Cancer stem cells in adult gliomas

Sunit Das
Division of Neurosurgery, Li Ka Shing Knowledge Institute, St. Michael's Hospital, University of Toronto, Canada (SD)

Published in Atlas Database: July 2011

DOI: 10.4267/2042/46077

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 2.0 France Licence.
© 2011 Atlas of Genetics and Cytogenetics in Oncology and Haematology

John Dick and colleagues demonstrated in the mid-1990s that only a small fraction of human acute myeloid leukemia tumour cells were capable of initiating and sustaining tumour growth following transplantation into an immunocompromised murine host (Lapidot et al., 1994; Bonnet and Dick, 1997). While the remaining tumour cells were able to proliferate, their proliferative potential was limited and they were incapable of sustaining tumour growth. In addition to their capability to proliferate, these leukemia-initiating cells possessed the ability to self-renew and were able to give rise to multiple heterogeneous progeny. Interestingly, these cells were identifiable by surface marker reminiscent of SCID-repopulating cells rather than committed precursors. Given their functional and morphologic similarity to normal hematopoietic stem cells, Bonnet and Dick called these cells leukemic cancer stem cells. Further, they hypothesized that the cellular heterogeneity found within this cancer reflected a hierarchy that recapitulates normal hematopoietic development and that cell identity within a cancer –as defined by a stem-like or a more committed state– had functional relevance for a cell's ability to drive tumour growth. Since then, numerous hematologic and solid tumours – including acute lymphoblastic leukemia (Cobaleda et al., 2000; Cox et al., 2004; Castor et al., 2005), breast (Al-Hajj et al., 2003), colon (O'Brien et al., 2007; Ricci-Vitiani et al., 2007) and lung cancer (Eramo et al., 2008), melanoma (Fang et al., 2005), and primary brain tumours (Singh et al., 2004)– have been found to harbor cancer stem cells. The cancer stem cell hypothesis has been forwarded as an alternative to the clonal evolution model of cancer development. In primary brain tumours, the cancer stem cell hypothesis has important ramifications for how we think about disease treatment and how we understand disease recurrence and progression. Here, I will review the current literature regarding cancer stem cells in primary brain tumours and discuss the relevance of the cancer stem cell hypothesis and clonal evolution model to their biology. Then, I will review the question of cell-of-origin in primary brain tumours. Finally, I will bring up questions regarding the implications of the cancer stem cell hypothesis for glioma biology that must be addressed with future studies.

Brain tumour stem cells: the evidence

The presence of cancer stem cells in adult glioma was established by concomitant independent work in the labs of Drs. Peter Dirks and Angelo Vescovi. Both groups applied techniques from the neural stem cell biology field to isolate brain tumour stem cells (BTSCs) from human surgical specimens, namely, by enrichment of BTSCs from dissociated tumour cells as gliomaspheres grown on non-adherent plates in serum-free media supplemented with the growth factors EGF and FGF (Galli et al., 2004; Singh et al., 2004). These cells, when exposed to growth factor withdrawal or serum, lost their stem-like features and gave rise to more differentiated progeny resembling normal committed neuroepithelial cells. In addition, when transplanted into the brains of immunocompromised mice, these cells gave rise to brain tumours that pathologically resembled the parent tumour and that could be propagated by serial dissociation and transplantation. Further, the Dirks lab found that the cell surface protein, CD133, could be used as a marker for the tumorigenic subpopulation in human tumours. Since that time, the Dirks group has shown that these cells can also be isolated and propagated as an adherent monolayer (Pollard et al., 2009). That the BTSC population can be enriched by growth in serum-free, growth factor-supplemented media
speaks to the hierarchical similarities between tumour cells in high-grade gliomas and stem and committed neuroepithelial cells in the normal brain. This technique is also used for enrichment of neural stem cells from the mouse subventricular zone (Reynolds and Weiss, 1992) and subgranular zone (Bonaguidi et al., 2008). In addition, normal neural stem cells differentiate into neurons, astrocytes and oligodendrocytes following withdrawal of growth factors or exposure to serum. Surprisingly, BTSCs appear capable of “differentiating” as well. BTSCs grown in these conditions stop expressing cellular markers of stemness, and instead take on the immunohistochemical and sometimes morphological properties of committed cells. What these cancer-derived “neurons”, “astrocytes”, and “oligodendrocytes” represent remains unclear. Some data suggest that these cells have exited the cell cycle and are in fact post-mitotic. Using murine xenotransplantation as the measure of tumourigenicity, these populations appear to be non-tumorigenic. Certainly, prolonged exposure to serum appears to give rise to or select for a cell population different from the original tumour, and these populations, when they do give rise to tumours following xenotransplantation, result in lesions that resemble the more mesenchymal tumours seen with implantation of many traditional glioma cell lines rather than a true glioblastoma (Lee et al., 2006). These findings would suggest that BTSCs, as reflected by the cells enriched by growth-factor supplemented, serum-free media culture, are the primary drivers of glioblastoma growth in vivo.

