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

Anita B. Hjelmeland and Jeremy N. Rich

A central critique of the cancer stem cell (CSC) hypothesis involves the robustness of CSC markers. Zorniak and colleagues suggest that different progenitor marker profiles can classify CSCs, and improved modeling of cellular hierarchies can be achieved by incorporating inter- and intratumoral diversity.

In this issue of Clinical Cancer Research, Zorniak and colleagues (1) report a study in which they classified patient-derived glioblastoma cells cultured under stem cell conditions based on neural lineage marker expression and invasive potential in vivo. Sphere-forming glioblastoma cells were divided into 3 classes according to whether they expressed markers of neural and oligodendrocyte progenitors, neural progenitors alone, or astrocyte progenitors. Astrocyte progenitor marker expression was associated with tumor cell 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 in animal models and improved survival in patients with glioblastoma. These data raise important unresolved questions for the field of neuro-oncology and tumor modeling at large.

What Is the Cancer Stem Cell Hypothesis and How Will the Present Study Affect Sources of Controversy?

With the advent of cell culture and genetically engineered models, dramatic advances in cancer biology have been made; however, these advances have translated into only a limited survival benefit for patients with 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, that is, tumors are characterized by location, morphology, and differentiation state. Further, no tumor is entirely homogeneous, with contributions from nonneoplastic tissues and diversity within the neoplastic compartment derived from the integration of genetic and nongenetic causes. The cancer stem cell (CSC) hypothesis holds that cancers are arranged in cellular hierarchies with self-renewing, tumorigenic CSCs at the apex (2). Although the CSC hypothesis remains controversial (3), it has been shown that CSCs in some cancers are preferentially resistant to conventional therapies, are invasive/metastatic, and promote tumor angiogenesis. Further, improved culture conditions designed to maintain putative CSCs can 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 subgroups within a cancer type display cellular hierarchies. In particular, during metastasis, the cellular hierarchy may collapse toward a more stem-like state. However, even in metastatic cancers the tumor 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 trypsin (4)] have made comparison of results across laboratories difficult. Zorniak and colleagues (1) used outgrowth of sphere-forming cells from glioblastoma patients to establish cultures termed glioblastoma stem-like cells (GSC). The GSC cultures had CSC hallmarks such as progenitor cell marker expression (which differs across cell lines), the capacity to form neurospheres, and tumorigenic potential in vivo. Outgrowth of sphere-forming cells avoids the limitations associated with prospective enrichment markers but precludes documentation of a cellular hierarchy. A comparison with non-GSCs isolated directly from the parental tumor is not possible, which makes it unclear whether markers are broadly expressed in the tumors or GSCs are specifically enriched for the observed phenotypes in comparison with 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 an individual patient, requiring enrichment for functional phenotypes. Although common core stem-cell
pathways likely contribute, the mechanisms that drive the CSC phenotype differ among patients, as highlighted by Zorniak and colleagues’ study. Indeed, tumor heterogeneity (both inter- and intratumoral) is clinically important, as evidenced by the use of age or neurosphere-formation potential (5) as independent predictors of survival in patients with glioma. At the DNA level, intertumoral heterogeneity reflects the diversity of the host’s genetic background as well as the mutations and epigenetic modifications driving tumor initiation and progression (Fig. 1). The cell-of-origin and genetic alterations likely influence the molecular and biologic CSC profiles. The originating cell will also determine the regional location of the resulting tumor, influencing intertumoral variations in the host microenvironment that regulate CSCs. Microenvironmental differences are also critical for intratumoral heterogeneity because CSCs exist in specific perivascular and perihypoxic niches that support their maintenance.

Figure 1. Factors that contribute to tumor heterogeneity. Differences between tumors in different patients as well as among tumor cells in an individual patient contribute to the complexity of cancer.
Does the Cell of Origin of Cancers Dictate CSC Phenotypes and Signals?

Although it is often a source of confusion, the CSC hypothesis does not indicate that a stem cell must be mutated to initiate a cancer. However, Zorniak and colleagues suggest that GSCs can be grouped according to the expression profiles of progenitor markers associated with specific lineages. It is not yet certain whether these classifications reflect a hierarchy that is initiated in an astrocyte, neural, or oligodendrocyte stem/progenitor cell, but the potential implications of such differences are intriguing. If true, it will be important to determine whether the core stem-cell signaling pathways that are activated in GSCs (e.g., Notch and Sonic hedgehog) differ depending on the progenitor marker profile or there are common signals that can be targeted for patient therapies across tumors. Evidence already suggests that activation of Sonic hedgehog signaling is not equivalent across GSCs isolated from different patients (6), although the association with distinct progenitor marker profiles is unclear.

Do the Classes of GSCs Identified Reflect Differences in the Glioblastoma Subtype of Parental Tumors?

Molecular glioblastoma profiling has informed classification into 3 subtypes associated with distinct genetic alterations: classical [epidermal growth factor receptor (EGFR) amplification or mutation], mesenchymal (NF1 and P53 mutation), and proneural [P53 and IDH1 mutation and platelet-derived growth factor receptor amplification (7, 8)]. In the study by Zorniak and colleagues (1), GSCs that expressed oligodendrocyte and neural progenitor markers (CD133, L1CAM, and OLIG2) expressed CNP but not EGFR, suggesting a link to a proneural phenotype. 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 subtype (9), which has been linked to better survival. In addition, the oligodendrocyte marker CNP was not expressed in cells that expressed EGFR (1), in similarity to findings by Hagerstrand and colleagues (10) in a study involving EGFR activation. Together, the data suggest that differences in the expression profiles of markers in isolated GSCs are indicative of classical versus proneural glioma subtypes in parental tumors. Thus, it will be important to compare the profiles of the progenitor markers used for classification in Zorniak and colleagues’ study of glioma stem and nonstem populations isolated from multiple glioblastoma patient specimens of known subtypes.

How Can We Best Use 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 glioblastoma (11). Although GSCs are more invasive than their nonstem counterparts, the ability to distinguish between highly invasive (CNP-low) and poorly invasive (CNP-high) GSCs may aid in the assessment of global tumor invasion. Although CNP is important for organization of paranodal adhesion proteins in the central nervous system (12), its function in glioblastoma is not well characterized. Zorniak and colleagues (1) suggest the utility of CNP as a glioblastoma biomarker; however, further studies are needed to determine whether 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 posed by 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: A.B. Hjelmeland, J.N. Rich
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