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The Role of Stem Cells in the Glioma Growth

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1. Introduction

Malignant glioma is the most common type of primary brain tumor and represents one of the most lethal cancers. In contrast to the long-standing and well-defined histopathology, the underlying molecular and genetic bases for gliomas are less known. (Collins, 2004; Dai & Holland, 2001).

As some other human cancers, particularly central nervous tumors are highly heterogeneous. Primarily because of its diffuse nature, relatively little is known about the processes by which they develop (Hulleman & Helin, 2005). Thus, the traditional evolution concept of tumors arising from a single mutated cell has limitations in explaining the heterogeneity observed in a single tumor nest.

Recent decades have seen only limited progress in treatment trials and basic research on human glioma, the most common central nervous malignancy (Huang et al., 2008). Unfortunately, for such gliomas, tumor recurrence after treatment is the rule due to the infiltrative nature of these tumors and the presence of cellular populations with ability to escape therapies and drive tumor recurrence and progression. At least in some cases, these resistant cells exhibit stem cell properties (Frosina, 2011). For these reasons the comprehension of the current knowledge of cancer stem cells (CSC) in relation to gliomas origin, growth and treatment is crucial. As the stem cells (for glioma, neuronal stem cells) are more susceptible to mutation, they become altered easily for their genetic composition and therefore act as the source of cancer/glioma cells. They are not actually a separate cell type and in most cases they are misinterpreted as cancer stem cells (in brain, they are glioma stem cells).

2. Glioma and the concept of cancer stem cells

For a long time it has been known that there are subpopulations of cells within solid tumors that contain different biological behaviors. Among these subpopulations, accumulating evidence supports the existence of the so-called cancer stem cells (CSCs), because these tumor cells possess stem cell properties, possibly being responsible for the initiation, growth and recurrence of tumors. Apparent similarities with non-transformed stem cells, including high self-renewal capacity and the ability to generate differentiated progeny of several cellular lineages, have led to the proposal that stem cell-like cancer cells may either originate from adult undifferentiated stem and progenitor cells or that these properties are being

expressed as an effect of the genetic alterations which drive tumorigenicity (Reya et al., 2001; Gilbertson, 2006; Das et al., 2008). Basically, the CSCs, which have also been described as tumor initiating cells or tumor propagating cells, are tumor cells that self-renew and propagate tumors phenotypically similar to the parental tumor (Li et al., 2009). Furthermore, recent studies have suggested that CSC cause tumor recurrence based on their resistance to radiotherapy and chemotherapy (Inoue et al, 2010).

Although considerable controversy still surrounds the existence, behaviors and even the nomenclature of CSCs, there is no doubt that populations of cells with stem-like properties do exist inside several solid and non-solid tumors, including brain cancers. So, despite the fact that CSCs in solid tumors have not yet been precisely identified, the "CSC hypothesis" opens a new paradigm in understanding the biology of cancers. For this reason, the search for the tumor stem cells that may originate and perpetuate the tumor growth has been receiving great attention in the literature (Sanchez-Martin, 2008), but the available knowledge on this issue with regards to the gliomas is scant. Particularly, the exact identity and cell(s) of origin of the so-called glioma stem cell remains elusive (Park & Rich, 2009). Vescovi (2006) offered a functional definition of brain tumor stem cells, namely: brain tumor cells should qualify as stem cells if they show cancer-initiating ability upon orthotopic implantation, extensive self-renewal ability demonstrated either ex vivo or in vivo, karyotypic or genetic alterations, aberrant differentiation properties, capacity to generate non-tumorigenic end cells, and multilineage differentiation capacity. Furthermore, parallels between normal neurogenesis and brain tumorigenesis have been proposed (Singh et al., 2004). It has been more recently confirmed that cancer stem cells from glioblastomas share some characteristics with normal neural stem cells including the expression of neural stem cell markers, the capacity for self-renewal and long term proliferation, the formation of neurospheres, and the ability to differentiate into multiple nervous system lineages (astrocytic, oligodendrocytic and/or neuronal differentiation) (Li et al., 2009).