Interestingly, many of the properties of BTSCs within the tumour microenvironment recapitulate what we know about neural stem cells. Like neural stem cells, BTSCs have been shown to be resistant to radiation- and chemotherapy-induced DNA damage (Bao et al., 2006a; Eramo et al., 2006; Liu et al., 2006). In vivo, BTSCs appear to reside within a vascular niche (Bao et al., 2006b; Calabrese et al., 2007) reminiscent of the normal neural stem cell niche (Shen et al., 2008; Tavazoie et al., 2008), and appear to respond to changes in the extracellular matrix (Lathia et al., 2010) —for example, to integrins— that are relevant to normal neural stem cell biology (Kazanis et al., 2010). Finally, many of the molecular pathways that are central to gliomagenesis —such as p53, Ras, PTEN, and Rb— are also relevant to normal development and maintenance of adult neural stem cell homeostasis (Meletis et al., 2006; Molofsky et al., 2006; Quinones-Hinojosa et al., 2006; Gil-Perotin et al., 2009; Gregorian et al., 2009).

The cell-intrinsic and micro-environmental similarities between BTSCs suggests that the study of neural stem cell biology might have resonance for our understanding of primary brain tumours, and these factors must be taken into account in our attempt to develop better and more effective therapies against them.

BTSCs and tumour recurrence

Expression of the polycomb group protein Bmi1 by neural stem cells has been found to enhance ATM recruitment to the chromatin in these cells and increase the rate of gamma H2AX foci resolution, resulting in resistance to radiation-induced DNA damage and cell death (Facchino et al., 2010). Neural stem cells also express high levels of ATP-dependent drug efflux pumps belonging to the superfamily of ATP-binding cassette (ABC) transporters such as ABCB1 (also known as MDR1) and ABCG2 (Islam et al., 2005a; Islam et al., 2005b). These transporters act as an effective salve against chemotherapeutic agents, which undergo rapid efflux from neural stem cells.

BTSCs have similarly been found to be preferentially resistant to radiation- or chemotherapy-induced cell death compared to non-stem glioma cells (Bao et al., 2006a; Eramo et al., 2006; Liu et al., 2006). Treatment of mice harboring a virally induced primary tumour with chemotherapy and radiation results in expansion of the side population following therapy (Blau et al., 2009). Conventional treatment of high-grade gliomas in humans similarly appears to result in expansion of the BTSC population, as measured by CD133-positivity, suggesting that these cells preferentially survive chemotherapy and radiation (Tamura et al., 2009; Pallini et al., 2011). Interestingly, analysis of glioblastomas within the TCGA database revealed an evolution of tumours toward a more mesenchymal phenotype on recurrence. In breast cancer, the epithelial-mesenchymal transition has been found to drive cells toward a more stem-like identity (Mani et al., 2008). It is intriguing to speculate that the mesenchymal transition in glioblastomas following chemoradiation also reflects an enrichment of stem-like cells in these tumours.

