Brain Tumours and Stem Cells: Similarities and Challenges

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Cancers are composed of heterogeneous cell populations ranging from highly proliferative immature cells to more differentiated cells of various cell lineages. Recent advances in stem cell research have demonstrated the existence of cancer stem cells in non-solid and solid tumours, such as those of the brain: glioblastoma multiforme (GBM), medulloblastoma (MDB) and ependymoma (EPM). These cells are defined as 'cancer stem cells', because they show some similarities with their normal counterpart in the corresponding organs, i.e. they are undifferentiated, self-sustaining transformed cells. In particular, glioblastoma-stem like cells (GBSCs) self-renew under clonal conditions, and differentiate into neuron- and glia-like cells, with aberrant, mixed neuronal/astroglial phenotypes. Following injection into immuno-deficient mice, these GBSCs are able to form secondary tumours that closely resemble the human pathology and retain their tumorigenic potential, even across serial transplantation. Many groups are working on identification of markers and molecular mechanisms that underpin the tumorigenic potential of these cells, particularly with the aim of defining new therapeutic approaches for the treatment of malignant brain tumours.

Keywords: central nervous system, cancer stem cells, glioblastoma multiforme, tumorigenicity

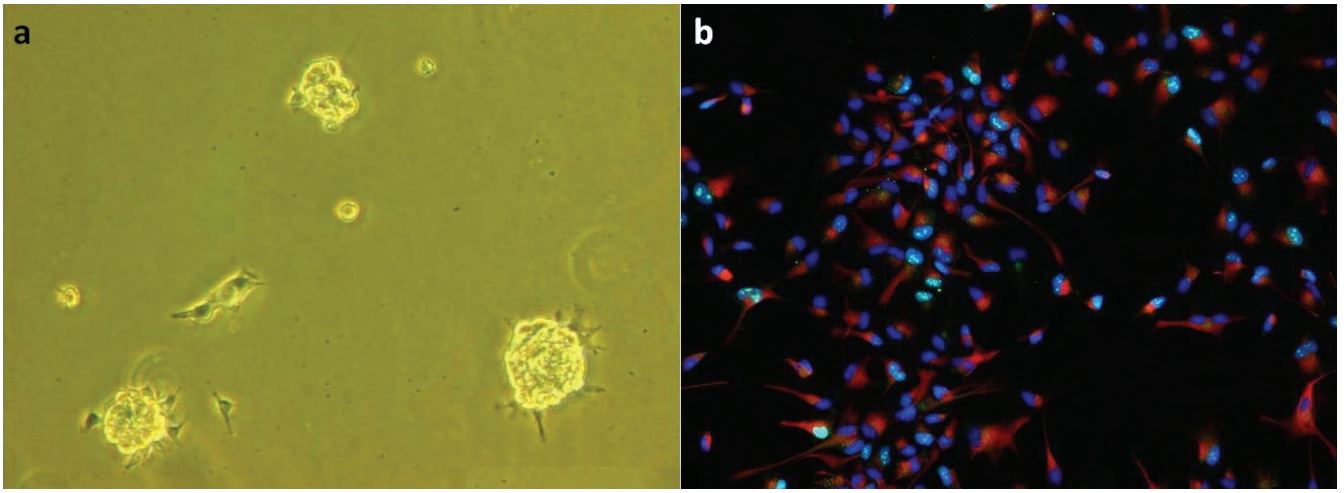
Introduction

Cancers are thought to derive from a single mutated cell that initiates malignant transformation. Cell division facilitates the transformation process because the cell progeny progressively accumulate additional mutations which lead to the development of a full neoplastic phenotype [1]. Somatic stem cells are undifferentiated cells which persist through adulthood and are endowed with self-renewal ability, long-term replication potential and multi-lineage differentiation. Although their properties are tightly controlled, it has been proposed that, when