the growth-factor-responsive

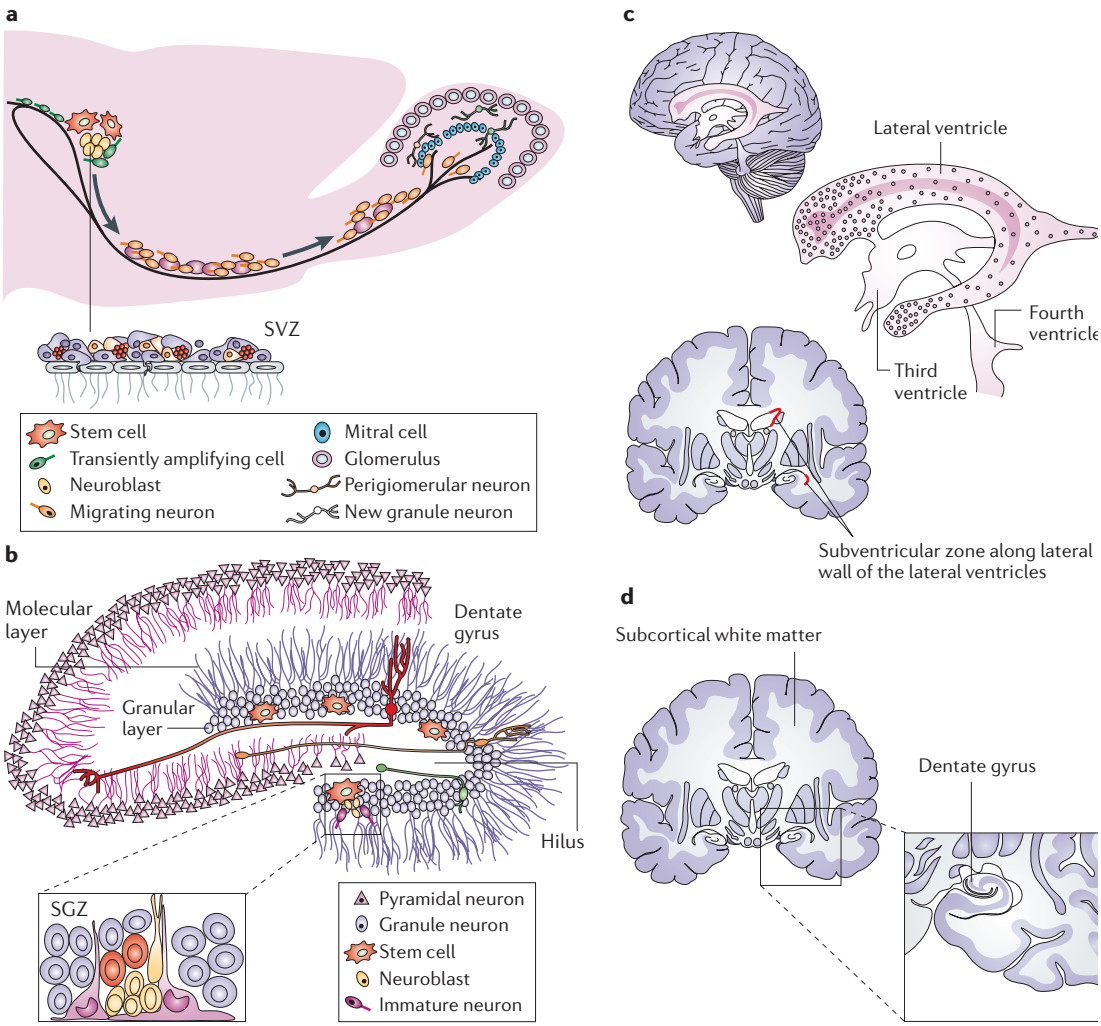


Figure 1 | **The anatomy and functioning of the subventricular zone and subgranular zone in rodents and humans. a** | A sagittal section through the lateral ventricle that shows the larger area of adult neurogenesis; that is, the subventricular zone (SVZ). This region lines the lateral ventricles of the forebrain and is comprised of three main cell types. The multipotent, type B astrocytes, that have been identified as the *bona fide* SVZ stem cells, give rise to fast-cycling transiently proliferating precursor cells that are called type C precursors and that, in turn, generate mitotically active type A neuroblasts. The type A cells, while dividing, migrate tangentially towards the olfactory bulbs where they integrate as new interneurons. **b** | An additional adult neurogenetic region is found in the subgranular zone (SGZ), which is located within the dentate gyrus of the hippocampus. A cellular hierarchy, somewhat similar to that of the SVZ, is seen in the SGZ in which the true stem cell is probably the type B astrocyte, which produces the intermediate type D precursor that eventually gives rise to the type G granule neurons. These neurons integrate functionally into the granule cell layer. **c** | In the adult human brain, a population of SVZ astrocytes that is organized as a periventricular ribbon has been identified as comprising neural stem cells. In contrast to the rodent SVZ, no signs of tangential neuronal chain migration were detected from the corresponding human area. **d** | The germinal zone of the adult human hippocampus is located within the dentate gyrus. Neurogenesis in this region has been demonstrated to take place in adult humans. Parts **a** and **b** are reproduced with permission from REF. 20 © (2005) Annual Reviews. Parts **c** and **d** are reproduced with permission from REF. 26 © (2005) Massachusetts Medical Society.

cells can be differentiated into neurons, astrocytes and oligodendrocytes. Under these conditions, growth-factor-responsive neural stem cells that are derived from the embryonic and adult CNS can be passaged and expanded indefinitely with little change in their growth or differentiation characteristics[32,33,35,36]. These results indicate that adult neural stem cells possess the fundamental stem-cell criteria of self-renewal, multipotency and the generation of many progeny[15,33,37–39].

The neurosphere assay[15] is a simple and robust assay for the isolation, expansion and identification of neural stem cells (FIG. 3), and has become the method of choice to study potential expanded neural stem-cell populations *in vitro*. Cells with stem-cell characteristics in culture have subsequently been found to line the entire mouse ventricular neuroaxis[40,41], to be present in the human fetal and adult CNS[25,36,42,43], and to participate in adult rodent neurogenesis[44–48]. Recently, the conditions of the