In examining the radioresistance of BTSCs, the Rich group found that CD133+ cells repaired DNA damage faster than CD133- cells (Bao et al., 2006a). The difference between these two groups was ameliorated by treatment with DBH, an inhibitor of CHK1/CHK2. Of note, they found that not all CD133+ cells were radioresistant, suggesting that this population is itself heterogeneous. Their results indicate that BTSC resistance to radiation-induced cell death is due at least in part to an elevated and more rapid DNA damage repair response, and that the epigenetic landscape necessary for this response to occur is intrinsic to BTSC identity.

The mechanisms underlying BTSC chemoresistance are less defined. Eramo et al. found human BTSCs to be resistant to cell death following treatment with multiple different chemotherapeutic agents in vitro (Eramo et al., 2006). In their model, chemoresistance appeared to be attributable to abnormalities in cell death pathways rather than to impaired drug uptake or enhanced drug efflux. Indeed, Liu et al. found
increased levels of expression of the DNA repair genes, MGMT and BCRP1, in CD133+ glioma cells (Liu et al., 2006). How to reconcile these findings with other data showing increased expression of drug efflux pumps in BTSCs (Bleau et al., 2009) is unclear. Regardless, these findings have tremendous import to our understanding of glioma recurrence and to our efforts to establish more effective treatment regimens for patients with this disease.

**Differentiation therapy for GBM**

Dirks has proposed that multi-potentiality is a defining element of stem-ness in glioma cells (Dirks, 2010). In vitro, BTSCs have been shown to be capable of giving rise cells resembling neurons, astrocytes, and oligodendrocytes, perhaps explaining the cellular heterogeneity once encounters in these tumours in vivo. If stem-ness confers treatment resistance to a subpopulation of glioma cells that seems responsible for disease recurrence and progression, then therapies directed against stem-cell identity may improve the efficacy of our current treatments. Effective cancer therapy may depend upon treating biologically distinct compartments within a glioblastoma that are sensitive to therapies may depend upon treating biologically distinct compartments within a glioblastoma that are sensitive to different types of therapies. It is unlikely that targeting of the BTSC subpopulation alone will lead to cancer remission. In other words, the goal for brain tumour treatment may need to be elimination or compromise of all tumour cells.

The clinical value of differentiation therapy has been best demonstrated by the use of retinoic acid in the treatment of acute promyelocytic leukemia (APL). APL is associated with a stereotypic chromosomal translocation event in which the PML gene is fused to the retinoic acid receptor α (RARα), resulting in the production of a PML-RARα chimeric protein. PML and RARα are both known to have fundamental roles in myeloid differentiation, and to have tumour-suppressor and cell-growth-suppressive activities. The PML-RARα fusion protein acts as a double dominant negative oncopgenic product, as is able to interfere with both the PML and RAR/RXR-RA pathways (Abbot et al., 1994; Dyck et al., 1994). Treatment of APL cells with RA results in inactivation of the PML–RARα fusion protein, myeloid differentiation of APL cells, and increased success rates with consolidation chemotherapy, presumably because of increased chemosensitivity of these “differentiated” cells.

Many pathways relevant to cell identity in neural stem cells—such as transforming growth-factor beta (TGF-β), leukemia inhibiting factor (LIF), sonic hedgehog (Shh), Notch and bone morphogenetic factor (BMP)—appear to have complementary roles in BTSC biology. Increased activation of the TGF-β, LIF and Shh pathways has been found to be associated with worse prognosis and increased stem-ness in patients with glioblastoma (Bruna et al., 2007; Xu et al., 2008; Penuelas et al., 2009; Anido et al., 2010; Carro et al., 2010). Inhibition of the Notch pathway by treatment of BTSCs with a γ-secretase inhibitor rendered these cells more sensitive to radiation (Wang et al., 2010) and temozolomide chemotherapy (Ulasov et al., 2011), suggesting that Notch signaling is necessary for maintenance of stem-ness in these cells. Similarly, the Vescovi group found that activation of the BMP pathway in BTSCs resulted in astrocytic differentiation and loss of tumorigenicity (Piccirillo et al., 2006). Whether these findings can be translated into patient care remains to be determined. In fact, inactivating mutations in these traditional developmental pathways may prove to be driver events in gliomagenesis, and a block in differentiation may be resistant to pathway activation, as is the case in APL in which the PML gene is lost (Wang et al., 1998; Collins, 2008) and in gliomas harboring methylation of the BMPR1b receptor (Lee et al., 2008). Regardless, adjuvant differentiation therapy could very well have a role in our future treatments of glioblastoma, and could improve the efficacy of our current therapies.

