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Authors
Muftuoglu, Yagmur
Pajonk, Frank

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Targeting Glioma Stem Cells

Yagmur Muftuoglu, MD, PhD[1], **Frank Pajonk, MD, PhD**[2,3]

[1]Department of Neurosurgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California USA

[2]Department of Radiation Oncology, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California USA

[3]Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, California USA

Keywords

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Introduction

Glioblastoma (GBM) remains to be the deadliest brain cancer in adults with almost all patients succumbing to the disease. The current standard of care, surgery followed by radiotherapy and temozolomide, prolongs the median survival from 2–3 months to 12–14 months[1]. Reasons for treatment failure in GBM are multiple and include the dispersion of cancer cells into the normal brain parenchyma far beyond the bulk tumor detected by clinical imaging modalities, the almost always incomplete surgical resection of the tumor, the lack of blood-brain-barrier penetration for many systemic therapies, and the normal tissue radiation tolerance of the brain. Mounting evidence suggests that GBMs contain a small number of glioma-initiating cells (GICs)[2–4] (often called glioma stem cells). The relative resistance of these GICs to chemo- and radiotherapy further contributes to the treatment resistance of GBM, making GICs an attractive target for novel treatment approaches against this disease[5,6].

CORRESPONDING AUTHOR: Phone: +1 310 206 8733, Fax: +1 310 206 1260, pajonk@ucla.edu.
Yagmur Muftuoglu, MD, PhD, Department of Neurosurgery, David Geffen School of Medicine at UCLA, 300 Stein Plaza Driveway suite 420, Los Angeles, CA 90095-1714
Frank Pajonk, MD, PhD, Department of Radiation Oncology, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, Los Angeles, CA 90095-1714

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The History of the Cancer Stem Cell Hypothesis

Tumor cell heterogeneity in cancer has been recognized since 1875 when Julius Conheim published a case report on a sarcoma of the kidney[7] and laid ground for the *cancer stem cell (CSC) hypothesis*. It argues that tumors are organized hierarchically with a small number of CSCs at the apex of this hierarchy, able to self-renew, repopulate a tumor after sublethal treatment, and give rise to the differentiated progeny, which lack these defining features of CSCs[8]. Given the resistance of CSCs to radiation[5,9] and chemotherapy[6,10] and their low frequency in many solid tumors including GBM[11], bulk tumor responses in these cancers do not necessarily reflect responses of CSCs to treatment. While this is, and always has been a well-understood phenomenon in radiation therapy, the efficacy of chemotherapeutic agents is often evaluated based on bulk tumor responses, and current classical radiosensitizers offer only marginal improvements in local control while adding significant toxicity[12]. With the lack of marker systems to identify CSCs, the CSC hypothesis was mainly a theoretical concept until in 2003 Michael Clarke, Peter Dirks, and Harley Kornblum independently reported surface marker combinations that could prospectively identify tumor cell populations, highly enriched for tumor-initiating cells in breast[13] and brain cancers[2,4].

In 1976, Peter Nowell introduced the *clonal evolution model* of tumor organization[14]. Similar to the CSC hypothesis, the model assumes a clonal origin of cancers, without proposing a hierarchical organization. The clonal evolution model postulates that the genetic instability of cancer cells leads to different clones of cells that contribute to the cellular heterogeneity of cancers; in turn, subsequent acquisition of additional mutations that favor cellular proliferation generate cells that outcompete other cell populations and become the driving cell population in a tumor. Considering the stochastic nature of acquiring additional genetic mutations, this model predicts that every cell in the tumor can acquire cancer stem cell traits through genetic changes, rather than epigenetic modifications. There is indisputable evidence supporting the genetically instable nature of solid cancers and its role on the genetic heterogeneity of solid tumors, even if they originate from specific cell clones. What is less clear is whether or not CSC traits are shifting from one clone to another in a stochastic manner. There is evidence that the clonal evolution model may hold true for some cancers[15], however the majority of solid tumors seem to follow a hierarchical model[11].

Finally, a lesser known interconversion model assumes multiple cellular states with differing tumorigenicities and growth rates depending on the context in which the process of differentiation is bidirectional. Evidence supporting interconversion of differentiated cells into leukemogenic cells has been reported for AML[16]. More recently, it has been increasingly recognized that these three models are not necessarily exclusive and that stemness is less of a binary state but rather a continuous variable contributing to the heterogeneity of tumors[17].