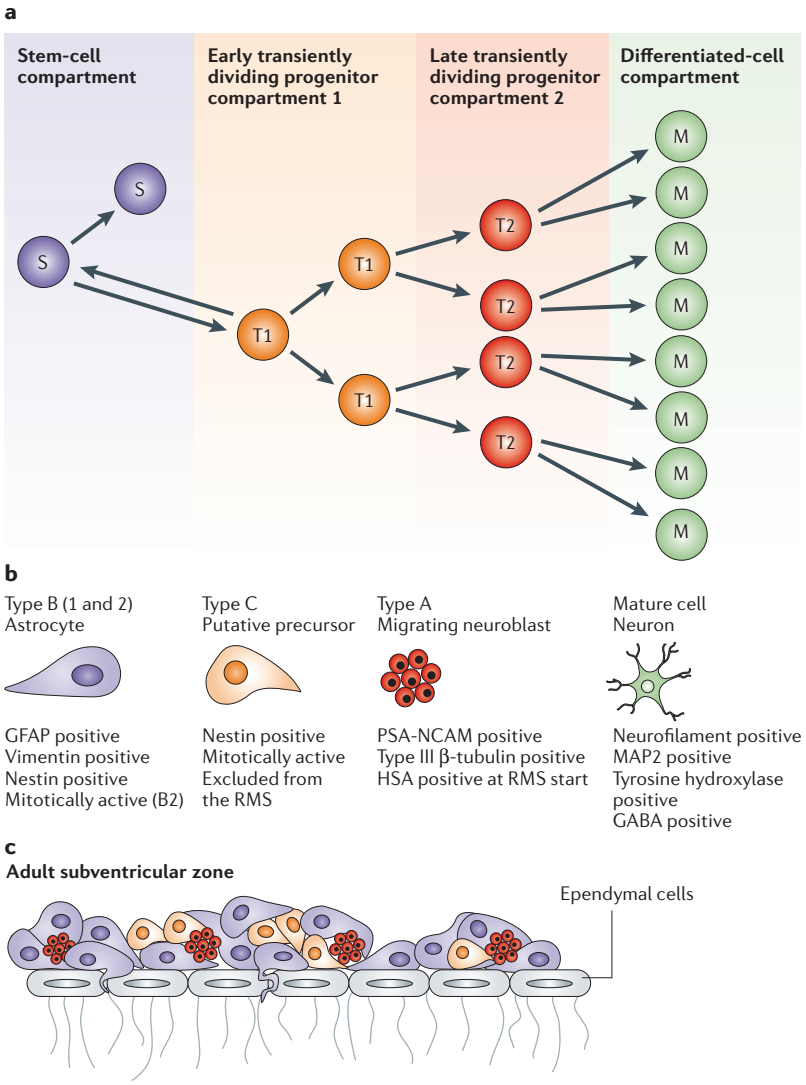


Figure 2 | **Hierarchical organization of the functional compartments in renewing tissues. a** | The stem-cell compartment (purple), early transiently dividing progenitor compartment (orange), late transiently dividing progenitor compartment (red) and differentiated-cell compartment (green) are schematically described. Cells in the stem-cell and transiently dividing progenitor compartments could be the target of the onco-transformation that leads to the formation of tumour stem cells. **b** | The neural precursors that make up similar functional compartments in the neurogenetic regions of the adult brain and that might be the source of brain tumour stem cells. **c** | The structure of the subventricular zone, showing how these precursors fit and are organized in the germinal neuroepithelium of the largest neurogenetic region of the adult brain. GABA, γ-aminobutyric acid; GFAP, glial fibrillary acid protein; HSA, heat-stable antigen; MAP2, microtubule-associated protein 2; NCAM1, neural cell adhesion molecule 1; PSA, polysialic acid; RMS, rostral migratory stream.

neurosphere assay have been used to isolate other types of candidate stem cell from various tissues, including skin[49], heart[50] and breast[51], and, significantly, to identify brain tumour stem cells.

Brain tumour stem cells. The identification and isolation of brain tumour stem cells remains a difficult and confusing issue, as the term 'brain tumour stem cell' is often applied to cells that have been identified by different methods and criteria, and that have varying characteristics. Although this is probably a consequence of the relatively recent identification of these cells, it also reflects the current state of flux in neurobiology in attempting to provide a general definition of neural stem cells and to identify neural stem-cell-specific markers. The most widely used neural lineage markers often label intracellular antigens[20], and adult neural stem-cell-specific antigens are still being identified. This shortcoming hinders the enrichment and purification of adult neural stem cells by flow cytometry. The situation is now improving with the identification of cell-surface antigens and methods that are used to identify putative neural stem cells in rodents[52–54] and humans[43] and that might eventually be used to purify brain tumour stem cells[55]. However, despite the modifications that cell culturing might bring about in tumour stem cells, to date all of the reports that describe the isolation and characterization of putative brain tumour stem cells have used the neurosphere assay (in some form) to help confirm the existence of a stem-cell population.

The first evidence of the existence of cells with stem-like characteristics in human brain tumours was reported by Steindler and colleagues[56], who isolated clonogenic, neurosphere-forming precursors from post-surgery specimens of human glioblastoma multiforme. These stem-like cells expressed both neuronal and astroglial markers on differentiation, together with several key determinants of neural stem-cell fate. Later, two independent groups extended these findings, showing that both glioblastoma multiforme and medulloblastoma contain neurosphere-forming cells that can give rise to neuronal and astroglial-like cells[57,58]. Medulloblastoma cells were also shown to express many genes that are characteristic of neural stem cells, including proteins such as SOX2, BMI1 and Musashi 1, and to give rise to dysplastic tissue following intracerebral transplantation[58].

The relevance of these findings is manifold. First, they confirm that different brain tumours contain transformed, undifferentiated neural precursors that respond to the same mitogens that activate adult neural stem cells. Second, they indicate that tumour stem-like cells possess some of the molecular features of neural stem cells. Third, they show that CD133, a 120 kDa cell-surface protein that is a marker of normal human neural precursors[43,59,60], can be used for the enrichment of tumour stem-like cells from brain tumours[55,57].

However, these observations do not show whether the stem-like cells that are derived from these tumours have the identifying characteristics of true stem cells (that is, long-term self-renewal, multipotency and generation of many progeny), and, most importantly, whether they have cancer-initiating capacity as would be expected of brain tumour stem cells.

By applying the same conditions that are used for the isolation of human neural stem cells, we were able to isolate clonogenic, neurosphere-forming progenitors from adult human glioblastoma multiforme[61]. These cells were isolated at a frequency comparable to that of the $CD133^+$ cells that were previously isolated from brain tumours[55,57], and they had the key *in vitro* stem-cell characteristics of extensive self-renewal, multipotency and generation

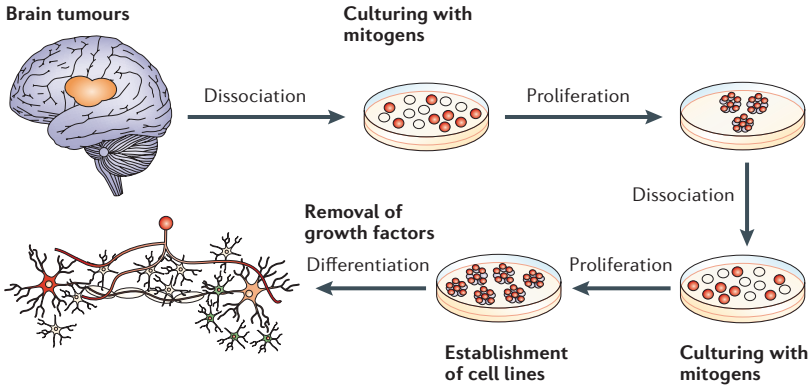


Figure 3 | **Isolation and perpetuation of brain tumour stem cells in culture.** The neurosphere assay is a defined serum-free culture system that allows the isolation and propagation of CNS-derived stem cells. Adult precursors are dissociated and plated in a liquid growth medium that contains the stem-cell mitogens epidermal growth factor and/or fibroblast growth factor 2. Because of the lack of serum and the low plating density, most cells die, except those that divide in response to the stem-cell mitogens. The growth-factor-responsive cells proliferate to form floating clusters of cells that are referred to as neurospheres. These can be further dissociated into a single-cell suspension and then re-plated in fresh medium to produce secondary neurospheres. The process can be repeated, resulting in a geometric expansion in the number of cells that are generated at each passage. Upon mitogen removal, the progeny of the proliferating precursors can be differentiated into neurons, astrocytes and oligodendrocytes, which are the three primary cell types that are found in the adult mammalian CNS. Under these conditions, the growth-factor-responsive precursors can be expanded indefinitely with little change in their growth or differentiation characteristics. These results indicate the existence of an adult neural stem cell as these cells possess the fundamental stem-cell features of extensive self-renewal, generation of many progeny and the ability to give rise to the primary cell types of the tissue from which they were obtained.