Among the so far evaluated stem cell markers, the transmembrane protein CD133 has been widely used to isolate putative CSC populations in several cancer types. In fact, CD133 is currently one of the best markers to characterize CSCs (Singh et al., 2004). In both human glioblastomas (GBMs) and medulloblastomas, the expression of the neural stem cell marker CD133 (also known as prominin 1) has been associated with both tumor initiation capacity and radioresistance (Pérez Castillo et al., 2008). Extensive computational comparisons with a compendium of published gene expression profiles revealed that the CD133 gene signature transcriptionally resembles human embryonic stem cells and in vitro cultured GBMs stem cells (GSC), and this signature successfully distinguishes GBMs from lower-grade gliomas. Moreover, the CD133 gene signature identifies an aggressive subtype of GBMs seen in younger patients with a shorter survival (Yan et al., 2011), confirming previous observations that Glioma stem cells are more aggressive in recurrent tumors (Huang et al., 2008). Nevertheless, it must be pointed out that the use of CD133 as a unique glioma stem cell marker is probably not sufficient to tag the whole self-renewing tumor cell reservoir (Clément et al., 2009).

Holmberg et al (2011) have recently characterized human gliomas in various malignancy grades according to the expression of stem cell regulatory proteins. These authors have shown that cells in high grade glioma co-express an array of markers defining neural stem cells (NSCs) and that these proteins can fulfill similar functions in tumor cells as in NSCs. In contrast to NSCs, the glioma cells co-express neural proteins together with pluripotent stem

cell markers, including the transcription factors as Oct4, Sox2, Nanog and Klf4. In line with these findings, in high grade gliomas, mesodermal- and endodermal-specific transcription factors were detected together with neural proteins, a combination of lineage markers not normally present in the central nervous system. These findings demonstrate a general deregulated expression of neural and pluripotent stem cell traits in malignant human gliomas.

3. Stem cells and the origin of gliomas

Primarily because of the diffuse nature of gliomas, relatively little is known about the processes by which they develop (Hulleman & Helin, 2005). The concept of stem cells originating gliomas is gaining increased recognition in neuro-oncology (Richj & Eyler, 2008). Until recently, the paradigm of a tumor-initiating stem cell was confined to hematopoietic malignancies where the hierarchical lineages of stem progenitor cells are well established. Nevertheless, the demonstration of persistent stem cells and cycling progenitors in the adult brain is coupled with the expansion of the cancer stem cell concept to solid tumors, leading to the exploration of "stemness" within gliomas. Emerging data are highly suggestive of the subsistence of transformed multipotential cells within a glioma, with a subfraction of cells exhibiting increased efficiency at tumor initiation stage. However, data in support of the true glioma stem cells are inconclusive to date, particularly in respect to the functional characterization of these cells. (Panagiotakos & Tabar, 2007).

Thus, it may be considered that currently it is conceivable thought that malignant gliomas may arise from neural stem cells and appear to contain tumor stem cells. It is thought that normal stem cells live in protected pockets of the body called *niches*, where they divide infrequently to avoid accumulating damaging mutations. Upon injury or in response to normal stimuli, stem cells are mobilized to divide (Gilbertson, 2006). Hence, parallel to the role that normal stem cells play in organogenesis, stem cells are thought to be crucial for tumorigenesis.

The normal adult neural stem cells (NSCs) arise from radial glia (RG) within the central nervous system (Weiner**,** 2008). The RG progeny includes all the main lineages of the CNS: neurons, astrocytes, oligodendrocytes, ependymocytes and adult neural stem cells (Malatesta et al. 2003). By comparing the gene expression profiles of ependymomas with those of cells in the normal developing nervous system, it was possible to identify the RG as candidate stem cells of this brain tumor (Gilbertson, 2006). Furthermore, RG cells produce neurons in addition to glia during central nervous system development in all vertebrates and are also involved in reparative process (Weiner, 2008).

Until recently, it was thought that ependymomas originated from neuroepithelial cells, glioblastomas from abnormal astrocytes, and medulloblastomas from primitive cells in the external granular layer, but there is now evidence that all tumors can originate from a special type of stem cell called "radial glial cell" (RGC). It is interesting to note that in the human brain, most of stem cells are located in the subventricular zones (SVZ). Both supra- and infratentorially and when stimulated with carcinogens, cells in the SVZ become tumorigenic faster than those located elsewhere. In the SVZ, stem cells exist in the form of RGCs, which remain quiescent until they receive transformational signals. It is not clear whether RGCs, after receiving transformation signals, return to their initial stem cell configuration and then become tumorigenic or they transform to tumor progenitor cells

directly. In the cerebellum, depending upon the signals received, RGCs and stem cells may give origin to either ependymoma or medulloblastoma.
Tumours with the highest incidence in humans— medulloblastomas and glioblastomas — both originate from abnormal brain stem cells. . Not surprising, both of these tumors are CD133-positive, containing great neuronal differentiation, which makes them prone to be diffuse and resistant to treatment (Castilo, 2010).

