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Brain Tumor Stem Cells as Therapeutic Targets in Models of Glioma

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At this time, brain tumor stem cells remain a controversial hypothesis while malignant brain tumors continue to present a dire prognosis of severe morbidity and mortality. Yet, brain tumor stem cells may represent an essential cellular target for glioma therapy as they are postulated to be the tumorigenic cells responsible for recurrence. Targeting oncogenic pathways that are essential to the survival and growth of brain tumor stem cells represents a promising area for developing therapeutics. However, due to the multiple oncogenic pathways involved in glioma, it is necessary to determine which pathways are the essential targets for therapy. Furthermore, research still needs to comprehend the morphogenic processes of cell populations involved in tumor formation. Here, we review research and discuss perspectives on models of glioma in order to delineate the current issues in defining brain tumor stem cells as therapeutic targets in models of glioma.

Key Words: Brain tumor stem cell, cancer stem cell, glioma, glioblastoma multiforme (GBM), neurosphere, PI3 kinase, Notch, Akt, Rapamycin

INTRODUCTION

The incidence of malignant brain tumors rose steadily over the last quarter of a century in both adults and children.[1-6] A proportion of this trend may be due to improved neuro-imaging techniques and access to medical care.[7] Familial gene mutations, immune disease, and high dose irradiation are known causes of brain tumors but are likely responsible for a minority of cases. Epidemiological studies and geographic variability in case numbers suggest that the etiology of brain tumors may be associated with environmental factors and exposure to carcinogens.[7-9] While brain tumors in the United States constitute a minority of cancer cases, with an incidence of 14.8 brain tumors per 100,000 person years, and roughly half diagnosed as benign, the malignant forms of brain tumor present a devastating prospect of morbidity and mortality.[10] The most common malignant brain tumors are gliomas, and within gliomas, glioblastoma multiforme (GBM) are the most common, representing 40% of all primary, malignant central nervous system tumors.[11] GBM have a median overall survival of approximately 1 year.[12] The term "multiforme" in GBM describes the heterogeneous nature of these neoplasms and their varied histological composition.[13] These tumors are characterized by diffuse infiltration into surrounding tissue that prevents complete surgical resection. Currently the standard treatment for GBM is surgical resection

followed by a combination of radiotherapy and chemotherapy.[14] Due to the poor prognosis of malignant tumors under this regimen of treatment, discovering effective new treatment is a crucial goal of further research. In order to design effective therapy it is imperative that ongoing research aims to understand the molecular pathways essential in tumor proliferation, survival, and invasion. Another emerging field of research is to develop an accurate model of the cell populations and morphogenic processes that produce the heterogeneous population of cells within a tumor. In both of these fields, brain tumor stem cells represent a central concept that remains to be fully determined and established.

BRAIN TUMOR STEM CELLS

Divergent perspectives on the fundamental nature of brain tumor biology fuel a debate that revolves around the theory of brain tumor stem cells (BTSC) as a model of glioma. The cancer stem cell theory posits that only a specific, minority of tumor cells possess the ability to produce a tumor and that these cells may arise from mutations in normal stem or progenitor cells.[15-17] The brain tumor stem cell theory holds that BTSC produce all the cells of a tumor and therefore represent the essential, specific targets of effective treatment necessary to prevent recurrence.[18] The notion that glioma tumors are caused by transformed neural stem cells was originally fueled by the discovery that brain tumors expressed nestin, an intermediate filament that can be expressed by neural stem cells,[19,20] although it is also expressed by more limited progenitors as well as by other cells within the body.[21] In this BTSC model, brain tumor stem cells arise from oncogenic mutations in neural stem cells. This hypothesis was supported by several observations: gliomas can arise near the lateral ventricles, a site housing neural stem cells that reside in the subventricular proliferative zone; neural stem cells proliferate enough to make them susceptible to transformation; and neural stem cells and BTSC share essential mechanisms for proliferation and survival.[22,23]

Evidence for the BTSC model of glioma first came from several laboratories.[24-26] These studies demonstrated biological similarities between brain tumor initiating cells and neural stem cells through the use of neurosphere cultures. Reynolds and Weiss, et al.[27] originally isolated and enriched neural stem cells from the adult brain through the use of neurosphere cultures.[28] Neural stem cells distinguished themselves from other cells in the brain by their ability to grow as neurospheres (floating spheres of cells) in relatively simple, serum free media with the addition of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or both. Neurospheres could subsequently differentiate into the multiple lineages of brain cells upon removal of growth factors. In like manner, cells derived from brain tumors form serially passaged clonal neurosphere cultures in serum free media, and, upon removal of growth factors, differentiate into multiple lineages to recapitulate tumor morphologies. In other words, *in vitro*, BTSC behave in a similar fashion to neural progenitor cells; they respond to the same mitogens, and they express similar markers.

