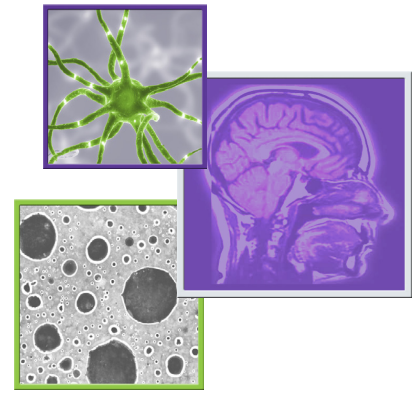
EDITORIAL



Cancer stem cells: moving past the controversy

Justin D Lathia*

"Much of the controversy around cancer stem cells is not due to their contribution to disease progression but rather observations made using suboptimal assays and the assignment of broad definitions."

It has long been recognized that tumors contain cellular heterogeneity and share histological features with developing tissues. These observations have recently been validated with functional assays to confirm the presence of a cancer stem cell (CSC) population in many advanced cancers, including glioblastoma (GBM) [1–4]. Although the CSC hypothesis remains contentious, CSCs have been well established in several advanced cancers, such as leukemia, breast and colon cancer (see the review by Visvader and Lindeman [5]), however, their existence is less clear in other cancers, including lymphoma [6]. Data identifying the glycoprotein CD133 as a putative CSC marker in GBM [3,4] have subsequently been challenged [7] and the evidence for a hierarchical organization, while indirectly implied from differentiation studies, is yet to be described using lineage-tracing approaches. Central to the controversy over the CSC hypothesis is the lack of standard functional assays, discrepancies in terminology, over-reliance on cell surface markers and plasticity between cell types. These issues will be discussed in detail below. To fully understand the contribution of CSCs to GBM progression and harness the therapeutic insight that they provide, the complexity associated with the CSC hypothesis needs to be reduced into standard definitions and assays.

Performing optimal assays

Why are these cells called CSCs? The key phenotype of normal stem cells is the capacity to self-renew and differentiate to form the tissue of interest. In the case of CSCs, this directly correlates with the capacity to recapitulate the parental tumor, including the original cellular heterogeneity found within, upon transplantation [8]. As such, the single most important assay required for the demonstration of a CSC population is tumor initiation *in vivo*. Just as embryonic and tissue-specific stem cells are defined on the basis of a function (teratoma formation, multilineage differentiation and organ reconstitution), CSCs must be validated by functional assays. The ability to grow GBM tissue as free-floating spheres does not necessarily mean the cells being studied are CSCs. In fact, long-term culture of many cell types induces selection, and over time the

"...cancer stem cells have been well established in several advanced cancers, such as leukemia, breast and colon cancer..."

*Department of Cellular & Molecular Medicine, Lerner Research Institute, Cleveland Clinic; Department of Molecular Medicine, Clinic Lerner College of Medicine of Case Western Reserve University (CCLCM); Case Comprehensive Cancer Center; Cleveland, OH, USA; lathiaj@ccf.org

Future Medicine part of fsg

population characteristics may drift away from the characteristics of the initially derived cells [9]. While it is now possible to culture patient-derived tumor tissue, these cells should not be continuously cultured and used as a replacement for serum-cultured high-passage GBM cell lines. Instead, limited *in vitro* culture and amplification of these cells via xenograft presents an opportunity to study CSCs and the development of heterogeneity. Using these approaches, in combination with rigorous *in vivo* dilution-limiting assays to demonstrate differential tumor initiation capacity between CSCs and matched non-CSCs, major advances are likely to emerge directly informing the development of more effective therapies.

Distinguishing tumor-initiating cells from CSCs

The terms used to describe self-renewing tumor cells are varied and include the following permutations: stem-like tumor cell, tumor-initiating cell, tumor-propagating cell and CSC. Among these, tumor-initiating cell and CSC are most frequently used, however, can they be used to describe the same population? The term tumor-initiating cell simply refers to the capacity for a given cell population to initiate a tumor upon transplantation. This does not necessarily mean that a CSC population is present and, in fact, while many high passage cell lines are capable of forming tumors *in vivo*, their pattern of growth does not resemble key features observed in patients (including invasion). Along with being efficient at tumor initiation, CSCs also generate cellular diversity and the tumors arising from CSCs exhibit cellular heterogeneity and a high degree of invasion. For the term CSC to be used, it must be accompanied by assays that demonstrate both tumor initiation and the generation of cellular heterogeneity.

"Central to the controversy over the cancer stem cell hypothesis is the lack of standard functional assays, discrepancies in terminology, over-reliance on cell surface markers and plasticity between cell types."

Moving beyond markers

The ability to prospectively enrich for CSCs using the expression of markers has enabled the demonstration of differential tumor formation between cell populations. However, no universal marker for CSCs in GBM has emerged, and while this has been used as an argument against the CSC hypothesis, one must consider the inherent diversity within human GBMs and the recent identification of multiple molecular subclasses [10]. At least seven markers (A2B5 [11], CD15 [12], CD44 [13], CD49f/integrin α6 [14], CD133 [3,4], EGFR [15] and L1CAM [16]) have now been demonstrated to enrich for cell populations with accompanying functional differences in tumor formation and self-renewal. While these markers are useful for the enrichment of CSCs for subsequent functional studies, they alone do not define CSCs. Marker expression is informative to understand the population being studied, but the functional capacity for self-renewal in the form of *in vivo* tumor initiation is what defines a CSC. There is no doubt additional CSC markers will be defined, but future efforts should be focused on understanding how signaling processes initiated at the cell surface impact CSC self-renewal and survival.