4. Gliomas and the field cancerization concept

It is universally accepted that tumors growth as a clonal evolution from a single cell (Nowell, 1976). The "field cancerization theory" was introduced more than fifty years ago by Slaugher et al (1953), when studying the presence of histologically abnormal tissue surrounding carcinomas. In a classic report on oral cancer, Slaughter called "field cancerization" – a process of repeated exposure of a region's entire tissue area to carcinogenic insult (e.g., tobacco and alcohol), which increases the tissue's risk for developing multiple independent premalignant and malignant foci. The field cancerization hypothesis states that multiple cells form independent tumors on one given tissue, since carcinogenic exposure affects multiple cells in the field (Slaughter *et al*., 1953), and predicts that second primary or synchronous tumours arise from independent genetic events (Garcia et al., 1999). The field cancerization theory may be explained by the concept that a given stem cell that acquires genetic alterations may form a "patch", a clonal unit of altered daughter cells. The proliferation of these patch cells forms expanding fields which gradually displace the normal tissue and, by clonal divergence, ultimately leads to the development of one or more tumors within a contiguous field of preneoplastic cells (Garcia et al., 1999). An important clinical implication is that fields often remain after surgery of the primary tumor and may lead to new cancers, designated presently by clinicians as "a second primary tumor" or "local recurrence," depending on the exact site and time interval (Braakhuis et al., 2003; Ryan, 2007). We had previously discussed how mutated clones from mutated stem cells may spread on tissues and that the field cancerization theory implies that the mutated genotype and molecular changes occur before the appearance of histopathological evidence of malignant cells (Garcia et al., 1999). Therefore, this "anomaly" might be due to changes that occur in a "premalignant" neoplastic condition that was histologically identified as "normal". In the clinical aspect, the field cancerization may have an etiologic role in a substantial number of recurrences. For example, a surgical resection margin that includes a genetically altered field can explain the occurrence of scar recurrence. This explanation suggests that molecular profiling of surgical margins will help reduce scar recurrences. Since multiple independent patches of cancer fields may be present in the same organ exposed to the same insults, clean molecular margins may not necessarily prevent recurrences in the residual organ (Dakubo et al., 2007). Similarly to gliomas, tumor recurrence is a major clinical concern for patients with urothelial carcinoma of the urinary bladder. Traditional morphological analysis is of limited utility for identifying cases in which recurrence will occur. However, recent studies have suggested that urothelial carcinogenesis occurs as a 'field effect' that can involve any number of sites in the bladder mucosa. Accumulating evidence supports the notion that resident urothelial stem cells in the affected field are transformed into cancer stem cells by acquiring genetic alterations that lead to tumor formation through clonal expansion (Cheng et al., 2009).

The available information in regards to the existence of a field phenomenon in gliomas is scant. In malignant gliomas, the high recurrence rates, the characteristically heterogeneous features and frequent diffuse spread within the brain have raised the question of whether malignant gliomas arise monoclonally from a single precursor cell or polyclonally from multiple transformed cells forming confluent clones (Inoue et al., 2008). To address this issue, Kattar et al (1997) have evaluated the clonality of low-grade and malignant gliomas by using polymerase chain reaction (PCR)-based assay for nonrandom X chromosome inactivation using surgical and autopsy material. The same pattern of nonrandom X chromosome inactivation was present in all areas of fifteen of 19 tumors, which were considered as monoclonal, suggesting that low-grade and malignant gliomas are, at least, usually monoclonal tumors, and extensively infiltrating tumors must result from migration of tumor cells.

Gliomatosis cerebri may shed some light in this issue. It is a rare condition in which the brain is infiltrated by an exceptionally diffusely growing of malignant glial cell population involving at least 2 lobes, though often more extensive, sometimes even affecting infratentorial regions. Kross et al (2002) have evaluated the existence of field cancerization in this affection, since *gliomatosis cerebri* may initiate as an oligoclonal process or result from collision of different gliomas. It was hypothesized that the presence of an identical set of genetic aberrations throughout the lesion would point to monoclonality of the process. In contrast, the finding of non-identical genetic changes in widely separated regions within the neoplasm would support the concept of collision of different mutated clones. For such, the authors used one autopsy case of *gliomatosis cerebri*, from which tissue samples were randomly taken from 24 locations throughout the brain and used for genetic investigation. With this aim, genome-wide screening for chromosomal aberrations was accomplished by comparative genomic hybridization (CGH). The authors found a wide distribution of particular sets of genetic aberrations, supporting the concept of monoclonal tumor proliferation (Kross et al., 2002). Nevertheless, it has been observed and well documented in one clinical case that on the long term, after initial treatment for *gliomatosis cerebri*, one glioblastoma multiforme has developed, and in a location separate from the initial lesion, suggesting that different clonal origin may had occurred (Inoue et al., 2008). More recently, Chen et al (2010) showed that the capacities for self-renewal and tumour initiation in GBM need not be restricted to a uniform population of stemlike cells.