of many progeny. Most importantly, when clonally derived, they could initiate new tumours when transplanted into the striatum of adult immunodeficient mice[61]. These tumours showed the classic *in vivo* features of human glioblastoma multiforme, having extensive migratory and infiltrative capacity, which indicated that the *in vitro* defined brain tumour stem cells could recapitulate the *in vivo* histological features of glioblastoma multiforme. In addition, stem-like cells could be re-isolated from these tumours and could re-establish secondary stem-cell lines with the same functional characteristics as the parental cell lines. Furthermore, following intracranial transplantation, these secondary brain tumour cells gave rise to new tumours, which demonstrated their self-renewal *in vivo*.

As this population of cells has the cardinal properties of stem cells, it seems likely that they are brain tumour stem cells[61]. Recently, a report from the Dirks group has described the presence of similar, *in vivo* self-renewing, $CD133^+$ cancer-initiating precursors in short-term neurosphere cultures that had been established from human glioblastoma multiforme and medulloblastoma[55]. Although it remains unclear which subset of the $CD133^+$ precursor pool can initiate tumour formation *in vivo*, the $CD133^-$ fraction was shown to lack any tumorigenic capacity. Despite the fact that the actual identity of the true tumour-initiating stem cells in both neurosphere cultures and $AC133^+$ populations remains to be established conclusively, these findings clearly support the existence of brain tumour stem cells in human glioblastoma multiforme.

This field is expanding rapidly and new studies[62,63] are corroborating the view that the cancer-initiating cells in glioblastoma multiforme and medulloblastoma are brain tumour stem cells. Interestingly, this concept has now been extended to include other brain tumours, such as ependymomas[64]. It has been reported that multipotent $CD133^+$ cells, which show features of radial glia — the progenitors that are known to give rise to both ependymal cells and adult neural stem cells in the postnatal subventricular zone[65] — can be cultured from human ependymomas using the neurosphere assay[64].

Several crucial considerations arise from these studies. The definition of brain tumour stem cells is often loosely applied, such that it includes cells of unknown or limited self-renewal and differentiation potential. Worryingly, the term might be applied to candidate cells in the absence of any reported cancer-initiating capability. This lack of rigour in the characterization of a putative brain tumour stem cell confuses the field as it groups cells with *bona fide* tumour stem-cell features together with those that do not satisfy these criteria. The undesired consequence of this situation is the allocation of divergent and contradictory properties to cells with different natures and identities that are then all called brain tumour stem cells.

We propose that the term brain tumour stem cell be applied only to cells with the cardinal features that are listed in BOX 3. The development of a rigorous definition and its application across the field would standardize and guide future work, allowing groups to compare and contrast their findings on the basis of reasonable and generally accepted criteria.

The origin of brain tumour stem cells. Different interpretations have been proposed about the nature of the neural cell type that is targeted by transformation and results in subsequent tumorigenesis. To date, evidence indicates that brain tumours might derive from the transformation of undifferentiated precursor cells, which are found not only in germinal regions of the developing and early-postnatal CNS — such as those that are involved in the genesis of the cerebellar external granular layer from which medulloblastoma develops[66] — but also in areas of the mature brain, in which neurogenesis persists throughout adulthood. Amongst these, the subventricular zone emerges as the most likely source of gliomas[26]. Many tumours develop near this region and exposure to oncogenic viruses or administration of carcinogens results in the preferential formation of tumours in germinal regions as opposed to the non-proliferative brain parenchyma[26]. Tumours arise with much greater frequency when carcinogens are administered in the subventricular zone rather than in the peripheral cortex[67]. This is further supported by the finding that tumours that are found in the white matter of dogs that have been exposed to avian sarcoma virus early in postnatal life originate in the subventricular zone and subsequently migrate to their final location[68].

Box 3 | **Definition of brain tumour stem cells**

Brain tumour cells should qualify as brain tumour stem cells if they are characterized by:

- Cancer-initiating ability upon orthotopic implantation (tumours should be a phenocopy of the tumour of origin)
- Extensive self-renewal ability, demonstrated either *ex vivo* (by showing both sequential-clonogenic and population-kinetic analyses[32,61]) or *in vivo* (by serial, orthotopic transplantation[55,61])
- Karyotypic or genetic alterations
- Aberrant differentiation properties
- Capacity to generate non-tumorigenic end cells
- Multilineage differentiation capacity*

* Not a defining characteristic in all circumstances.

Recently, Parada and colleagues established a double-mutant mouse in which they combined mutations in tumour suppressor p53 (*Trp53*) and the neural-specific neurofibromatosis type 1 gene (*Nf1*), which activates Ras and therefore increases the formation of astrocytomas in humans. They suggested that some of the astrocytoma-like lesions that they observed developed within the subventricular zone[69]. Similarly, a series of classic studies proposed that deletion of tumour suppressors and/or activation of oncogenes, such as Ras and Akt, in undifferentiated nestin-expressing cells results in a higher frequency of tumour formation than such alterations in GFAP-expressing astrocytes[70,71]. Likewise, overexpression of platelet-derived growth factor-β (PDGFβ) in either nestin-expressing progenitors or GFAP-expressing astrocytes caused a significant increase in the rate of glioma formation, with the highest frequency observed following nestin-directed targeting[72]. However, mature astroglia have also been shown to be more susceptible to oncogenic transformation when a more immature phenotype is targeted. The combined loss of the *CDKN2A* locus (which encodes the INK4a and ARF tumour suppressors) and the expression of constitutively active EGF receptor (EGFR) in mature astrocytes led to the formation of glioma-like lesions following intracranial transplantation[73].

However, the observation that, in patients, the site of glioma origin is often distinct from the site where the tumour eventually develops might be explained by the hypothesis that a brain tumour stem cell, through asymmetric divisions, generates another brain tumour stem cell that remains within the subventricular zone, and a progenitor cell that migrates away to give rise to the tumour mass[74].

In the same way, persistence of neurogenesis in the hippocampus throughout life entails the persistence of undifferentiated, mitotic neural precursors within this area. As these precursors are a probable target of tumorigenic transformation[20], it remains unclear why there is currently no clear evidence of increased tumour frequency in the regions that are adjacent to the hippocampus. A possible explanation can be found in the recent observation that the actual neural stem cells that give rise to subgranular-zone neurons might reside outside the subgranular zone itself[75,76].

Nestin-expressing cells
Undifferentiated neural precursors that express the neuroepithelial intermediate filament nestin.