The theory of BTSC was further substantiated when Galli, et al.[29] demonstrated that GBM derived neurosphere cultures were tumorigenic upon xenotransplantation into immunodeficient mice and Singh, et al.[30] demonstrated that tumor cells expressing CD133 (a putative marker of human neural stem cells), when sorted from patient samples, formed tumors in immunodeficient mice while the CD133 negative fraction did not. The theory of BTSC gained acceptance and the model developed that BTSC may originate from transformed neural stem or progenitor cells, and furthermore, are unique amongst other tumor cells in that BTSC possess the capacities to extensively self renew, initiate tumors upon orthotopic transplantation, and give rise to a heterogeneous population of cells such as those found in their parent tumors. More recent studies demonstrated that the ability of glioma tumors to form neurosphere cultures is an independent predictor of clinical outcome.[31,32] These data provide further evidence that BTSC play a central role in tumor progression and aggressiveness. However, BTSC remains a hypothesis and both the definition and terminology are still debated. Some scientists prefer the less declarative terms “brain tumor initiating cells” or “brain tumor stem like cells”. For the purposes of this paper, we shall use the term brain tumor stem cells.

MOLECULAR DIAGNOSIS AND THERAPY

In order to discover effective treatment for malignant glioma, one must seek to characterize and target specific molecular pathways and mechanisms employed by brain tumor stem cells. While gliomas are classified on the basis of histopathological criteria into four grades, in ascending order of malignancy, molecular expression profiling has also been effective at distinguishing subclasses of glioma.[33] Molecular expression profiles provide an advantage by offering valuable insights into the specific oncogenic pathways that drive tumor proliferation and, thereby, produce a more specific characterization of each tumor. Classification of high grade glioma based on molecular expression profiles have classified 3-5 distinct types of malignant tumors that resemble different stages in neurogenesis, predict pa-

tient prognosis, and indicate that activation of the Akt and Notch canonical oncogenic pathways reflect the aggressiveness of these neoplasms.[34-36]

Many cellular processes involved in regulating neural stem cells are also essential in glioma brain tumor stem cells. For example, certain cell cycle regulators and transcription factors involved in the regulation of neural progenitors, such as c-MYC, OCT-4, BMI-1, Olig-2, and MELK, also regulate brain tumor and putative BTSC proliferation and survival.[18,37-43] Similarly, multiple secreted growth factors involved in neural stem cell proliferation, such as EGF, and IGF (insulin like growth factor), bind with receptor tyrosine kinases to activate downstream proliferation and survival pathways in brain tumor initiating cells.[42]

Noteworthy is the PI3 kinase/Akt pathway, a key regulator of signaling via different pathways, including those regulated by EGF and IGF receptors. The PI3 kinase/Akt pathway has received a lot of attention as a target for cancer treatment.[44,45] Recently, expression of Akt and PI3 kinase activity has been shown to be associated with glioma tumor grade.[46] There are many agents available to researchers that specifically target this pathway, and this area of research promises to change therapeutic strategies utilized in glioma treatment.[47] Rapamycin is a microbial derived therapeutic that acts specifically on mTOR.[48-51] mTOR is one downstream effector of the Akt pathway which can also act via a feedback loop to influence Akt signaling.[52] Recently, rapamycin has made its way to clinical trials for the treatment of glioma.[53] However, rapamycin treatment in clinical trials as well as in laboratory trials produces cellular resistance.[53,54] Further research is necessary to determine whether this resistance is due to the many feedback loops inherent in the pathway[55,56] or to some other biological process. A recent study has suggested that improved inhibitors of mTOR may decrease resistance.[57,58]

Another critical signaling pathway involved in both neural progenitor and glioma proliferation and survival is the Notch pathway.[59-62] Notch is a family of transmembrane receptors that interact with adjacent cells. Upon ligand binding to Notch, gamma secretase cleaves the intracellular portion of the Notch receptor (NICD), thereby releasing NICD to translocate to the nucleus where it acts as a transcription factor promoting proliferation and inhibiting differentiation. Evidence is accumulating that the Notch pathway plays a crucial role in the formation and growth of glioma tumors[63] and drugs that inhibit gamma secretase are gaining interest as therapeutic agents in the treatment of glioma.

