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Review Article

Characterisation of Cancer Stem Cell Populations in Primary Glioblastoma Cultures: A Review

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Abstract

Background: Glioblastoma contains cells that initiate tumours at low dose and resist therapy. How that population should be defined, and whether it is a fixed compartment, remains contested.

Objective: To review how cancer stem cell populations are characterised in primary glioblastoma culture, and what the markers and assays establish.

Methods: Narrative review of primary studies on glioma stem cell isolation, culture conditions, marker validation, functional assays and heterogeneity.

Findings: As few as 100 CD133-positive cells initiated a serially transplantable tumour where 100,000 negative cells did not, but stem-like CD133-negative cells were later shown to sustain a subset of tumours, so the marker is neither necessary nor sufficient. Serum-cultured lines diverge from the parent tumour, while serum-free bFGF and EGF culture preserves genotype and phenotype. Single-cell sequencing describes four interconverting states rather than fixed types.

Conclusions: Functional assays, not surface markers, define the population, and plasticity makes that definition conditional.

Keywords: glioblastoma; cancer stem cells; CD133; limiting dilution; tumour heterogeneity

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Introduction

Glioblastoma recurs. Surgery, radiotherapy and temozolomide together extend median survival by months, and the tumour returns in almost every patient, usually within the irradiated field. That fact motivated the search for a resistant subpopulation able to regenerate the tumour after treatment, which the cancer stem cell model was adopted to describe.

The model arrived from neural biology. The demonstration that neurons and astrocytes could be generated from isolated cells of the adult mammalian central nervous system [1] established the neurosphere assay, and the same technique in paediatric brain tumours yielded cells with stem-like properties [2]. Two 2004 papers established it for glioma: cells selected on CD133 initiated tumours in immunodeficient mouse brain [3], and tumorigenic stem-like neural precursors were isolated from human glioblastoma [4].

Two decades later the biology is better described but the definition is less settled, and the disease has been redefined around molecular rather than histological criteria, glioblastoma now being an IDH-wildtype diagnosis [5]. This review asks what the markers and assays in primary culture support, and why the field has moved toward interconverting states.

Materials and Methods

Studies were retrieved from PubMed, Europe PMC and Crossref for the terms *glioma stem cell*, *glioblastoma*, *CD133*, *neurosphere*, *limiting dilution* and *tumour heterogeneity*, without date restriction. Priority went to primary studies testing marker and assay validity in patient-derived material. Bibliographic records were confirmed against Crossref or the publisher, and quantitative values taken from the source, not secondary citation.

Culture conditions determine what is studied

Before markers, the medium: the most consequential methodological point in the field, and the most often taken for granted.

Glioblastoma lines maintained in serum diverge from the tumours they came from: cells cultured in bFGF and EGF without serum mirror the phenotype and genotype of primary tumours far more closely [6]. Serum drives differentiation and selects for genetic changes over passage, so an older literature based on serum-adapted lines describes cells whose relationship to the patient's tumour is uncertain.

Serum-free culture has two configurations. Free-floating neurospheres are the historical default, but they aggregate as well as proliferate, confounding any measure based on sphere number. Adherent culture on laminin gives lines with tumour-specific phenotypes suitable for chemical and genetic screening [7], in a monolayer where individual cells can be observed.

Markers and their limitations

CD133 was the founding marker and remains the most used, on the strength of one result: as few as 100 CD133-positive cells produced a tumour in NOD-SCID brain that could be serially transplanted and reproduced the patient's phenotype, while 100,000 CD133-negative cells engrafted but formed no tumour [3].

The marker did not survive scrutiny intact. CD133-positive and CD133-negative glioblastoma-derived cells differ in growth characteristics and molecular profile, and CD133-positive cells maintain only a subset of tumours, the remainder arising from CD133-negative cells that are themselves stem-like [8]. A marker that identifies the initiating population in some tumours and not others is an enrichment tool, not a definition.

Alternatives have accumulated without resolving this. Integrin α6 regulates glioblastoma stem cells and co-enriches with CD133 [9]; L1CAM is required for their

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maintenance and its targeting suppresses glioma growth [10]; a CD44-high, Id1-high population is targeted by TGF-β receptor inhibitors [11]; and aldehyde dehydrogenase 1A3 marks and metabolically sustains mesenchymal glioma stem cells [12]. Each identifies a functionally relevant population; none the same one as the others.

Functional assays

Because no marker suffices, the operational definitions are functional.

The limiting dilution sphere-forming assay estimates the frequency of cells that initiate a clonal sphere, and extreme limiting dilution analysis standardises its statistical treatment, providing the framework for comparing depleted and enriched populations [13]. Its principal weakness is that spheres can form by aggregation as well as by clonal growth.

Serial passaging tests self-renewal over time, and orthotopic xenograft tumour initiation is the reference standard. Genome-wide CRISPR dropout screens extend this to dependency, nominating SOX factors, SOCS3, USP8 and DOT1L as growth requirements [14]. Fate mapping revealed a conserved hierarchy in which slow-cycling stem-like cells generate rapidly cycling progenitors that yield non-proliferative progeny [15], and in a murine model a restricted population propagated tumour growth after chemotherapy [16] — the strongest evidence that a hierarchy exists.