These studies provide compelling evidence that mitotically active precursors are the most likely source of many brain tumours; however, the specific identity of these cells within the neurogenetic regions of the brain remains unresolved. It has been suggested that between four and seven independent mutations must occur within a normal somatic cell before a cancerous phenotype results[5]. Some of these alterations might be acquired early in development, predisposing certain adult cells to cancer[77]. Nonetheless, oncogenic mutations are rare, stochastic events, that are thought to accumulate over a considerable period of time. Transiently dividing progenitors exist only briefly before differentiating into cells that are often quiescent or die because of normal turnover or damage. Therefore, mutagenic events might not have the opportunity to accumulate in transiently dividing progenitors and their terminally differentiated progeny, as time spent in the transformation-prone phase is short and mutated cells might be discarded through differentiation and normal cell loss. Conversely, somatic stem cells are perennial cells with a significant proliferation and self-renewal potential that lasts the lifetime of the animal, making them a preferential target for tumorigenesis. It is thought that this might be the case in acute myeloid leukaemia and several solid-tissue malignancies[78]. However, as the transiently dividing progenitors are the immediate descendants of somatic stem cells, which will pass down their mutations, it is arguable that the rapidly and transiently dividing cells could accumulate additional tumour-promoting events, eventually leading to the transformation of these cells into cells with the properties of tumour stem cells (FIG. 2).

The role of transiently dividing progenitors (type C cells) as the possible source of brain tumour stem cells is supported by the finding that in the adult subventricular zone these progenitors express EGFR[31]. The activity of this receptor is altered in more than 50% of human gliomas[79] and its constitutive activation can cause glioma formation in the CNS[73]. Strikingly, *in vivo* exposure to EGFR ligands, such as transforming growth factor-α (TGFα) or EGF[31,80], produces the formation of tumour-like structures that protrude into the ventricle, and an aberrant and invasive pattern of neural cell proliferation. This leads to many progeny being displaced, throughout the CNS parenchyma, at significant distances away from the periventricular region[80,81]. *In vitro*, transiently dividing progenitors that are derived from the subventricular-zone population can revert to a stem-cell-like phenotype as they form neurospheres and show some proliferative ability. This supports the potential of this population to be a target for further oncogenic transformation[31].

Intuitively, given the lack of an intrinsic self-renewal capacity and the ephemeral nature of transiently dividing progenitors, this indicates that the frequency with which they give rise to tumour stem cells is much lower than that of somatic stem cells, which retain a proliferative capacity throughout the lifetime of an animal. But this might not be the case — the size of the transiently dividing progenitor compartment is at least one order of magnitude larger than that of the somatic stem-cell compartment (FIG. 2), which

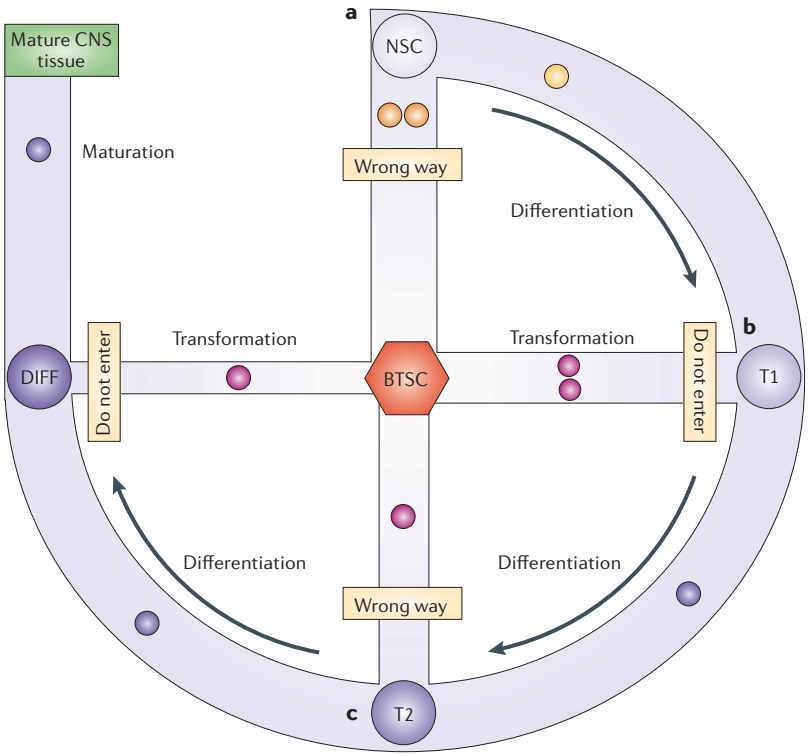


Figure 4 | **Neurogenetic compartments as developmental 'beltways'.** Brain tumour stem cells (BTSCs), despite the fact that they have stem-cell properties, might be derived from many of the mostly undifferentiated cells that are found in the neurogenetic areas of the adult brain. During this process, sequential cell lineages are generated that progressively acquire properties of greater differentiation. Therefore, cells in the neurogenetic process can be envisioned as rolling along a developmental 'beltway'. They start as neural stem cells (NSCs), which give rise to progressively maturer, transiently dividing progenitors (T1 and T2) until they leave the beltway (terminally differentiate; DIFF) to become either functional mature neurons or other cells. Throughout this process and at different stages of differentiation mistakes can occur, and cells on the beltway can enter some of the 'forbidden' beltway exits that, regardless of where they start, all lead to the same result: the formation of BTSCs. The widths of the horizontal and vertical arms that take cells from the beltway to their final BTSC fate become progressively narrower closer to the exit to the compartment of differentiated cells. This represents the fact that the more mature a cell is, the less likely it will turn into a BTSC.

increases the overall probability of cell transformation within the former population. This is particularly true in complex tissues that contain many mature cell types, as the dimensions of the transiently dividing progenitor compartment — and therefore the size of the pool of mitotically active cells — increase with the number of terminally differentiated cell lineages that are generated in a given tissue[82]. Most importantly, the de-differentiation of transiently dividing progenitors into somatic stem cells has been shown to occur under physiological conditions, both *in vivo* and *in vitro*, without the need for transformation[31,83]. Furthermore, although somatic stem cells have a lifelong proliferation potential, the global number of divisions that they undergo might not be any greater than that of the transiently dividing progenitor population, which expresses its full proliferative potential over a relatively short time. This concept is strengthened by the observation that forced cycling of haematopoietic stem cells, either through serial transplantation or genetic disruption of key negative cell-cycle regulators, affects the functional integrity of stem cells over time. This indicates that, similar to transiently dividing progenitors, the total number of divisions that is allowed for somatic stem cells might be limited[84,85].

Additional factors decrease the actual frequency at which somatic stem cells undergo transformation. For instance, Cairns proposed that somatic stem cells retain their original, or template, DNA strands at each cycle, thereby protecting themselves effectively against DNA-replication errors[86]. This immortal-strand hypothesis, as it has become known, has now found experimental support in studies of small-intestinal and neural stem cells[87,88].

Therefore, it seems plausible that the anatomical origin of brain tumour stem cells might be in regions of the CNS that have the greatest degree of cell proliferation, such as the subventricular zone. The transformation of normal somatic stem cells or of their progeny, the transiently dividing cells, are equally conceivable hypotheses for the cellular origin of brain tumour stem cells within these proliferative zones (FIG. 4).