**Glioma recurrence and progression**

Recent work from the Morrison lab demonstrated that xenotransplantation might not be an appropriate proxy for tumourigenicity (Quintana et al., 2008). Using freshly dissociated human melanoma cells, Quintana et al. found much higher engraftment and tumour formation rates following transplantation into NOD/SCID Il2rg−/− rather than NOD/SCID mice. Tumour engraftment could be further enhanced by co-injection with Matrigel. Many of the cells that gave rise to tumours following transplantation in Matrigel did not possess the phenotypic characteristics of melanoma stem cells, and in fact, many of them instead resembled committed melanocytes, leading the authors to conclude that they were unable to identify any phenotypic differences to distinguish tumourigenic from non-tumourigenic melanoma cells. They postulated that the limited or absent tumourigenic potential ascribed to non-stem cancer cells is in fact an artifact of the assay system employed to measure tumourigenicity.

What relevance do these findings have for our understanding of the cancer stem cell hypothesis in glioblastoma? First, these data do show that tumourigenic potential is graded and varies between phenotypically heterogeneous cells—some melanoma cells were capable, for example, of invasion into the normal brain, rather than cells able to drive growth within the tumour mass. It seems overly optimistic to believe that only a
small population of tumour cells in a glioblastoma is
capable of driving tumour growth within this relatively
familiar environment. I would propose instead that the
cellular hierarchy in glioblastoma is associated with a
graded difference in tumourigenicity, and that the stem-
cell identity that defines BTSCs is one –and certainly
not the only– mechanism of treatment resistance in this
disease.

While CD133+ cells appear to be enriched at
glioblastoma recurrence, even recurrent tumours are
heterogeneous in nature. Does this heterogeneity reflect
a repopulation of the tumour by treatment-resistant
BTSCs? Or are the remaining cells reflective of
numerous clones –among them, a BTSC
subpopulation– that have survived treatment? If the
latter, then stem-ness is only one of many mechanisms
by which tumour cells evade radiation- and
chemotherapy- induced cell death, and studies examining genetic drift in tumour cells remain
necessary and very relevant to cancer biology.

**BTSCs and the cell-of-origin**

Numerous historical observers have speculated that
glioblastoma is a disease of neural progenitor cells.
Experimental models of brain tumours in the
developing mouse implicated known brain precursor
zones as the site of origin of brain tumours induced by
viral or chemical oncogenesis (Globus and
Kuhlenbeck, 1944; Copeland and Bigner, 1977; Vick et
al., 1977; Barnett et al., 1998; Holland et al., 2000;
Abel et al., 2009). While it is attractive as an extension
of the cancer stem cell hypothesis to postulate that
gliomas originate from mutations within the neural
stem cell compartment, the cancer stem cell hypothesis
does not actually speak to a cell-of-origin. There is
ample evidence to suggest that cells within the
progenitor compartment of the brain are more
susceptible to transformation than committed
neuroepithelial cells (Holland et al., 2000; Holland,
2001; Uhrbom et al., 2002). Using a comprehensive
murine genetic screen in which Rb, p53, and PTEN
function were abolished, Jacques et al. found that
gliomas arose only when mutations were directed to the
neural stem cell compartment and not when these same
mutations were present in mature astrocytes (Jacques et
al., 2010). In this system, the combination of driver
mutations present was relevant to the identity of the
resulting tumour, specifically in this case, whether
mutant mice developed gliomas or primitive
neuroectodermal tumours. However, the possession of
a non-committed state may not be necessary for a cell
to undergo gliomagenesis. Non-stem hematologic cells
have been shown to dedifferentiate and reacquire the
property of self-renewal as part of the transformation
process (Krivtsov et al., 2006). Further, the Weiss lab
has postulated that oligodendrogliomas arise from
transformation of oligodendrocyte precursor cells
rather than neural stem cells (Persson et al., 2010). In
breast cancer and leukemia, founder mutations in
different cell populations within the mammary tissue or
hematopoietic cell hierarchy have been found to result
in the development of divergent breast cancer subtypes
or different types of leukemia, respectively. Similarly,
may be the case that glioma grade and histology are
dependent not only upon the types of mutations that
occur during transformation but also on the identity of
the cell in which transformation initially occurs.