5. The contribution of studies in animal models: Unifying the cancer stem cells and field cancerization concepts

Many genetic alterations have been identified in human gliomas, however, establishing unequivocal correlation between these genetic alterations and gliomagenesis requires accurate animal models for these cancers (Dai & Holland, 2001). Indeed, it is useful and necessary to have animal models for CNS tumors studies allowing to be carried out in different stages of tumor growth, especially in early stages, rare to be detected and observed in clinical practice (Bulnes-Sesma, 2006).

Experimental models of gliomagenesis most commonly used alkylating agents such as N-ethyl N-nitrosourea (ENU), which has been considered as a suitable model to study malignant changes. These changes were reported to appear firstly as early neoplastic proliferation (ENP) center, which continues in following stages subsequently progressing to ''microtumors'' until a tumor in itself. (Koestner et al., 1971; Naito et al., 1984).

By using the experimental model of gliomagenesis induced by the N-ethyl N-nitrosourea, we were able to detect putative tumor stem cells in early oncogenesis, yielding to analyze a field cancerization process and observe a close morphological relationship between metallothionein (MT) positive cells and blood vessels. With this aim, we have developed an experimental model to track putative mutated stem cells, using the ENU experimental model and metallothioneins (MT) immunostaining. MTs are metal binding proteins that take part in the homeostasis of the ions of the metals which are necessary for the proper metabolism of the organism (zinc, copper), disintoxication of metals and protect the tissues from the effects of free radicals, radiation and from mutagens (Thirumoorthy et al., 2007). MT expression is present in a significant portion of especially malignant brain tumors. In astrocytic tumors an acquired enhanced ability to produce MT has been observed as the malignant potential of a tumor increases (Hiura et al., 1998), and MT might be involved in poor response to antineoplastic drugs (Maier et al., 1997). In the murine colonic mucosa, the crypt restricted immunopositivity for MT has been shown to be reliable marker of stem cell mutation that may be induced early after mutagen treatment and that can be assayed in paraffin-fixed tissue sections (Cook et al., 2000). We have observed that 30 days after the treatment of rats with ENU, the main location of the MT positive cells have striking similarity to that of the RG cells and that the frequency of these cells (a) is strongly correlated with the increased appearing of ENP centers and new blood vessels, (b) is augmented at higher levels in long-term observation, i.e., 180 days after the carcinogen administration, (c) is related to a high staining intensity in both nucleus and cytoplasm, and (d) is very similar to the pattern of immunostaining that was observed in the nervous tissue surrounding gliomas, which were originated at an average of 321 days after the ENU administration (Fernandes-da-Silva et al., 2009). The mechanisms and reasons why MT is expressed in the preneoplastic and neoplastic lesions remain to be fully elucidated. It has been hypothesized that mutation-induced MT overexpression may interfere with the function of zinc finger DNA binding transcription factors (Zeng et al., 1991), which have been implicated in transcriptional control of various genes, including TP53, involved in cell proliferation and apoptosis. These MT-mediated effects on gene transcription are thought to confer a selective growth or survival advantage (or both) on the mutated cells (Bruewer, 2002).

6. Glioma, stem cells niche and angiogenesis

Recently in a review article, Gilbertson & Rich JN (2007) address a number of key questions which remain to be answered: do all cancer stem cells require the support of aberrant niches? Are cancer stem cell niches the primary drivers of tumor development, or are they recruited by pre-formed cancer stem cells? How do cancer stem cells and their niches subvert the tight regulatory conditions that characterize normal stem cell niches?

The stem cells of glioblastoma seem to be dependent on signals from aberrant vascular niches that mimic the normal neural stem cell niche (Gilbertson & Rich, 2007). Stem cells of various tissues are tightly regulated by the immediate microenvironment or stem cell niche (Moore & Lemischka, 2006), which is provided by capillaries in specific locations (Riquelme et al., 2008). This organization places the stem cells in close proximity to endothelial and other vascular cells, facilitating cross-talking among these cell types and affecting stem cell

fate choices (Gilbertson, 2006). It is well-known that stem cells and their microenvironments may influence each other (Scadden, 2006). In fact, Cues within the niche, from cell–cell interactions to diffusible factors, are spatially and temporally coordinated to regulate proliferation and neurogenesis, ultimately (Riquelme et al., 2008).

