Review

Neuro-oncogenesis and the adult human sub-ventricular zone in high grade glioma

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ABSTRACT

The last fifteen years have seen the application of the cancer stem cell hypothesis to tumors of the central nervous system, in particular to high grade glioma (HGG), the most aggressive and common brain cancer in adults. Seminal studies have shown that cancer stem cells (alternatively named tumor-initiating cells) are capable of self-renew and multipotency, similar to their normal counterpart. More importantly they give rise to tumors that closely mimic the phenotype and genotype of human HGG. The identification of neurogenic niches in adult rodent and human brain has further reinforced the hypothesis that HGG might derive from the malignant transformation occurring in these areas, especially in the sub-ventricular zone (SVZ), the largest and most well characterised stem cell niche. Following from evidence of animal model studies supporting this hypothesis, recently we investigated the role of the SVZ in neuro-oncogenesis using tissue material derived from HGG patients. We also described response to conventional chemo-therapies of cancer stem cells isolated from the SVZ and the tumor mass (T) of the same patients and reconstructed tumor evolution. In this review, such findings will be discussed in the context of the current literature on the biology of the SVZ in the normal and disease brain.

Key words: High grade glioma; tumor-initiating cells; sub-ventricular zone; tumor development

INTRODUCTION

High grade glioma (HGG) are aggressive and lethal brain tumors whose prognosis remains dismal despite advances in neurosurgical techniques and combination of radio- and chemo-therapy. The recent years have seen two major directions of investigation: firstly, the evidence from stem cell biology showing that cancer stem-like populations exist in HGG and other brain tumors and secondly, the application of high-resolution genomics to study HGG genetic heterogeneity. However, the existence of cancer stem cells in tumors does not prove *per se* that the disease originates from normal stem cells.

In the brain, the sub-ventricular zone (SVZ) is a germinal niche where neurogenesis persists throughout adulthood. In the last twenty years, seminal studies have described the cellular organisation and functional properties of this niche, mainly composed of neural stem, precursor cells and migrating neuroblasts. Given

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the capacity of stem cells to self-renew and generate differentiating cells while also maintaining their pool, it has been proposed that SVZ stem cells could play a role in tumorigenesis. This hypothesis has been supported by studies using genetically-engineered animal models where the key genetic alterations of HGG occur only in neural stem/precursor cells of the SVZ.

The advent of high-resolution genomic techniques gave us the unique opportunity to overcome the challenges associated with studies in the human brain of HGG where only a small amount of tumor tissue is available and longitudinal studies to assess tumor development are not possible. We developed a real-time fluorescence-guidedtiple-sampling (FGMS) strategy based on 5-aminolevulinic acid to identify cancer stem cells in different tumor regions[1] and we used this approach to describe the extent of spatial genetic intra- tumor heterogeneity in HGG[1,2] and to reconstruct tumorigenesis.[2] In parallel, we derived cancer stem cells from the tumor mass and the SVZ of the same patients and we showed that drug-resistant cells are present in this niche.[3] These findings have implications for the development of new therapeutic approaches targeting the SVZ.