Definitions/Background

Normal neural stem/progenitor cells in the CNS have been rigorously defined, and stem cell state and differentiation steps into neurons can be followed by well-defined marker combinations that have been carefully validated in functional assays[18]. The definition of

GICs is less uniform in the literature and ranges from correlating rigorous functional testing of patient-derived cells with marker profiles[4] to less stringent studies that establish gliomaspheres from cell lines that have been cultures as monolayers for decades and labeling them glioma stem cell lines. The functional identification of tumor-initiating cells, including GICs, is traditionally performed using *in vivo* limiting dilution assays in which the frequency of GICs can be calculated retrospectively[19]. Time-intensive and resource-consuming, this assay is now often replaced or complemented with *in vitro* limiting dilution assays, where cells form clonal gliomaspheres from single cells under conditions that favor cells able to grow serum-free and where cells prone to die from anoikis are eliminated[20]. Gliomaspheres in this assay do not necessarily derive exclusively from GICs but can give an approximation on the self-renewal capacity of GBM cell subpopulations.

In many studies, the prospective identification of GIC relies on the surface protein CD133 (AC133 or prominin-1), a pentaspan transmembrane glycoprotein that was first described on cellular protrusions of hematopoietic stem cells[21]. Its validity to identify GICs in GBM is discussed controversially[22,23] with some studies viewing it as a measure of a bioenergetic stress, unrelated to stemness[24]. Despite the controversy surrounding CD133, it is widely applied to enrich for tumorigenic GBM cells. Additional surface markers for GIC enrichment include stage-specific embryonic antigen-1 (SSEA-1)[23], a surface antigen expressed by glia and neural progenitors called A2B5[25], stem cell markers like nestin[26] and CD15[27]. and integrin-α6, often co-expressed with other markers and thought to contribute to both tumorsphere generation *in vitro* and tumor growth *in vivo*[28]. Functional markers include high ALDH1 activity and lack of proteasome activity[29]. An excellent review of surface and functional markers of GICs can be found in[30].

Discussion

Clinical relevance of Glioma-initiating cells

GICs constitute a small fraction of the tumor bulk[31] but quietly play multiple critical roles in promoting relentless tumor progression. They resemble non-cancerous progenitor cells normally found in brain tissue and can produce neurons and other cell types, both *in vitro* and *in vivo*. By definition, they promote development of the initial lesion by producing differentiated cancer cells that possess the ability to rapidly divide. Additionally, when injected into immunologically deficient mice via secondary transplantation, GICs cause development of tumors phenotypically similar to the donor tissue[31]. Recent studies have demonstrated the role of GICs in promoting invasiveness, as well, especially subpopulations expressing A2B5 with or without CD133 expression[25,32].

In addition to their direct actions, GICs also manipulate and benefit from their microenvironment for optimal tumorigenesis. Residing in the perivascular space allows GICs to retain their stem cell properties via a steady supply of nutrients and vascular-derived signaling factors that promote self-renewal[33], and GICs further secrete extracellular matrix proteins to develop a specific microenvironment conducive to their proliferation and differentiation[34]. Cross-signaling between GICs and other cell types also propagates tumor development[35]. As another example, GICs effectively evade the immune system by inhibiting the proliferation of T-cells, by expressing defective antigen-processing machinery,

and by only weakly expressing MHC-I, MHC-II, and NKG2D ligand, important players in antigen recognition by T-cells and natural killer cells[36].

Finally, GICs remain infamous for their ability to survive both chemotherapy and radiation. Traditional chemotherapies target rapidly-dividing cells by taking advantage of unstable DNA repair mechanisms, but GICs remain rather quiescent relative to these typical tumor cells. GICs also overexpress important players in the DNA repair pathway like O-6 methylguanine-DNA-methyltransferase (MGMT)[37]. This allows them to more efficiently correct DNA damage caused by temozolomide, which functions by methylating guanine moieties at the O-6 position, normally resulting in serious DNA damage. In fact, protein expression of MGMT in GIC cell lines predicts resistance to chemotherapy, though MGMT methylation status curiously does not strongly correlate with resistance[38]. GICs may also express drug transporters - like multi-drug resistance 1 (MDR1) of the ABC transporter family - at higher levels to pump out therapeutic molecules[39]. Interestingly, radiation seems to actively enrich the percentage of CD133+ GICs remaining after conventional fractionation[5], and the GIC population that remains serves to heighten DNA repair efforts, via more efficient homologous recombination and aberrancies in the checkpoints that govern cell growth[40]. As such, slowly dividing GICs allow for the universal recurrence that ultimately dampens patient survival.