Plasticity & the stem cell state

The recent observation that CSCs contain a high degree of plasticity has been another argument against the CSC hypothesis. How can a CSC truly be a stem cell if non-CSCs can become CSCs? The fundamental flaw with this argument is that it is based on the stipulation that lineage commitment is a one-way process. The 2013 Nobel Prize was awarded to investigators who demonstrated that cells can be reprogrammed and in the case of induced pluripotency, oncogenes were the key factor enabling the reprogramming process. With this in mind, it should come as no surprise that key tumor microenvironmental factors, such as hypoxia [17], acidic stress [18] and nutrient availability [19], have the capacity to revert non-CSCs to functional CSCs. This in itself brings into question whether a stem cell is a fixed entity or a transition state. The capacity for reversion would support the hypothesis that CSCs represent an adaptive state and that external stimuli have the ability to move a cell from one state to another. This perspective adds complexity to both CSC regulation and cancer in general, however it provides a viewpoint that takes into account key components present within a tumor that have yet to be fully recapitulated in many model systems.

Final thoughts

The association between development and cancer has long been recognized and progress regarding the understanding of stem cell regulation has provided new insights into cancer. The CSC hypothesis does not simplify cancer but rather adds an additional layer of complexity onto our ever-evolving understanding

of this diverse class of diseases. Much of the controversy around CSCs is not due to their contribution to disease progression but rather observations made using suboptimal assays and the assignment of broad definitions. By utilizing patient-derived CSCs and interrogating their biology and therapeutic response in appropriate models, it is very likely that the next generation of therapies for many advanced cancers will emerge.

Acknowledgements

The author would like to thank J Rich and the members of his laboratory for comments on this editorial as well as for a continuing dialog that assisted in its development. The author would also like to acknowledge the members of the 2013 Glioma Invasion Forum (Bergen, Norway) for many stimulating discussions and specifically R Berkvig and C Watts for many insightful comments on this editorial.

Financial & competing interests disclosure

This work was supported by the NIH (NINDS [R01NS083629]), Pathway to Independence Award (NCI K99 CA157948), V Scholar Award from the V Foundation for Cancer Research, Voices Against Brain Cancer, Ohio Cancer Research Associates, American Cancer Society (grant via Case Comprehensive Cancer Center) and the Lerner Research Institute. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Ignatova TN, Kukekov VG, Laywell ED *et al.* Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers *in vitro*. *Glia* 39(3), 193–206 (2002).

2 Galli R, Binda E, Orfanelli U *et al.* Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res.* 64(19), 7011–7021 (2004).

3 Singh SK, Hawkins C, Clarke ID *et al.* Identification of human brain tumour initiating cells. *Nature* 432(7015), 396–401 (2004).

4 Bao S, Wu Q, McLendon RE *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444(7120), 756–760 (2006).

5 Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. *Cell Stem Cell* 10(6), 717–728 (2012).

6 Kelly PN, Dakic A, Adams JM *et al.* Tumor growth need not be driven by rare cancer stem cells. *Science* 317(5836), 337 (2007).

7 Beier D, Hau P, Proescholdt M *et al.* $CD133^+$ and $CD133^-$ glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res.* 67(9), 4010–4015 (2007).

8 Clarke MF, Dick JE, Dirks PB *et al.* Cancer stem cells – perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res.* 66(19), 9339–9344 (2006).

9 Lee J, Kotliarova S, Kotliarov Y *et al.* Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell* 9(5), 391–403 (2006).

10 Phillips HS, Kharbanda S, Chen R *et al.* Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9(3), 157–173 (2006).

11 Ogden AT, Waziri AE, Lochhead RA *et al.* Identification of $A2B5^+CD133^-$ tumor-initiating cells in adult human gliomas. *Neurosurgery* 62(2), 505–514 (2008).

12 Son MJ, Woolard K, Nam DH *et al.* SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell* 4(5), 440–452 (2009).

13 Anido J, Sáez-Borderías A, Gonzàlez-Juncà A *et al.* TGF-β receptor inhibitors target the CD44(high)/Id1(high) glioma-initiating cell population in human glioblastoma. *Cancer Cell* 18(6), 655–668 (2010).

14 Lathia JD, Gallagher J, Heddleston JM *et al.* Integrin α6 regulates glioblastoma stem cells. *Cell Stem Cell* 6(5), 421–432 (2010).

15 Mazzoleni S, Politi LS, Pala M *et al.* Epidermal growth factor receptor expression identifies functionally and molecularly distinct tumor-initiating cells in human glioblastoma multiforme and is required for gliomagenesis. *Cancer Res.* 70(19), 7500–7513 (2010).

16 Bao S, Wu Q, Li Z *et al.* Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res.* 68(15), 6043–6048 (2008).

17 Heddleston JM, Li Z, McLendon RE *et al.* The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle* 8(20), 3274–3284 (2009).

18 Hjelmeland AB, Wu Q, Heddleston JM *et al.* Acidic stress promotes a glioma stem cell phenotype. *Cell Death Differ.* 18(5), 829–840 (2011).

19 Flavahan WA, Wu Q, Hitomi M *et al.* Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. *Nat. Neurosci.* doi:10.1038/nn.3510 (2013) (Epub ahead of print).