A central concern with utilizing specific drug targets is whether the redundancy of multiple oncogenic pathways confers resistance to single-pathway-targeted therapy.[64,65] For example, the Notch pathway and Akt pathway have been shown to interact in multiple ways[66-70] and this interaction may confer chemoresistance.[66] Hence, many investigators purport that combinatorial therapy provides a more robust therapeutic strategy.[71] Besides the Notch and PI3 kinase pathways, other pathways implicated in glioma survival are the hedgehog, Wnt, and bone morphogenic protein pathways. In addition, the post-transcriptional modification of miRNA's have also been shown to regulate glioma.[23,42,72] To address the multiplicity of oncogenic pathways, future treatment for glioma patients may include molecular expression characterization of tumor biopsies followed by a tailored regimen of combinatorial, targeted therapy. Efforts in pharmacology research include the search for synergistic combinations of specific drugs. Another aim of research is to determine molecular and genetic diagnostic criteria for tumor biopsies that are predictive of which oncogenic pathways are the essential targets for tailored therapy.

Chemoresistance represents a known challenge to glioma therapy. In addition to mechanisms of resistance that are dependent on specific signaling pathways, brain tumors possess other mechanisms of chemo-resistance. One example is the ability of O-6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, to reverse the DNA damage caused by alkylating agents such as temozolomide that target rapidly dividing cancer cells.[73] In fact, MGMT-positive expression status in a tumor biopsy predicts specific resistance to treatment with alkylating agents. Even more challenging, chemoresistance to a broad spectrum of cytotoxic agents, termed multi drug resistance (MDR), is a characteristic of glioma and represents a major obstacle in effective treatment.[74] MDR may be the result of genetic evolution, an adaptation through mutations that occurs during chemotherapy, or it may be an a priori property of certain tumor cells. In both cases increased expression of drug transporters, such as the ATP binding cassette super-family (ABC transporters), act to pump cytotoxic agents out of the cell.[75] Evidence that a distinct "side population" exists within tumors with enhanced drug efflux capacity suggests that MDR may be the intrinsic property of a minority of distinct tumor cells with enhanced drug transporting capacity.[76] This multi drug resistant "side population" is believed to be enriched for brain tumor stem cells. It is unknown whether MDR is due to an intrinsic property of BTSC, or to ongoing mutational evolution, or is the result of a systemic response to treatment.

MODELS OF GLIOMA

Many models exist to explain the etiology and function of the heterogeneous cell populations that form glioma tu-

mors.[23] The hierarchical model of BTSC contrasts with the more established stochastic model of cancer in which variegated cell populations possess equivalent capabilities to form tumors. In the stochastic model of tumors, different populations undergo clonal evolution in competition with each other in a process driven by mutation to form the tumor bulk.[77] It is thought that multiple mutations are required to transform a normal cell into a malignant, cancer cell.[78] Possibly, a mutator phenotype is a requirement to produce malignant cells.[79] In this model, a primary mutation causes genetic instability that drives further mutations; this mutator phenotype eventually produces cancerous cells. In this clonal evolution model of tumors, the diversity of cells within a tumor is not caused by a single BTSC but by a heterogeneous population of genetically distinct cancer cells.