Given their role in promoting recurrence, studies have shown prognostic value in the characterization of tumor GIC populations[41,42]. A recent massive meta-analysis proved that higher levels of CD133 expression correlate with worse progression-free survival and worse overall survival, particularly among patients with grade IV - but not grade II or III - glioma, while higher levels of Nestin expression correlate with worse overall survival among patients with only grade II or III glioma[43]. In addition, GICs can serve as a model for high-throughput experiments to identify molecules that target this quiescent population. One such study successfully cultured GICs as neurospheres with heterogeneity matching that of the original tumor and developed a high-throughput proliferation assay to identify multiple compounds with anti-GIC activity from a pilot experiment. Not all results of *in vitro* assays translate well *in vivo*, however, partly because multiple GIC lines have been identified and developed over time[44].

In reality, the phenotype of these powerful stem cells varies among patients, and a single tumor can harbor a handful of different phenotypes, as well. For example, GIC subpopulations even without CD133 expression also demonstrate similar properties. The A2B5+/CD133− subpopulation retains motility, invasiveness, and tumorigenic properties[22,25,32], as alluded above. The SSEA-1/CD15+ subpopulation of GICs reportedly can also form tumor spheres, albeit typically smaller than CD133+ tumor spheres and yet positive for Ki-67, rendering these smaller formations likely more proliferative than those positive for CD133[45]. Such a diversity offers a mere glimpse into the complexity of various GIC subpopulations and harkens back to the heterogeneous nature of GBM, further encouraging the development of personalized combinatorial therapies for combating inevitable recurrence. The challenge of targeting GICs may very well present the most impactful existing opportunity to improve the standard-of-care for GBM, elucidated in greater depth below.

Glioma-initiating cells and the standard-of-care

The standard-of-care, surgery followed by radiotherapy and temozolomide, fails to provide cure for GBM patients and only prolongs median survival by about 12 months[46].

The benefit of surgery in patients with GBM is well-established and gross-total resection undeniably prolongs survival[47]. Like all surgeries, every brain tumor resection creates a wound and sets a repair program into motion that is triggered by cytokines and hypoxia. However, hypoxia in particular is a key feature of GBM, associated with the niche requirements of GICs[48,49] and known for supporting phenotypic plasticity of GBM cells[50]. It remains to be seen if pathways engaged in GBM cell in those hypoxic resection margins can be employed against GICs.

One of the many reasons for treatment failure in GBM is that the standard-of-care differentially affects GICs and their progeny. First reported by Jeremy Rich's laboratory, GICs exhibit relative radioresistance as a result of a more efficient repair of DNA double strand breaks[5]. It is important to point out that this resistance is relative to that of more differentiated GBM cells, that GICs still respond to radiation and that radiotherapy prolongs median survival in a dose-dependent manner[51] by about 6 to 9 months, thus making it the most effective agent against GBM so far. The dose-dependency of the response of GBM to radiation would imply that further dose escalation or alternative fractionation schemes could improve treatment outcome. However, standard fractionation schemes applying 2 Gy fractions, five times per week were empirically established and do not necessarily present an optimum. In fact, experimental evidence suggests that taking the heterogeneity of GBM into consideration, more unconventional fractionation schemes could improve the efficacy of radiotherapy against GBM[52]. Yet, clinical attempts at improving the impact of radiotherapy have so far largely failed[53] and dose escalation up to a total dose of 90 Gy did not improve survival[54,55].

A large number of groups have demonstrated resistance of GICs to many commonly used chemotherapeutic agents,[6,56] including temozolomide[38,57]. Several different mechanisms contribute to this resistance including the overexpression of ABC transporter proteins, which can be exploited to enrich GICs in side-population assays[58] and are responsible for an active efflux of chemotherapeutic agents, the preference to reside not only in a perivascular niche but also inside the hypoxic microenvironment of the tumor core[59,60], in which the efficacy of chemotherapy is drastically reduced[61,62] and overexpression of free radical scavenging systems[63] able to detoxify drugs. Furthermore, a large number of targeted therapies against bulk tumor cell populations in GBM have largely failed to improve outcome[64].

Taken together, the standard-of-care against GBM and many of its iterations have hit a critical barrier and their inefficacy to eliminate GICs suggests that specific targeting of GICs could further improve treatment outcome for GBM patients.