**Gliomas and the cancer stem cell hypothesis: next steps**

Whether the cancer stem cell hypothesis is relevant to
glioma biology is finally important because of the
human cost of our incomplete understanding of these
diseases. So far, the treatments that we have employed
for patients with glioblastoma have been relatively
nonspecific measures to relieve mass effect (i.e.
surgery) and to cause tumour cell dysfunction or death
(i.e. radiation and alkylating chemotherapy). They have
failed. While biological agents have yet to show benefit
as therapies for glioblastoma, I suspect that it is only
through biology that we are going to make meaningful
advances in the treatment of this disease.

Numerous questions remain. For example, what should
we make of the cellular heterogeneity seen in
glioblastoma? Even among BTSCs, tumours appear to have multiple BTSC populations,
each possessing different properties in vitro (Beier et
al., 2007; Chen et al., 2010). Are different cell
compartments responsible for different aspects of
tumour behavior? For example, are some cells
responsible for local growth within or just adjacent to
the tumour mass, and others responsible for distant
invasion? What is the import of non-cancerous cells –
such as endothelial cells, microglia, immune cells and
astrocytes– that are present within the tumour mass?
Are these cells reactive? Or are they recruited to the
tumour by glioma cells? How do interactions between
cancer cells and the surrounding cellular stroma effect
tumour behavior?

Second, where is the niche for glioma cells in vivo? Are
there in fact, as is the case in the bone marrow, multiple
niches? Are glioma cells within a hypoxic niche
different from those within a vascular niche? Do they
both house cancer stem cells? Are the cells residing in
these divergent niches resistant to cell death because of
divergent biological properties? Does the niche itself
provide additional protections to glioma cells that are
independent of DNA damage response?

Third, is the hierarchical relationship among glioma
cells implied by the cancer stem cell hypothesis
unidirectional in nature? In other words, can non-stem
glioma cells give rise to BTSCs? Work from the
induced pluripotency field has demonstrated that
minimal genetic changes can initiate whole-genome
programmatic changes that can reverse fate
commitment in mature cells (Hanna et al., 2010). Not
inconsequentially, many of the transcription factors that
have been found to be relevant to induced pluripotency
are mutated in cancer, and specifically in glioblastoma. In breast cancer cells, epigenetic modifications driven by extrinsic signaling cues appear capable of directing cells from a non-stem to a stem-like phenotype (Chaffer et al., 2011). Could BTSCs and non-stem glioma cells be fluid in their relationship? If so, what implications would this fluidity have for interventions directed against each of these cell compartments?

Finally, the cancer stem cell hypothesis, if it indeed explains the biology of glioblastoma beyond the clonal evolution model, should have deep implications for how we care for patients with this disease. Beyond the need to develop new therapies to target BTSCs, it is likely that delineating the biology of these cells will allow us to refine the manner in which we deliver chemotherapy and radiation. What is the appropriate treatment scheme for radiation delivery? Should chemotherapy be given as an adjunct or following its completion? We are far from fully understanding glioblastoma, and as a result, must still work to develop the foundation from which to approach its treatment.

**BTSCs and glioma: conclusion**

Whether the cancer stem cell hypothesis is the key to understanding the basic biology of glioblastoma or simply one of many formulations by which we can explain glioma cell evasion of treatment-induced cell death, it is likely that we have only begun to delineate the manner in which lessons from the stem cell field can enhance our understanding of gliomas.

**References**


Das S

Cancer stem cells in adult gliomas


Cancer stem cells in adult gliomas

Das S


This article should be referenced as such: