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The Quest for Self Identity: Not All Cancer Stem Cells Are the Same

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SUMMARY

A central critique of the cancer stem cell hypothesis centers on the robustness of cancer stem cell (CSC) markers. In this issue, Zorniak et al. suggest that different progenitor marker profiles can classify CSCs and improved modeling of cellular hierarchies will be informed by incorporating intertumoral and intratumoral diversity.

In this issue of Clinical Cancer Research, Zorniak et al.¹ classified patient-derived glioblastoma cells cultured under stem cell conditions based on neural lineage marker expression and the invasive potential in vivo. Three classes of sphere-forming glioblastoma cells expressed markers of neural and oligodendrocyte progenitors, neural progenitors alone, or astrocyte progenitors. Astrocyte progenitor marker expression was associated with invasion and reduced survival of mice bearing human glioma xenografts. In contrast, the oligodendroglial marker, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), was associated with less aggressive tumor growth and informed favorable survival among glioblastoma patients. These data raise important unresolved questions for neuro-oncology and tumor modeling at large.

What is the CSC hypothesis and how will the present study impact sources of controversy?

With the advent of cell culture and genetically engineered models, dramatic advances in cancer biology have occurred but translated into only limited survival benefit for advanced or metastatic cancers. The identification of cancer-associated mutations led to the conclusion that cancer is a genetic disease, but this simplistic notion fails to account for cell state: tumors are characterized by location, morphology, and differentiation state. Further, no tumor is entirely homogeneous with contributions from non-neoplastic tissues and diversity within the neoplastic compartment derived from the integration of genetic and non-genetic causes. The CSC hypothesis holds that cancers are arranged in cellular hierarchies with self-renewing, tumorigenic CSCs at the apex². While the CSC hypothesis remains controversial³, CSCs in some cancers are preferentially resistant to conventional therapies, invasive/metastatic, and promote tumor angiogenesis. Further, improved culture conditions designed to maintain putative CSCs better preserve gene expression patterns and some genetic mutations (e.g. isocitrate dehydrogenase, IDH).

Despite the enthusiasm for the CSC concept, a number of challenges have developed as researchers have delved into its intricacies. First, it is apparent that not all tumor types or

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subgroups within a cancer type display cellular hierarchies. In particular, during metastasis the cellular hierarchy may collapse towards a more stem-like state. However, even in these cancers the biology may be informed by shared regulatory programs with stem cells. Differences in methodology including the use of established cell lines that have been subjected to artifact induced by cell culture, tumor dissociation and methods for isolation (e.g. problems with trypsin⁴) have made comparison of results across laboratories difficult. Zorniak et al.¹ utilized outgrowth of sphere forming cells from glioblastoma patients to establish cultures termed “glioblastoma stem-like cells” or GSCs. The GSC cultures had CSC hallmarks, including progenitor cell marker expression (differs across lines), neurosphere formation capacity, and tumorigenic potential in vivo. Outgrowth of sphere forming cells avoids limitations associated with prospective enrichment markers, but precludes documentation of a cellular hierarchy: comparison to non-GSCs isolated directly from the parental tumor is not possible, making it unclear whether markers are broadly expressed in the tumors or GSCs are specifically enriched for the observed phenotypes in comparison to other tumor cells.

What factors contribute to tumor heterogeneity and how does this inform the CSC hypothesis?

The CSC hypothesis addresses differences among tumor cells within the same patient, requiring enrichment for functional phenotypes. While common core stem cell pathways likely contribute, mechanisms driving the CSC phenotype differ between patients as highlighted by the present study. Indeed, intertumoral and intratumoral heterogeneity are both clinically important as evidenced by the use of age or neurosphere formation potential⁵ as independent predictors of glioma patient survival. At the DNA level, intertumoral heterogeneity reflects the diversity of the host genetic background as well as the mutations and epigenetic modifications driving tumor initiation and progression (Figure 1). The cell-of-origin and genetic alterations likely influence the molecular and biological CSC profiles. The originating cell will also determine the regional location of the resulting tumor, impacting intertumoral variations in the host microenvironment that regulate CSCs. Microenvironmental differences are also critical for intratumoral heterogeneity as CSCs exist in specific perivascular and perihypoxic niches supporting their maintenance.

Does the cell-of-origin of cancers dictate CSC phenotypes and signals?

Although often a source of confusion, the CSC hypothesis does not indicate that a stem cell must be mutated to initiate a cancer. However, the current work suggests that GSCs can be grouped according to expression profiles of progenitor markers associated with specific lineages. It is not yet certain if these classifications reflect a hierarchy that was initiated in an astrocyte, neural, or oligodendrocyte stem/progenitor cell, but this potential implication is intriguing. If true, it will be important to determine whether the core stem cell signaling pathways activated in CSCs (Notch, Sonic hedgehog, etc.) differ depending on the progenitor marker profile or if there are common signals which could be targeted for patient therapies across tumors. Evidence already suggests that activation of Sonic hedgehog signaling is not equivalent across GSCs isolated from different patients⁶, although association with distinct progenitor marker profiles is unclear.

Do the classes of GSCs identified reflect differences in the glioblastoma subtype of the parental tumors?

Molecular glioblastoma profiling has informed classification into subtypes associated with distinct genetic alterations: classical (EGFR amplification or mutation), mesenchymal (NF1 and P53 mutation), and proneural (P53 and IDH1 mutation and PDGFRA amplification)^{7,8}.

In the current study, GSCs that expressed oligodendrocyte and neural progenitor markers (CD133, L1CAM, OLIG2) did not express EGFR but expressed CNP suggesting a link to a proneural phenotype¹. Proneural tumors may express relatively higher levels of markers currently associated with the GSC phenotype, explaining the association of CD133 expressing GSCs with a proneural subtype⁹, which has been linked to better survival. In addition, the oligodendrocyte marker CNP was not expressed in cells expressing EGFR¹, data similar to that of Hagerstrand et al.¹⁰ involving EGFR activation. Together the data suggest that differences in the expression profiles of markers in the isolated GSCs are indicative of classical vs. proneural glioma subtypes in the parental tumors. Thus, it will be important to compare the profiles of the progenitor markers used for classification in the Zorniak et al. study¹ in glioma stem and non-stem populations isolated from multiple glioblastoma patient specimens of known subtypes.

How can we best utilize information linking regulators of an invasive GSC phenotype to poor patient outcome?

Invasion into normal brain prevents curative surgical resection, and molecular regulators of invasion are important prognostic factors in glioblastoma¹¹. As GSC models are invasive, distinguishing highly invasive (CNP-low) and poorly invasive GSCs (CNP-high) may inform global tumor invasion. CNP is important for organization of paranodal adhesion proteins in the central nervous system¹², although its function in glioblastoma is not well characterized. Zorniak et al.¹ suggest the utility of CNP as a glioblastoma biomarker, and further studies will need to determine if CNP regulates GSC invasion.

In conclusion, tumors differ not only in the frequency of CSCs and genetic background but also in the inherent molecular characteristics of CSCs. The current challenges in the CSC hypothesis may reflect the inadequacies of our scientific methods rather than failure of the concept. The development of CSC markers and targeting strategies will be informed by the recognition of tumor complexities.

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Figure 1. Factors Contributing to Tumor Heterogeneity

Differences between tumors arising in individual patients as well as differences in among tumor cells within the same patient contribute to the complexity of cancer.