Targeting Glioma-initiating cells

From the identification of tumor-initiating cells came considerable enthusiasm for novel therapies aimed at this rare population. With this in mind, the discussion of how gliomas are organized becomes of less academic and more of practical importance as it dictates possible

intervention points to target GICs. A hierarchical model of GBM implies a finite number of GICs, responsible for the repopulation of the tumor, and suggests that their successful elimination will improve treatment outcome. The understanding that those tumor-initiating cells potentially derive from normal stem cells implied to target developmental signaling pathways including the Wnt, BMP, and c-Met pathways in GICs[65], known to govern stem cell traits in normal neural/progenitor stem cells. However, if GICs are driven by the same pathways that maintain neural stem cells or progenitor cells, every attempt to target these pathways for therapeutic gain will then rely on the existence of a therapeutic window[66] that limits current established anti-tumor therapies. E.g., while CD133+ GBM cells can be targeted by inhibition of the Notch pathway by γ-secretase inhibitors in patients[67], normal stem cell compartments like that of the gastrointestinal tract rely on the same pathway and exhibit significant toxicity. In fact, clinical results fall short of the encouraging experimental findings that motivated the clinical trials[68].

If in fact the clonal evolution model more accurately describes the organizational structure of GBM, targeting GICs becomes more complicated. Different driver mutations in individual clones will emerge over time[69], leading to dysregulation of multiple pathways that potentially can maintain stemness. And even though the number of these mutations is most likely finite, surgical specimens will only provide snapshots of the evolutionary landscape of these mutations and will not be informative enough to select treatments against individual clones over time unless an overarching feature of GICs can be identified and targeted.

The problem of targeting GICs is further complicated when one factors the effects of cancer therapies[70] and the response of surviving tumor cells into the equation. Microenvironmental stress[71], chemotherapy, and radiation[72] induce interconversion of non-GICs into induced GICs, thus replenishing the pool of treatment-resistant GICs and fueling recurrences. In the case of ionizing radiation, this process involves re-expression of developmental transcription factors and global epigenetic changes[73]. In a recent unbiased high-throughput screen, we were able to identify compounds that can interfere with the process of radiation-induced phenotype conversion[74]. One group of compounds identified in this screen was that of dopamine receptor antagonists that easily cross the blood-brain barrier and not only prevent the induction of GICs but also target intrinsic GICs and non-GICs[73], thereby significantly prolonging survival in a mouse model of GBM. With novel dopamine receptor antagonists harboring more favorable side effect profiles in clinical development[75], this strategy could offer a novel approach to improve the efficacy of the standard-of-care in GBM.

Summary

GICs accomplish several tasks critical to tumor growth and recurrence by promoting invasiveness, immune system evasion, and resistance to existing therapeutic options via conversion of non-GICs into new GICs following radiation. While GICs can be identified by a handful of different markers (CD133, SSEA-1, A2B5, nestin, integrin-α6), the scientific community still lacks a complete understanding of different such subtypes. Furthermore, translating the excitement surrounding experimental therapies into successful clinical outcomes has proved difficult. Hampering the effect of GICs may be possible, however, by

inhibiting the treatment-induced phenotype conversion that replenishes residual tumor of its GIC population and helps promote recurrence.

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KEY POINTS

- The concept of tumor-initiating cells is an old concept in oncology that was recently revived with the discovery of markers, able to enrich for tumor-initiating cells
- Intratumoral heterogeneity and plasticity during undisturbed growth and in response to anti-cancer affect the efficacy of the standard-of-care in glioblastoma
- Targeting cellular plasticity in glioblastoma emerges as a means to improve treatment outcome in glioblastoma

SYNOPSIS

Only a small fraction of the tumor cell population, glioma-initiating cells (GICs) help glioblastoma propagate, invade, evade immune recognition, repair DNA in response to radiation, remodel the microenvironment for optimal growth, and actively pump out chemotherapies. Recent data hints that efforts toward GIC characterization and quantification can help predict patient outcomes, and yet the different sub-populations of GICs remain incompletely understood. A better understanding of GIC sub-types and functions proves critical for engineering targeted therapies. Challenges for doing so are discussed, and dopamine receptor antagonists are introduced as new means to enhance the efficacy of the current standard-of-care against GICs.

CLINICS CARE POINTS

- GICs drive recurrences and are a potential target against GBM
- Prospective identification of GICs is hampered by the fact that marker systems only enrich for GICs
- Intratumoral heterogeneity and plasticity of GBM allow tumors to escape therapies aimed at GICs