Although we think that brain tumour stem cells are best defined on the basis of functional competence rather than on the identity of the cell that underwent the original oncogenic transformation, the need to identify accurately the cellular origin of such stem cells is not superfluous. In fact, brain tumour stem cells are likely to retain many of their parental characteristics[55,57,58,61,63]. As adult neural stem cells and neural transiently dividing progenitors have specific functional, molecular and antigenic attributes (FIG. 2), these might be used to develop better classification and diagnostic assays, as heterogeneity exceeds the sensitivity of the current classification system[89]. These differential antigenic and molecular attributes might also be exploited to devise cell-enrichment procedures to establish nearly pure preparations of tumour-initiating cells, which might enable the identification of a more specific pathway to target for brain tumour therapy and the devising of exclusive immunotherapy approaches. Furthermore, they might lead to a more reliable identification of tumour-initiating stem cells and the development of novel and accurate assays that enable the number of true tumour stem cells in different brain tumours, or in the same brain tumour type from different patients, to be identified. The experimental and clinical benefits of these findings could be considerable. It is also imperative to define the functional, regulatory, molecular and antigenic features that distinguish normal from tumour stem cells. This will allow us to devise therapeutic strategies that specifically target the tumour population, but leave normal stem and precursor cells unharmed.

Regulation of neural stem-cell division: insights into tumour stem-cell biology. The finding that adult germinal, neural precursors might provide the origin of brain tumour stem cells indicates the presence of common regulatory pathways. There is evidence that the same key mechanisms that control the activity of

normal neurogenetic progenitors are altered in brain tumours. As this topic has recently been covered in a series of excellent articles[26], here we provide only a brief overview of the most relevant pathways.

EGFR expression is upregulated during tumour formation, is correlated with malignancy and response to treatment[79], and is considered a reliable marker for primary glioblastoma multiforme[1]. Similarly, FGFs, which have been shown to regulate neural stem-cell proliferation[44,32] and cell-fate commitment[90], have been proposed as glioma-promoting autocrine factors that are involved in the proliferation of tumour cells[91] and the induction of angiogenesis[92].

Likewise, overexpression of Notch receptors and their ligands Delta-like 1 (DLK1) and Jagged 1 (JAG1) correlates with the proliferative capacity of human glioma cells[93]. Therefore, Notch has been suggested to function as a positive effector of self-renewal in adult neural stem cells[94,95].

Another gene that has recently been implicated in the regulation of self-renewal of normal neural stem cells[96,97] and in CNS tumorigenesis[98] is BMI1. This belongs to the Polycomb group of genes and has been shown to positively regulate cell proliferation and self-renewal through repression of the tumour suppressors INK4a and ARF[96]. Intriguingly, BMI1 expression is significantly increased in human medulloblastomas, directly linking the deregulation of BMI1-dependent self-renewal to tumour development[98].

Similarly, Sonic Hedgehog (SHH) and its downstream effectors glioma-associated oncogene homologue 1 (GLI1), GLI2 and GLI3 have been shown to specifically regulate neurogenesis and self-renewal within the external granular layer of the early postnatal cerebellum and to control precursor proliferation in the adult subventricular zone[99]. Therefore, mutations in this pathway are directly implicated in the genesis of medulloblastoma[100,101].

Other genes, the expression of which links normal regulation of neural stem cells to aberrant function in brain tumours, include PTEN and elements of the canonical Wnt–β-catenin signalling pathway[102,103]. PTEN is a tumour suppressor with an important function in the control of proliferation of neural stem cells and progenitor cells *in vivo* and *in vitro*[104]. It has also been shown to control migration in the adult subventricular zone[105]. Importantly, *PTEN* is often inactivated in glioblastomas[66,106] and, as a result, maintenance of its expression has been closely associated with a favourable prognosis in patients with glioblastoma multiforme[79].

Finally, the Wnt–β-catenin pathway has recently been implicated in the regulation of adult neurogenesis[107,108]. Although a direct correlation between the deregulation of the Wnt regulatory system has been documented in the pathogenesis of medulloblastomas[109], evidence for its involvement in gliomagenesis is less obvious. However, interfering with Wnt signalling through the ectopic expression of soluble Wnt modulators such as the secreted Frizzled-related proteins (sFRPs) has been shown to promote the growth of malignant glioma cells while inhibiting their invasive ability[110].

Perspectives

A new system for drug/target discovery and validation. The hallmarks of tumour cells are exacerbated proliferation and aberrant self-renewal, which indicates that cells from CNS cancers could be used to establish brain tumour stem-cell lines from adult human specimens. This possibility is true for brain tumour stem cells from glioblastoma multiforme and, to a much lesser extent, from medulloblastoma[61], and it has considerable practical implications. Brain tumour stem cells, even at the single-cell level, have the genetic information that is required to generate a faithful phenocopy of the original tumour from which they are derived[55,61]. The increasing number of copies of the original brain tumour stem cells that are produced during serial subculturing generates a renewable and stable source of cancer-initiating cells that retain patient-specific features, such as growth characteristics, an aberrant differentiation phenotype and a specific molecular fingerprint[61]. These personalized brain tumour stem-cell lines can be used both as a means to identify novel molecular markers and as a source of material for patient-specific drug-screening protocols. We predict that these brain tumour stem-cell-based assays will result in a more meaningful and predictive model of the actual therapy responsiveness of brain tumours than the standard glioma cell lines that are currently available.

A new target. The discovery of putative brain tumour stem cells identifies a new cellular target that might be amenable to novel or traditional treatments.

One feature that contributes to the ability of a stem cell to survive throughout the lifespan of an animal is its inherent resistance to drugs and toxins. A peculiarity of many stem-cell populations is their relatively high expression of ATP-binding cassette (ABC) drug transporters, which can protect cells from cytotoxic agents[111]. Haematopoietic stem cells have been isolated on the basis of their expression of these transporters and their ability to efflux the fluorescent dye Hoechst 33342, which give them a unique profile, referred to as the side population, when analysed by flow cytometry. Side-population cells have also been isolated from the CNS[54]. Although somatic and tumour stem cells can be identified within the side population, not all of the stem-cell population is contained within this group, nor does the side population contain a pure stem-cell population, therefore negating the use of this technique as a method to purify tumour stem cells. However, the presence of drug-resistance genes in the tumour population and their non-selective expression in a putative tumour stem-cell population indicates the hypothetical possibility that drug resistance and, in particular, tumour recurrence might be related to the inability of cytotoxic compounds to eliminate the tumour-initiating cells. The next generation of chemotherapy agents might be a combination of chemosensitizers and cytotoxic agents that alters ABC-transporter activity, leading to better clinical outcomes[111].