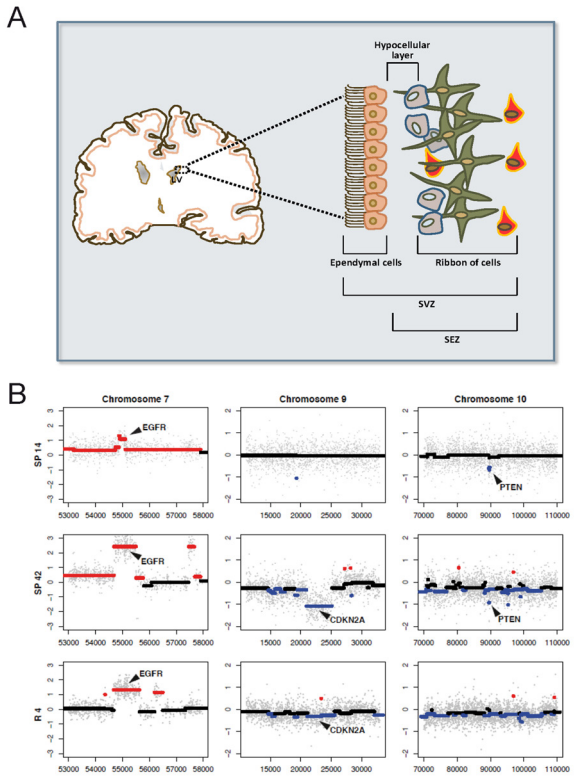

Figure 1: The anatomy of SVZ and the genetic alterations in HGG patients. A: Anatomical structure of the human brain SEZ and SVZ; B: copy number aberrations of three SVZ samples from different patients share hallmarks of HGG such as amplification of EGFR and loss/deletion of PTEN. SP42 and R4 display also loss of CDKN2A, another hallmark of the disease. SP14 also exhibits amplification of AKT3 whereas SP42 shows amplification of CDK6 and MET as well. This clearly confirms the aberrant nature of SVZ cells. HGG: high grade glioma; SEZ: sub-ependymal zone; SVZ: sub-ventricular zone

THE SVZ IN THE ADULT HUMAN BRAIN

The identification of neurogenic niches in rodents[4] has challenged the long-standing notion that the mammalian brain was a quiescent organ characterized by lack of neurogenesis postnatally.[5] In the adult mammalian brain, neurogenesis occurs in 2 germinal regions: the SVZ[6] and the subgranular layer (SGL) of the dentate gyrus of the hippocampus.[7] Several works on the cellular organisation of the SVZ in rodents have revealed the existence of neural stem cells that express the astrocytic marker glial fibrillary acidic protein (GFAP) and give rise to neurons. When compared to the SGL, the SVZ represents the most abundant source of neurons.[8-11] More recently, studies on the adult human brain have shown that the SVZ retains the same functional properties of the rodent brain, but the GFAP+ve cells are organised in a ribbon.[12,13] However, important differences exist between the human and rodent SVZ: (1) in humans, the SVZ is positioned in the wall of the lateral ventricles and is characterized by 4 layers. SVZ astrocytes are organised in ribbons separated from the ependymal layer by a hypocellular gap, that is a reminiscence of the neuronal formation and migration occurring at embryonic stages[14] [Figure 1]. Interestingly, the terms SVZ and SEZ have been used interchangeably, however they describe specifically these layers with the inclusion or not of the ependymal layer [Figure 1]; (2) the number of actively proliferating cells in human SVZ is very low in comparison to rodents;[12,15] and (3) the evidence of the existence of neural stem cells *in vivo* is still missing in humans, whereas it is well established in rodents.

Accumulating evidence points out to the influence of pathological conditions on neurogenesis. These include infections, inflammations, stroke, epilepsy, tumors and neurodegenerative disorders.[16,17] For instance, in Huntington's disease an increase in cell proliferation and neurogenesis occur in the SVZ of disease brains.[18] Extending our understanding of the biology of the human SVZ might lead to the identification of novel therapeutic interventions against the large spectrum of diseases affecting the brain.

THE SVZ AS INFLAMMATORY RESERVOIR

In HGG, the onset of malignant transformation can be seen as a traumatic event that can initiate inflammation. This can then persist during the subsequent phases of tumor growth: promotion and progression.[19] Inflammatory cells, particularly tumor-associated macrophages and microglia, are abundant in HGG and pro-inflammatory genes are overexpressed in the tumor core.[20,21] Most importantly, in HGG inflammation promotes radioresistance.[22] However, so far no study

responsible for tumor re-initiation following chemo-therapy.[54] In support of these findings it was also noted that p53 mutations preferentially occur in the SVZ.[55]

Collectively, these results raise the question on whether cancer stem cells directly derive from SVZ stem cells. Although mouse model studies have indicated that this is the case, these findings have been severely hampered by a limited representation of the aberrant genetic landscape of HGG and the use of markers that poorly discriminate between stem cells and precursor cells.[32] More recently, the same question has been addressed by using a transgenic cell-labelling system known as mosaic analysis with double markers.[56] Using this model, it has been proposed that the cells of origin in HGG are oligodendrocyte precursor cells, thus challenging the notion that HGG may originate from transformation and expansion of the neural stem cell pool.