Evidence is accruing that tumors are in a state of genetic flux. Analysis of lymphoblastic leukemia patients revealed that cancer recurrences differed in DNA copy number from their original, primary cancers.[80] Similarly, recurrences of breast cancer tumors were shown to have different mutational profiles than their original, primary tumors.[81] This evidence suggests that tumors possess a heterogeneous population of genetically distinct cells that undergo clonal evolution. The ongoing debate between the cancer stem cell model and the clonal evolution model has been reviewed by Shackleton, et al.[82] Glioma seem to fit well within the cancer stem cell model because tumorigenic capacity is a relatively rare trait among glioma tumor cells and not a uniform trait as would be predicted by the clonal evolution model. Indeed, glioma BTSC have been shown to demonstrate a hierarchical model,[29] capable of generating a diversity of cells. However, genetic diversity has also been discovered within glioma tumors. A study by Shapiro, et al.[83] in 1981 performed karyotypic analysis of different glioma tumors and the cultures derived from them and discovered 3-21 genetically distinct subpopulations within the average glioma tumor with varying chemosensitivities.[84] As this study was done in an age before neurosphere cultures, one cannot determine from this experiment how many genetically distinct, tumorigenic cultures were derived from each tumor. Recently, Piccirillo, et al.[85] isolated two genetically distinct populations of cells from distinct regions of a GBM tumor. However, only one population was tumorigenic, so one cannot assume that multiple populations of cancer stem cells existed in that particular tumor. However, this data does suggest the possibility that genotypically distinct BTSC may coexist within the same tumor.

It has been demonstrated that there is considerable genetic variability within populations of neural stem cells in the brain.[86,87] In fact, it can even be assumed that some genetic variation and instability found in neurosphere cultures represents the genetic variation and instability within the brain.[88] A systems based approach may syncretize the disparate models of glioma in order to address the manifest complexity of these tumors. In contrast to clonal evolution, the complex system model we shall discuss considers the features of adaptive and resistant behavior exhibited by malignant brain tumors to be the emergent properties of a complex adaptive system consisting of multiple brain tumor stem cells. In this model, both genetic and potentially reversible epigenetic changes may explain not only the cellular diversity, but also the increased plasticity these tumors exhibit upon therapeutic intervention.

BRAIN TUMOR STEM CELLS AS A COMPLEX ADAPTIVE SYSTEM

Cancer has been characterized as a robust, complex system[71,89,90] and tumors have been described as a cooperative system of interacting cells.[91,92] Therefore it is worthwhile to assess cancer as a complex adaptive system.[93,94] A complex adaptive system is characterized by emergent, global properties that are produced by a requisite diversity of local interactions.[95] These emergent properties are only ascribed to the complex system itself and cannot be reduced to the properties of the individual components of the system.[89,94,95] Emergent properties confer the hallmarks of a complex adaptive system: organization, adaptability, and survival.

Gliomas fit the essential criteria for a complex adaptive system, they are heterogeneous, self adaptive and self organized. Evidence exists for interactions between BTSC and local environmental cues that play a role in BTSC survival and proliferation.[96] Autocrine and paracrine factors are secreted by brain tumor stem cells to enhance infiltration and migration into surrounding brain tissue.[97] Diffusible factors and adherence cues emitted from surrounding vasculature exert an influence on BTSC proliferation and survival.[98] With all these factors involved in BTSC proliferation, survival, and infiltration, it is conceivable that a diversity of brain tumor stem cells may arise as a complex adaptive system that interacts through diffusible factors and adherence cues. Recently it has been shown in a drosophila model that diverse, adjacent tumor cells can cooperate to produce emergent properties of tumorigenesis and infiltration.[99] To what extent this occurs in human glioma has yet to be determined.

In order to model the tumorigenic process of glioma, it is necessary to ascertain which processes are involved. Besides the brain tumor stem cell model and the clonal evolution model are more complex systems whose roles in glioma are in the realm of possibility (Fig. 1). In order to prioritize therapeutic targets of glioma, it is important to have