Normal stem cells to target tumour stem cells? A particularly innovative approach indicates that normal neural stem cells can be used to target and deliver molecules to CNS tumours[112]. Endogenous neural stem cells[113], as well as neural stem cells that have been engineered to secrete interleukin 4 (IL-4)[114], IL-12 (REF. 115) or a fragment of metalloproteinase-2 called PEX[116], can produce tumour regression and increase survival in experimental intracranial gliomas. Similarly, immortalized neural stem cells that were placed near the tumour or at a distant site through intravascular delivery could migrate towards and integrate with the previously implanted tumour cells[117]. The transplanted neural stem cells surrounded the expanding tumour mass, even seeking out and attaching to the infiltrating tumour cells. Furthermore, migratory, tumour-seeking neural stem cells could be genetically engineered to deliver a therapeutically relevant molecule. This intriguing approach has been confirmed with other neural precursors[118,119] and the tropism of these cells for glioblastoma multiforme cells has been shown to be dependent on the vascular endothelial growth factor (VEGF) that is secreted by these cells[120] and on their expression of the chemokine receptor CXCR4 (REF. 121). It should be noted that the strategy of using neural stem cells and progenitor cells as 'Trojan horses' with anti-glioma homing capacity has, to date, only been shown using experimentally implanted cell lines and that the same phenomenon ought also to be demonstrated with tumours that arise spontaneously in the brain. This notwithstanding, this innovative approach might prove useful not only for the site-specific delivery of cytotoxic agents or virally mediated genetic elements, but also for the widespread release of molecules that regulate the proliferation, differentiation and migration of the tumour stem cells.

Conclusions

The discovery that brain tumours, particularly glioblastoma multiforme, contain tumour-founding cells with the defining features of neural stem cells strongly indicates that immature precursors within neurogenetic areas represent the cellular origin of brain tumour stem cells. The latter ought not to be exclusively viewed as the result of transformation of normal neural stem cells, as our knowledge of the mechanisms that regulate adult neurogenesis indicates that it is equally likely that brain tumour stem cells might derive from transiently dividing progenitors. According to this view, and while we await the identification of specific brain tumour stem-cell-lineage antigens, it seems reasonable to define brain tumour stem cells as cancer-initiating precursors that show defining stem-cell properties and, principally, a long-term capacity for self-renewal *in vitro* or *in vivo*. The tumours that are generated from these cells must be a faithful, possibly patient-specific, phenocopy of the original neoplasia (BOX 3). The capacity to culture brain tumour stem cells *in vitro* might give us an opportunity to devise new and more-specific therapeutic approaches to target incurable brain tumours.

There is hope that the identification of brain tumour stem cells as possibly the long-sought culprit in brain cancers might revolutionize the way that we approach brain tumour physiology, diagnosis and therapy.

1. Behin, A., Hoang-Xuan, K., Carpentier, A. F. & Delattre, J. Y. Primary brain tumours in adults. *Lancet* **361**, 323–331 (2003).
2. Stupp, R. *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **352**, 987–996 (2005).
3. Holland, E. C. Glioblastoma multiforme: the terminator. *Proc. Natl Acad. Sci. USA* **97**, 6242–6244 (2000).
4. Ellison, D. Classifying the medulloblastoma: insights from morphology and molecular genetics. *Neuropathol. Appl. Neurobiol.* **28**, 257–282 (2002).
5. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
6. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **414**, 105–111 (2001).
7. Bonnet, D. & Dick, J. E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Med.* **3**, 730–737 (1997).
Describes for the first time the identification of stem-like cells in haematopoietic malignancies.
8. Hope, K. J., Jin, L. & Dick, J. E. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nature Immunol.* **5**, 738–743 (2004).
9. Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J. & Clarke, M. F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl Acad. Sci. USA* **100**, 3983–3988 (2003).
10. Altman, J. & Das, G. D. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124**, 319–335 (1965).
11. Altman, J. Proliferation and migration of undifferentiated precursor cells in the rat during postnatal gliogenesis. *Exp. Neurol.* **16**, 263–278 (1966).
12. Dacey, M. L. & Wallace, R. B. Postnatal neurogenesis in the feline cerebellum: a structural-functional investigation. *Acta Neurobiol. Exp. (Wars.)* **34**, 253–263 (1974).
13. Sell, S. Stem cell origin of cancer and differentiation therapy. *Crit. Rev. Oncol. Hematol.* **51**, 1–28 (2004).
14. Nottebohm, F. The road we travelled: discovery, choreography, and significance of brain replaceable neurons. *Ann. N. Y. Acad. Sci.* **1016**, 628–658 (2004).
15. Reynolds, B. A. & Weiss, S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707–1710 (1992).
Reports the initial evidence that multipotent neural stem cells that reside in the adult rodent brain can be identified and isolated *in vitro* following mitogen stimulation.
16. Gould, E., McEwen, B. S., Tanapat, P., Galea, L. A. & Fuchs, E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J. Neurosci.* **17**, 2492–2498 (1997).
17. Gould, E., Tanapat, P., McEwen, B. S., Flugge, G. & Fuchs, E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc. Natl Acad. Sci. USA* **95**, 3168–3171 (1998).
18. Eriksson, P. S. *et al.* Neurogenesis in the adult human hippocampus. *Nature Med.* **4**, 1313–1317 (1998).
19. Lie, D. C., Song, H., Colamarino, S. A., Ming, G. L. & Gage, F. H. Neurogenesis in the adult brain: new strategies for central nervous system diseases. *Annu. Rev. Pharmacol. Toxicol.* **44**, 399–421 (2004).
20. Ming, G. L. & Song, H. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250 (2005).
21. Loeffler M. and Potten, C. in *Stem Cells and Cellular Pedigrees — A Conceptual Introduction.* (ed. Potten, C.) Ch. 1 (Academic Press, London, 1997).
22. Passegue E. J. C., Ailles L. E. & Weissman I. L. Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *Proc. Natl Acad. Sci. USA.* **100**, 11842–11849 (2003).
23. Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703–716 (1999).
Results in this paper show that the *bona fide* subventricular-zone stem cell is the type B cell, which shows features of a differentiated astrocyte.
24. Morshead, C. M. *et al.* Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* **13**, 1071–1082 (1994).
25. Sanai, N. *et al.* Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**, 740–744 (2004).
Provides the first evidence of the existence of neural stem cells in the subventricular zone of the adult human brain, and highlights the differences from the same compartment in rodents and the implications for the origin of brain tumours.
26. Sanai, N., Alvarez-Buylla, A. & Berger, M. S. Neural stem cells and the origin of gliomas. *N. Engl. J. Med.* **353**, 811–822 (2005).
27. Quinones-Hinojosa, A. *et al.* Cellular composition and cytoarchitecture of the adult human subventricular zone: A niche of neural stem cells. *J. Comp. Neurol.* **494**, 415–434 (2006).
28. Seri, B., Garcia-Verdugo, J. M., McEwen, B. S. & Alvarez-Buylla, A. Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* **21**, 7153–7160 (2001).