altered, the mechanisms of stem cell maintenance can contribute to tumorigenesis. In fact, many pathways that are associated with cancer, such as the Bmi-1, Notch, Hh and Wnt pathways, are also implicated in stem cell development [2-5]. In leukaemia and multiple myeloma, it was found that a small subset of cancer cells was capable of long-term proliferation and tumour-founding ability and this population was defined as cancer stem cells [6]. It is important to emphasise that the adoption of this term does not imply cancer stem cells directly derive from the transformation of normal stem cells but, rather, underlines a situation in which the cancer cell has acquired stem-like properties. In particular, it has yet to be convincingly demonstrated whether cancer stem cells are normal stem cells that have undergone transformation or differentiated cells that have become de-differentiated and stem-like [3,4]. This latter hypothesis has recently been supported for brain tumours by a study showing that inactivation of specific tumour suppressors p53, Nf1 and Pten in neural stem/progenitor cells is both necessary and sufficient to induce astrocytoma formation in a somatic tumour suppressor mouse model [7].

Brain cancer stem cells

Starting from the initial findings in leukaemia and multiple myeloma, the cancer stem cell hypothesis has been extended to several solid tumours (breast,

Figure 1. a. GBSCs grow as neurospheres in neural stem cell medium. b. Immunofluorescent detection of the neurodevelopmental antigen Nestin (red) was used to characterise GBSCs. Ki67 (green) was used to detect proliferating cells.



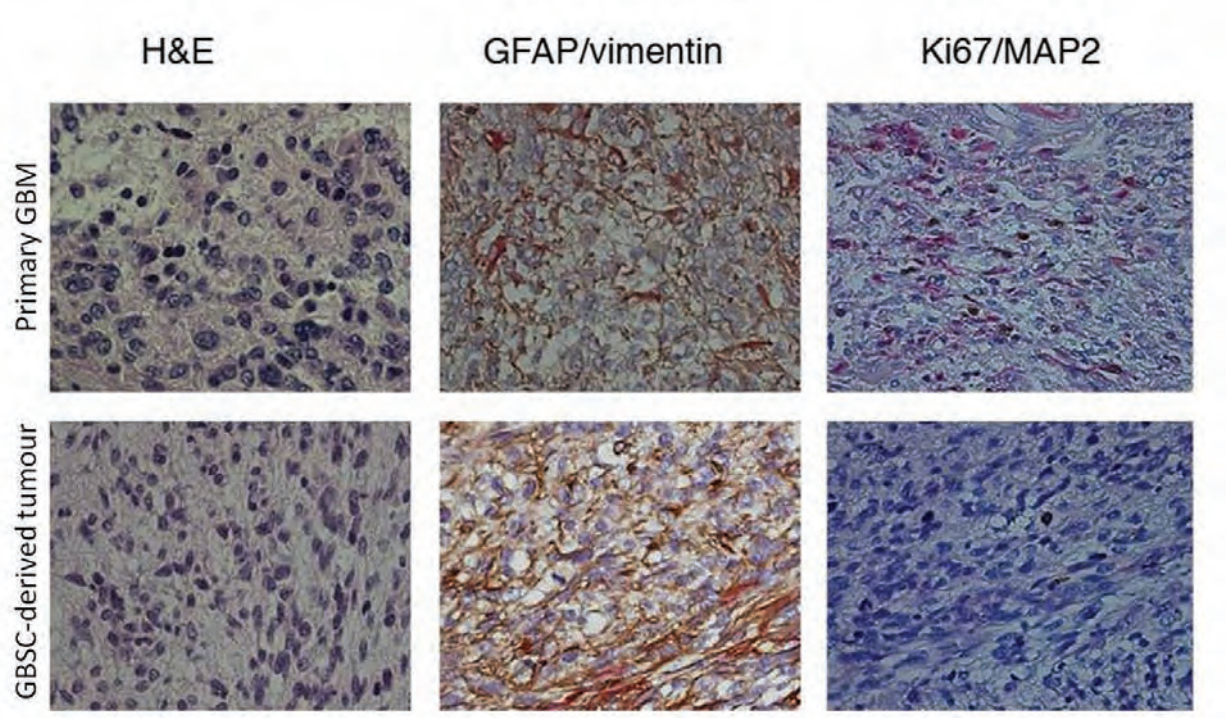


Figure 2. Hematoxylin and eosin staining, anti-GFAP/-vimentin and anti-Ki67-/mMAP2 immuno-histochemistry (brown/purple colour, respectively for each immuno-histochemistry) on the primary surgery specimen and on GBSC-derived tumours in mice brain show comparable morphology, GFAP immunoreactivity, mitotic index and nuclear atypia in the two samples. Adapted from Piccirillo S.G.M. et al., 2009.