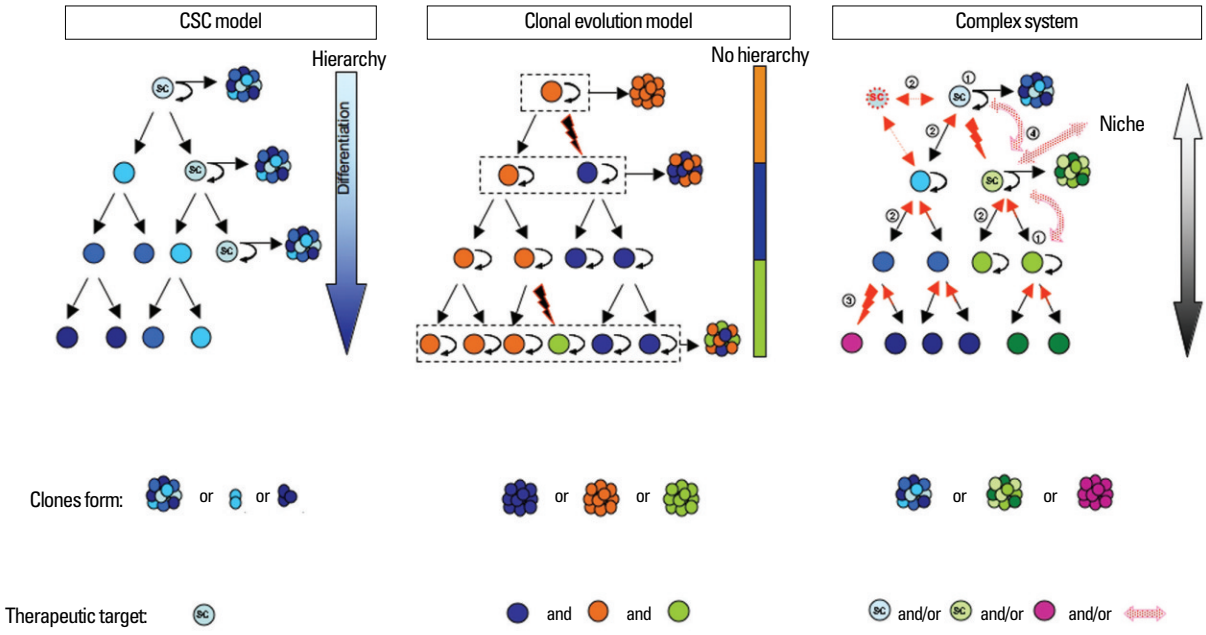

Fig. 1. Different tumor models of GBM. According to the cancer stem cell model (left panel), a subpopulation of cancer cells possesses the capacity of self-renewal, clonal sphere formation and *in vivo* tumor formation, as well as the capability to form progeny with a more restricted fate (darker colors). This forms a hierarchical lineage system where the primary therapeutic cell target is the CSC itself. The clonal evolution model (middle panel) exhibits no lineage hierarchy, as the multiple cell populations are the result of different genetic mutations (broken arrows). There is no cell hierarchy, because most of these cell subtypes self-renew and are capable of tumor formation, which makes them all targets of therapeutic interventions. In a complex system (right panel), both genetic and epigenetic changes might occur within a single tumor, resulting in a multifaceted cell system where several tumor-initiating cell types may coexist. While genetic mutations may produce new tumor cell populations (#3), epigenetic changes (#2) might enable cells to produce progeny with a more or less restricted fate and also to temporarily adopt different states characterized by therapy resistance and expression of different cell markers. Another important feature of a complex system is that the individual cell populations interact (red arrows, #4). While all potential tumor forming cells have to be targeted for successful therapy in this model, the interruption of the cell-cell and cell-niche interactions may also weaken the tumor system as a whole. GBM, glioblastoma multiforme; CSC, cancer stem cell.

the most informative model of glioma tumorigenesis. Further research is needed to determine whether de-differentiation occurs, whether BTSC can adapt to treatment by switching between different phenotypic states that confer either resistance or growth, whether multiple, genetically distinct brain tumor stem cells exist within each tumor, whether a mixture of the clonal model and the BTSC model co-exist, and to what extent signaling between BTSC, tumor cells, and the niche provides additional therapeutic targets.

FUTURE AIMS OF GLIOMA RESEARCH

To produce a model of glioma with improved diagnostic and therapeutic prediction-value may require a thorough understanding of both the essential molecular and morphogenic processes involved in tumor survival and proliferation. To devise tailored treatment, predictive molecular and genetic diagnostic criteria must be ascertained. Furthermore, it is important to discover whether treatment resistance in glioma is due to intrinsic characteristics of cancer stem cells, mutational evolution, redundant molecular pathways, or to the adaptation of a complex system of multiple brain tumor stem cells. Through the elucidation of glioma tumor biology, research aims to overcome treatment resistance and devise appropriate therapeutic approaches. BTSC may represent an essential target of therapy. Targeting the essential pathways and transcription factors of BTSC may deliver the next generation of improved therapeutic options.

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