29. Seri, B., Garcia-Verdugo, J. M., Collado-Morente, L., McEwen, B. S. & Alvarez-Buylla, A. Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J. Comp. Neurol.* **478**, 359–378 (2004).
30. Roy, N. S. *et al. In vitro* neurogenesis by progenitor cells isolated from the adult human hippocampus. *Nature Med.* **6**, 271–277 (2000).
31. Doetsch, F., Petreanu, L., Caille, I., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron* **36**, 1021–1034 (2002).
32. Gritti, A. *et al.* Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. *J. Neurosci.* **16**, 1091–1100 (1996).
33. Gritti, A. *et al.* Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. *J. Neurosci.* **19**, 3287–3297 (1999).
34. Reynolds, B. A. & Rietze, R. L. Neural stem cells and neurospheres — re-evaluating the relationship. *Nature Methods* **2**, 333–336 (2005).
35. Reynolds, B. A. & Weiss, S. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. *Dev. Biol.* **175**, 1–13 (1996).
36. Vescovi, A. L. *et al.* Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp. Neurol.* **156**, 71–83 (1999).
37. Galli, R. *et al.* Emx2 regulates the proliferation of stem cells of the adult mammalian central nervous system. *Development* **129**, 1633–1644 (2002).
38. Parras, C. M. *et al.* Mash1 specifies neurons and oligodendrocytes in the postnatal brain. *EMBO J.* **23**, 4495–4505 (2004).
39. Soria, J. M. *et al.* Defective postnatal neurogenesis and disorganization of the rostral migratory stream in absence of the *Vax1* homeobox gene. *J. Neurosci.* **24**, 11171–11181 (2004).
40. Weiss, S. *et al.* Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J. Neurosci.* **16**, 7599–7609 (1996).
41. Gritti, A. *et al.* Multipotent neural stem cells reside into the rostral extension and olfactory bulb of adult rodents. *J. Neurosci.* **22**, 437–445 (2002).
42. Svendsen, C. N., Caldwell, M. A. & Ostenfeld, T. Human neural stem cells: isolation, expansion and transplantation. *Brain Pathol.* **9**, 499–513 (1999).
43. Uchida, N. *et al.* Direct isolation of human central nervous system stem cells. *Proc. Natl Acad. Sci. USA* **97**, 14720–14725 (2000).
44. Palmer, T. D., Markakis, E. A., Willhoite, A. R., Safar, F. & Gage, F. H. Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J. Neurosci.* **19**, 8487–8497 (1999).
45. Zhao, M. *et al.* Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc. Natl Acad. Sci. USA* **100**, 7925–7930 (2003).
46. Zhang, X., Klueber, K. M., Guo, Z., Lu, C. & Roisen, F. J. Adult human olfactory neural progenitors cultured in defined medium. *Exp. Neurol.* **186**, 112–123 (2004).
47. Consiglio, A. *et al.* Robust *in vivo* gene transfer into adult mammalian neural stem cells by lentiviral vectors. *Proc. Natl Acad. Sci. USA* **101**, 14835–14840 (2004).
48. Markakis, E. A., Palmer, T. D., Randolph-Moore, L., Rakic, P. & Gage, F. H. Novel neuronal phenotypes from neural progenitor cells. *J. Neurosci.* **24**, 2886–2897 (2004).
49. Toma, J. G., McKenzie, I. A., Bagli, D. & Miller, F. D. Isolation and characterization of multipotent skin-derived precursors from human skin. *Stem Cells* **23**, 727–737 (2005).
50. Messina, E. *et al.* Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ. Res.* **95**, 911–921 (2004).
51. Dontu, G. *et al. In vitro* propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev.* **17**, 1253–1270 (2003).
52. Rietze, R. L. *et al.* Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature* **412**, 736–739 (2001).
53. Capela, A. & Temple, S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron* **35**, 865–875 (2002).
54. Kim, M. & Morshead, C. M. Distinct populations of forebrain neural stem and progenitor cells can be isolated using side-population analysis. *J. Neurosci.* **23**, 10703–10709 (2003).
55. Singh, S. K. *et al.* Identification of human brain tumour initiating cells. *Nature* **432**, 396–401 (2004). **Shows that only CD133$^+$ human brain tumour cells are endowed with tumour-initiating capacity *in vivo*.**
56. Ignatova, T. N. *et al.* Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers *in vitro*. *Glia* **39**, 193–206 (2002).
57. Singh S. K. *et al.* Identification of a cancer stem cell in human brain tumors. *Cancer Res.* **63**, 5821–5828 (2003).
58. Hemmati, H. D. *et al.* Cancerous stem cells can arise from pediatric brain tumors. *Proc. Natl Acad. Sci. USA* **100**, 15178–15183 (2003).
59. Corbeil, D., Roper, K., Weigmann, A. & Huttner, W. B. AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? *Blood* **91**, 2625–2626 (1998).
60. Tamaki, S. *et al.* Engraftment of sorted/expanded human central nervous system stem cells from fetal brain. *J. Neurosci. Res.* **69**, 976–986 (2002).
61. Galli, R. *et al.* Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res.* **64**, 7011–7021 (2004).
62. Tunici, P. *et al.* Genetic alterations and *in vivo* tumorigenicity of neurospheres derived from an adult glioblastoma. *Mol. Cancer* **3**, 25 (2004).
63. Yuan, X. *et al.* Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* **23**, 9392–9400 (2004).
64. Taylor, M. D. *et al.* Radial glia cells are candidate stem cells of ependymoma. *Cancer Cell* **8**, 323–335 (2005).
65. Merkle, F. T., Tramontin, A. D., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. Radial glia give rise to adult neural stem cells in the subventricular zone. *Proc. Natl Acad. Sci. USA* **101**, 17528–17532 (2004).
66. Wechsler-Reya, R. & Scott, M. P. The developmental biology of brain tumors. *Annu. Rev. Neurosci.* **24**, 385–428 (2001).
67. Hopewell, J. W. & Wright, E. A. The importance of implantation site in cerebral carcinogenesis in rats. *Cancer Res.* **29**, 1927–1931 (1969).
68. Vick, N. A., Lin, M. J. & Bigner, D. D. The role of the subependymal plate in glial tumorigenesis. *Acta Neuropathol.* **40**, 63–71 (1977).
69. Zhu, Y. *et al.* Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer Cell* **8**, 119–130 (2005).
70. Holland, E. C. *et al.* Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. *Nature Genet.* **25**, 55–57 (2000). **Shows that cell-specific deregulation of oncogenic pathways leads to the development of glial tumours that arise from nestin-positive progenitors but not from differentiated astrocytes.**
71. Holland, E. C., Hively, W. P., Gallo, V. & Varmus, H. E. Modeling mutations in the G1 arrest pathway in human gliomas: overexpression of CDK4 but not loss of INK4a-ARF induces hyperploidy in cultured mouse astrocytes. *Genes Dev.* **12**, 3644–3649 (1998).
72. Dai, C. *et al.* PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes *in vivo*. *Genes Dev.* **15**, 1913–1925 (2001).
73. Bachoo, R. M. *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* **1**, 269–277 (2002).
74. Berger, F., Gay, E., Pelletier, L., Tropel, P. & Wion, D. Development of gliomas: potential role of asymmetrical cell division of neural stem cells. *Lancet Oncol.* **5**, 511–514 (2004).
75. Seaberg, R. M. & van der Kooy, D. Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. *J. Neurosci.* **22**, 1784–1793 (2002).
76. Bull, N. D. & Bartlett, P. F. The adult mouse hippocampal progenitor is neurogenic but not a stem cell. *J. Neurosci.* **25**, 10815–10821 (2005).
77. Frank, S. A. & Nowak, M. A. Cell biology: Developmental predisposition to cancer. *Nature* **422**, 494 (2003).
78. Huntly, B. J. & Gilliland, D. G. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nature Rev. Cancer* **5**, 311–321 (2005).
79. Mellinghoff, I. K. *et al.* Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N. Engl. J. Med.* **353**, 2012–2024 (2005).
80. Kuhn, H. G., Winkler, J., Kempermann, G., Thal, L. J. & Gage, F. H. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J. Neurosci.* **17**, 5820–5829 (1997).
81. Craig, C. G. *et al. In vivo* growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J. Neurosci.* **16**, 2649–2658 (1996).
82. Potten, C. S., Booth, C. & Hargreaves, D. The small intestine as a model for evaluating adult tissue stem cell drug targets. *Cell Prolif.* **36**, 115–129 (2003).
83. Potten, C. S. & Loeffler, M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* **110**, 1001–1020 (1990). **Provides a fundamental introduction to the general concept of stem cells by describing the properties of the stem-cell compartment that is localized within the intestinal crypt.**
84. Ross, E. A., Anderson, N. & Micklem, H. S. Serial depletion and regeneration of the murine hematopoietic system. Implications for hematopoietic organization and the study of cellular aging. *J. Exp. Med.* **155**, 432–444 (1982).
85. Hock, H. *et al.* Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. *Nature* **431**, 1002–1007 (2004).
86. Cairns, J. Mutation selection and the natural history of cancer. *Nature* **255**, 197–200 (1975).
87. Potten, C. S., Owen, G. & Booth, D. Intestinal stem cells protect their genome by selective segregation of template DNA strands. *J. Cell Sci.* **115**, 2381–2388 (2002).
88. Karpowicz, P. *et al.* Support for the immortal strand hypothesis: neural stem cells partition DNA asymmetrically *in vitro*. *J. Cell Biol.* **170**, 721–732 (2005).
89. Louis, D. N., Holland, E. C. & Cairncross, J. G. Glioma classification: a molecular reappraisal. *Am. J. Pathol.* **159**, 779–786 (2001).
90. Vescovi, A. L., Reynolds, B. A., Fraser, D. D. & Weiss, S. bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells. *Neuron* **11**, 951–966 (1993).
91. Joy, A. *et al.* Nuclear accumulation of FGF-2 is associated with proliferation of human astrocytes and glioma cells. *Oncogene* **14**, 171–183 (1997).
92. Auguste, P. *et al.* Inhibition of fibroblast growth factor/fibroblast growth factor receptor activity in glioma cells impedes tumor growth by both angiogenesis-dependent and-independent mechanisms. *Cancer Res.* **61**, 1717–1726 (2001).
93. Purow, B. W. *et al.* Expression of Notch-1 and its ligands, Delta-like-1 and Jagged-1, is critical for glioma cell survival and proliferation. *Cancer Res.* **65**, 2353–2363 (2005).
94. Hitoshi, S. *et al.* Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. *Genes Dev.* **16**, 846–858 (2002).
95. Shen, Q. *et al.* Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* **304**, 1338–1340 (2004).
96. Molofsky, A. V. *et al.* Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425**, 962–967 (2003).
97. Bruggeman, S. W. *et al.* Ink4a and Arf differentially affect cell proliferation and neural stem cell self-renewal in Bmi1-deficient mice. *Genes Dev.* **19**, 1438–1443 (2005).
98. Leung, C. *et al.* Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. *Nature* **428**, 337–341 (2004).
99. Palma, V. *et al.* Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. *Development* **132**, 335–344 (2005).
100. Goodrich, L. V., Milenkovic, L., Higgins, K. M. & Scott, M. P. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**, 1109–1113 (1997).