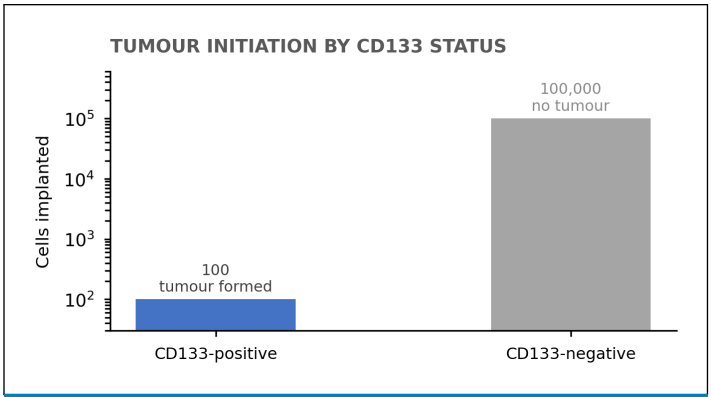

Figure 1: Tumour initiation in NOD-SCID brain by CD133-positive and CD133-negative cells from human glioblastoma. Note the logarithmic scale: a thousandfold larger negative population formed no tumour. Data from Singh et al. [3].

Plasticity and cellular states

The hierarchy is not one-way, the finding that most complicates the model. Core transcription factors are sufficient to reprogram differentiated glioblastoma cells into stem-like cells [17], so the state is inducible rather than inherited. NF- B-mediated mesenchymal differentiation promotes radiation resistance [18], meaning treatment itself drives cells toward the resistant phenotype.

Single-cell sequencing reframed the question. Intratumoral heterogeneity in primary glioblastoma spans the expression subtypes defined at bulk level [19,20], and an integrative model describes four interconverting cellular states whose relative proportions are influenced by genetic drivers [21]. On this account the tumour is not a hierarchy of fixed types but a distribution over states, and a cancer stem cell is one currently occupying one of them.

Table 1: Markers used to enrich glioma stem cell populations, and what each has been shown to do.

Marker	Established role	Limitation	Ref.
CD133 / PROM1	100 cells initiate a serially transplantable tumour	CD133-negative cells sustain a subset of tumours	[3,8]
Integrin α6	Regulates and co-enriches stem cells	Overlaps but is not identical to CD133	[9]

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Marker	Established role	Limitation	Ref.
L1CAM	Required for maintenance; targeting suppresses growth	Marks a functional dependency, not a compartment	[10]
CD44 / Id1	Defines a TGF-β-dependent initiating population	Associated with the mesenchymal state	[11]
ALDH1A3	Sustains mesenchymal glioma stem cells	State-specific rather than universal	[12]

Niche, resistance and models

The state is partly environmental. Brain tumour stem cells occupy a perivascular niche that maintains them [22], and hypoxia-inducible factors regulate their tumorigenic capacity [23], so oxygen tension and vascular contact belong in any culture system meant to represent them.

Resistance is the model's clinical justification. CD133-positive cells promote radioresistance through preferential activation of the DNA damage response, reversible with checkpoint kinase inhibition [24]. Chemoresistance is dominated by MGMT: silencing predicts benefit from temozolomide [25] within the regimen that defines standard care [26].

Culture systems have moved toward preserving this context. Three-dimensional organoids derived from human glioblastoma recapitulate the hypoxic gradients and stem cell heterogeneity found in vivo [27]. Organoids generated from surgical tissue without dissociation preserve the parent tumour's histology, cellular diversity and mutational profile, and infiltrate on transplantation [28].

Discussion

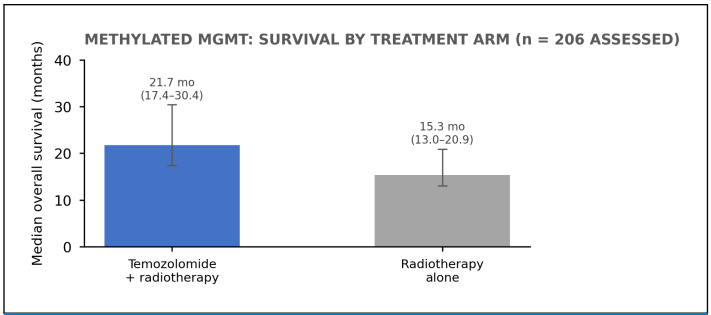

Figure 2: Median overall survival with a methylated MGMT promoter, by treatment arm, with 95% confidence intervals; 206 tumours were assessable for methylation status. Data from Hegi et al. [25].

Three conclusions follow. First, culture conditions are not a technical preliminary but the primary determinant of what is characterised: serum-adapted lines are not representative of the tumour.

Second, no surface marker defines the population. CD133 enriches for tumour-initiating capacity in some tumours and not others, and every alternative marks an overlapping but non-identical population. Characterisation therefore rests on functional assays: limiting dilution for frequency, orthotopic xenograft for the property itself.

Third, plasticity undermines the compartment model without eliminating its utility. Cells interconvert, treatment pushes them toward resistant states, and reprogramming can restore stem-like potential. What remains is a reproducible hierarchy of proliferative behaviour, a weaker but better-supported claim than a fixed stem cell compartment.

This is a narrative review without systematic search or risk-of-bias assessment. Marker studies use different antibody clones, dissociation protocols and xenograft strains, which may explain part of the disagreement, and several conclusions rest on murine models whose correspondence to human disease is assumed, not demonstrated.

Conclusion

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Cancer stem cell populations in primary glioblastoma culture are best characterised functionally, in serum-free conditions that preserve the parent tumour's genotype, by limiting dilution and orthotopic transplantation rather than phenotype alone. CD133 remains useful for enrichment but defines neither a necessary nor a sufficient population. The state is plastic, environmentally conditioned and inducible by treatment, so what is characterised depends on when and how it is examined. Studies should report culture medium and format, passage number and the functional assay alongside marker data, and treat marker-defined populations as operationally rather than biologically distinct.

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Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this review.

Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

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