Although the debate about the cell of origin in HGG is still open, the above studies have helped define the potential targets of malignant transformation that

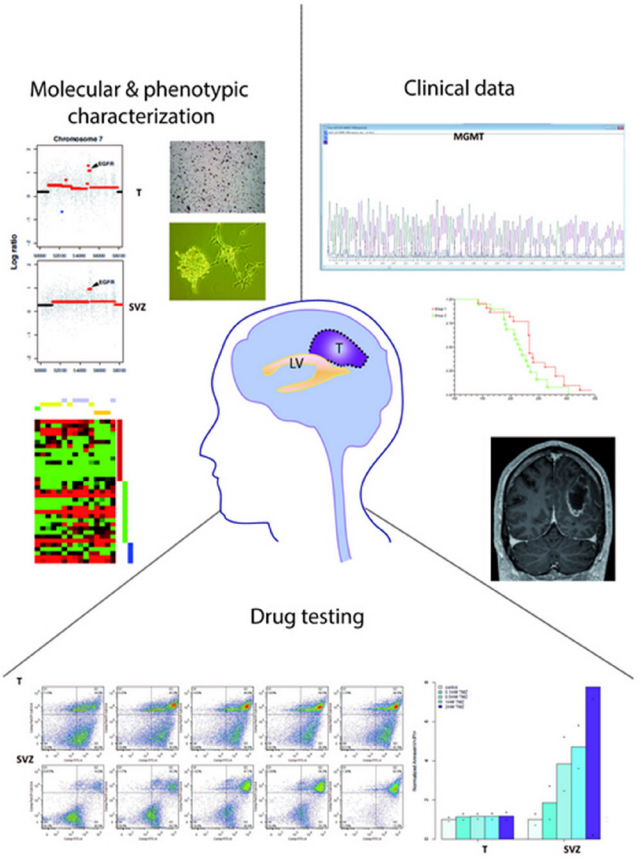

Figure 2: Drug treatments of cancer stem cells derived from SVZ and T combined with molecular and phenotypic characterization of corresponding tissues can help classify HGG patients and develop personalized therapeutic approaches. A better understanding of drug resistance can be achieved by a systematic comparison of drug screening analyses between cancer stem cells isolated from the SVZ and T of the same HGG patient. This will be integrated with molecular profiling of the matched SVZ and T tissues, clinical data and the phenotypic characterization of the derived cells. This approach has the potential to impact on clinical decisions as the molecular/phenotypic characterization and phylogenetic reconstruction may allow a personalized therapeutic approach based on a better understanding of tumor heterogeneity and potentially may lead to the identification of novel targets in the SVZ and in the T. LV: lateral ventricle; T: tumor mass; HGG: high grade glioma; SVZ: sub-ventricular zone

can be further investigated to elucidate the process of oncogenesis in HGG patients. The limited availability of tissue samples and the clinical complex scenario at the time of surgery make it difficult to reconstruct the initial steps of tumor development and alternative methods are needed. Given the critical functional role of the SVZ in the adult human brain, it has been speculated that this niche might play a role in neuro-oncogenesis. This has been the focus of our recent study on HGG patients.[3]

THE SVZ AS A SOURCE OF TUMOR CELLS IN HGG PATIENTS

The identification of cancer stem cells from human HGG has represented a novel tool to develop therapeutic strategies[57,58] and these cells have been proposed as a model that more closely represents the human disease.[59] We took advantage of these findings to objectively interrogate primary HGG in humans using a neurosurgical techniques based on FGMS. In the clinic fluorescence-guided resection has resulted in enhanced cytoreduction and improved progression-free survival in patients in a randomized Phase III trial.[60] We have adapted this technology to allow the objective identification of tumor tissue based on combining fluorescence emission and neuroanatomical landmarks and we have recently demonstrated that this technique can be successfully employed to characterize cancer stem cells derived from fluorescent and non-fluorescent material in HGG patients.[1]

Quite unexpectedly, we observed for the first time that fluorescent material is present in the SVZ of 42 out of 65 HGG patients who underwent surgery using fluorescence-guided resection and we isolated tissue from the tumor mass and the SVZ. Using these samples we reported that the SVZ contains malignant cells that contribute to tumor growth.[3] This has never been demonstrated in humans, but similar observations have been reported in mouse models of HGG.[46,53-55,61]

Importantly, the phylogenetic relationship between SVZ and tumor in these patients identifies the SVZ as a reservoir of tumor cells (either early tumor clones or late-emergent clones that develop during HGG growth) that need to be therapeutically targeted. Thus, we investigated responses to chemo-therapeutic agents using cancer stem cells from SVZ and T of the same patients. Surprisingly, we found that such cells respond differently to therapies, which represent the standard of care for HGG patients. Our data also suggest that a large fraction of cells is resistant to chemo-therapy even at supra-maximal doses[3] providing a possible explanation for the treatment failure seen in HGG patients.