101. Pomeroy, S. L. *et al.* Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* **415**, 436–442 (2002).
102. Baker, S. J. & McKinnon, P. J. Tumour-suppressor function in the nervous system. *Nature Rev. Cancer* **4**, 184–196 (2004).
103. Reya, T. & Clevers, H. Wnt signalling in stem cells and cancer. *Nature* **434**, 843–850 (2005).
104. Groszer, M. *et al.* Negative regulation of neural stem/progenitor cell proliferation by the *Pten* tumor suppressor gene *in vivo*. *Science* **294**, 2186–2189 (2001).
105. Li, L. *et al.* PTEN in neural precursor cells: regulation of migration, apoptosis, and proliferation. *Mol. Cell. Neurosci.* **20**, 21–29 (2002).
106. Rasheed, B. K., Wiltshire, R. N., Bigner, S. H. & Bigner, D. D. Molecular pathogenesis of malignant gliomas. *Curr. Opin. Oncol.* **11**, 162–167 (1999).
107. Chenn, A. & Walsh, C. A. Increased neuronal production, enlarged forebrains and cytoarchitectural distortions in β-catenin overexpressing transgenic mice. *Cereb. Cortex* **13**, 599–606 (2003).
108. Lie, D. C. *et al.* Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370–1375 (2005).
109. Marino, S. Medulloblastoma: developmental mechanisms out of control. *Trends Mol. Med.* **11**, 17–22 (2005).
110. Roth, W. *et al.* Secreted Frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. *Oncogene* **19**, 4210–4220 (2000).
111. Dean, M., Fojo, T. & Bates, S. Tumour stem cells and drug resistance. *Nature Rev. Cancer* **5**, 275–284 (2005).
112. Shah, K. *et al.* Glioma therapy and real-time imaging of neural precursor cell migration and tumor regression. *Ann. Neurol.* **57**, 34–41 (2005).
113. Glass, R. *et al.* Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. *J. Neurosci.* **25**, 2637–2646 (2005).
114. Benedetti, S. *et al.* Gene therapy of experimental brain tumors using neural progenitor cells. *Nature Med.* **6**, 447–450 (2000).
Shows that implantation in established glial tumours of neural stem/progenitor cells that have been engineered to secrete IL-4 elicits a strong anti-tumour effect.
115. Ehtesham, M. *et al.* The use of interleukin 12-secreting neural stem cells for the treatment of intracranial glioma. *Cancer Res.* **62**, 5657–5663 (2002).
116. Kim, S. K. *et al.* PEX-producing human neural stem cells inhibit tumor growth in a mouse glioma model. *Clin. Cancer Res.* **11**, 5965–5970 (2005).
117. Aboody, K. S. *et al.* Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc. Natl Acad. Sci. USA* **97**, 12846–12851 (2000).
The first demonstration that distally implanted somatic neural stem cells can migrate considerable distances and target previously established tumours, including tumour cells that have infiltrated surrounding tissue.
118. Zhang, Z. G. *et al.* Magnetic resonance imaging and neurosphere therapy of stroke in rat. *Ann. Neurol.* **53**, 259–263 (2003).
119. Tang, Y. *et al. In vivo* tracking of neural progenitor cell migration to glioblastomas. *Hum. Gene Ther.* **14**, 1247–1254 (2003).
120. Schmidt, N. O. *et al.* Brain tumor tropism of transplanted human neural stem cells is induced by vascular endothelial growth factor. *Neoplasia* **7**, 623–629 (2005).
121. Ehtesham, M. *et al.* Glioma tropic neural stem cells consist of astrocytic precursors and their migratory capacity is mediated by CXCR4. *Neoplasia* **6**, 287–293 (2004).

Acknowledgements
We are grateful to S. Piccirillo for valuable help with the preparation of this manuscript and to R. Rietze and M. De Palma for their useful suggestions and comments.

Competing interests statement
The authors declare competing financial interests: see web version for details.

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