In ENU treated rats, we have observed the existence of a close morphological relationship between MT positive cells and blood vessels. What is the relationship between them? It is known that MT is involved in the regulation of the functions of endothelial cells as well as in their protection against cytotoxic agents (Kaji et al., 1993). MT knock-out (MT-KO) mice presented dramatically decreased IL-6-induced angiogenesis caused by cortical freeze injury, suggesting that the MT have major regulatory functions in the angiogenesis process (Penkowa et al., 2000). In fact, human CD133+ Glioma CSCs are capable of producing vascular endothelial growth factor (VEGF) and thus may play an important role in glioma angiogenesis (Yao et al.,2008).

7. The concept of stemness, modulation of csc and glioma treatment

Understanding the characteristics and function of CSCs has shed light on their roles in glioma progression, including the implications for prognosis and treatment resistance. The original use of the term stemness was derived from a number of articles aimed to look for genes that could be expressed in general stem cell populations. The *stemness hypothesis* states that all stem cells use common mechanisms to regulate self-renewal and multi-lineage potential. This hypothesis has been debated and so far no conclusive evidence for a set of genes expressed in all stem cells. Certainly, identifying genes regulating stem cell properties will greatly improve our understanding of the molecular mechanisms regulating stem cell functions, our ability to manipulate stem cell fate, and the roles of stem cells in cancer (Koeva et al., 2011). Interestingly, overexpression of the transcription factor NANOG in gliomas and its close relationship with the undifferentiated state of glioma cells in vivo and in vitro indicated that NANOG may contribute to the existence of brains CSCs and may be related to tumorigenesis of the cerebrum by maintaining the undifferentiated state of glioma cells (Niu et al., 2011).

The new concept stemness is closely related to the observation that there are tissue environment factors that are able to influence or modulate CSCs. The main one is hypoxia, which activates the Hypoxia Induced Factor alpha number 1 (HIFα-1) alpha to enhance the self-renewal activity of CD133-positive cells and to inhibit their differentiation (Soeda et al., 2009). This and other signaling systems drive the transformation of normal stem cells, and perhaps of the bulk of tumor cells to cancer stem cells or to maintain the CSC phenotype (Katoh, 2011). For instance, the oxygen level of 7% has been observed to enhance the stem cell–like phenotype of $CD133^+$ in GBM cells (McCord et al., 2009). Furthermore, it has been observed that human glioblastoma cells from tumor biopsies, which were engrafted intracerebrally into nude rats, that CD133 negative glioma cells were tumorgenic in nude rats, and that CD133 positive cells can be obtained from these tumors. Upon the passing of the cell tumors in vivo, CD133 expression is upregulated, coinciding with the onset of angiogenesis and a shorter patient survival (Wang et al., 2008). Furthermore, the bone morphogenic protein BMP4 effectively reduces proliferation of CD133 positive cells in vitro and the tumor growth in vivo. BMP4 may act as a key inhibitory regulator of cancer initiation and therefore may be used in combined stem cell-based therapy as a non-cytotoxic therapeutic agent (Altaner, 2008).

If one accepts that there is a subpopulation of cancer cells with stem cell properties, which is responsible for tumor maintenance and progression, and may contribute to the resistance to anticancer treatments, it is very reasonable to deduce that compounds that target cancer stem-like cells could be effective to impair or even to destroy a neoplasm and nas important therapeutic implications. Various compounds have been investigated as putative influencers of stemness and malignancies in glioma stem-like cells, leading the proposal that stem cell regulatory factors may provide significant targets for therapeutic strategies (Holmberg et al., 2011). Ongoing work aims the identification of unique pathways governing self-renewal of these putative stem cells and their validation as ultimate therapeutic targets (Panagiotakos & Tabar, 2007). Additionally, it is possible to conceive that epigenetic-based drugs that modulate gene expression in CSC possibly constitute a promising alternative resource for target therapy in the treatment of these, thus far, incurable malignancy.

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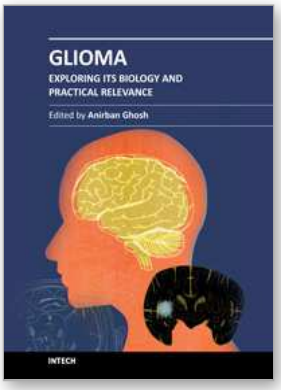

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The tittle 'Glioma - Exploring Its Biology and Practical Relevance' is indicative of its content. This volume contains 21 chapters basically intended to explore glioma biology and discussing the experimental model systems for the purpose. It is hoped that the present volume will provide supportive and relevant awareness and understanding on the fundamental advances of the subject to the professionals from any sphere interested about glioma.

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