brain, prostate, ovary, colon, skin, lung) (reviewed in Piccirillo, 2007[8]). In particular, for brain neoplasia, the most convincing and frequent results come from studies on GBMs [9-12] (Figure 1). When implanted intracranially, GBSCs form highly invasive tumours that are histologically identical to GBMs [12,13] (Figure 2). GBSCs have subsequently been shown to resemble more closely glioblastomas, genotypically and phenotypically, than do traditionally cultured GBM cell lines and were also found to be very similar to normal neural stem cells (NSCs) [13]. There are, however, essential differences between these cell types: the gene expression profile of GBSCs does not match the pattern of NSCs [14], their propagation rate in vitro and in vivo is higher, they show a partial mitogen independence [15] and their tumorigenicity does not correlate with expression of specific NSC markers [16].

Brain cancer stem cells as therapeutic targets

Glioblastomas are highly invasive, malignant lesions and by the time of diagnosis glioblastoma cells have already migrated great distances from the primary tumour; it has also been proposed that these highly migratory cells are, in fact, GBSCs [17]. Surgical resection, while effective in removing the primary lesion, cannot remove all of the micro-deposits seeded by the migrating glioblastoma cells. Genetic, mutational, and proteomic profiling of these glioblastoma stem cells will provide therapeutic targets unique to this small subpopulation. Very recently, it has been shown that more than one GBSC type resides in human GBM [18]. Notably, this represents an unprecedented model by which it will be possible to investigate, by differential screening assays, the mutations that underpin malignancy. Furthermore, since these cells derive from the same surgical sample, this will be feasible while avoiding the issue of the "background noise" usually determined in these kind of studies by the use of cells derived from different patients, ages and sites and by tumour heterogeneity.

GBSCs have been shown to exhibit increased chemoresistance [19,20] and radioresistance [21], thus indicating that a more targeted, multi-pronged approach is needed. To that end, gene expression profiling and proteomic analysis will be instrumental in identifying targets unique to GBSCs that can then be used to screen various libraries of therapeutic molecules. Recently it has been shown that treatment of GBSCs with cyclopamine, a blocking agent of the Hh pathway, and the bone morphogenetic protein BMP4, which induces the Smad signaling cascade and thus causes terminal differentiation, results in the inhibition of the tumorigenicity in vivo [22,23]. More inhibitors need to be identified in order to maximise the dream of identifying chemotherapeutic agents with few side effects for the patient, while counteracting tumour progression and recurrence.

Conclusions

The discovery that brain tumours, in particular GBM, contain cancer stem cells presents us with new opportunities to develop innovative therapeutic approaches. Unfortunately, to date, the identity of the normal cells that acquire the first genetic 'hits' that lead to tumour initiation has remained elusive. For this reason, the term 'cancer stem cell' is a term defining a cancer cell that has the ability to self-renew, dividing to give rise to another malignant stem cell and a cell that gives rise to the phenotypically diverse tumour cell population.

It is certainly reasonable to argue that tumours of the same family may also share common features at the level of cancer stem cell populations. This could facilitate diagnosis and the development of novel generic therapeutic strategies. However, in many cases cancer stem cells from different

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patients bear distinctive molecular genetic characteristics. This suggests that the development of more specific, individualised approaches to patients may be a necessary prerequisite to successfully treating this disease [18]. The challenge will be to identify ways of rapidly identifying and interrogating this cell population to uncover specific targets and screen libraries of small molecular therapeutic agents and collate this data into a tailor-made treatment plan for each patient. A parallel challenge will be to pinpoint the differences between cancer stem cells and normal stem cells to avoid indiscriminate injury on non-pathological tissues, such as is imposed by current therapies. ■

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