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The Implications of the Cancer Stem Cell Hypothesis for Neuro-Oncology and Neurology

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Abstract

The cancer stem cell hypothesis posits that cancers contain a subset of neoplastic cells that propagate and maintain tumors through sustained self-renewal and potent tumorigenicity. Recent excitement has been generated by a number of reports that have demonstrated the existence of cancer stem cells in several types of brain tumors. Brain cancer stem cells – also called tumor initiating cells or tumor propagating cells – share features with normal neural stem cells but do not necessarily originate from stem cells. Although most cancers have only a small fraction of cancer stem cells, these tumor cells have been shown in laboratory studies to contribute to therapeutic resistance, formation of new blood vessels to supply the tumor, and tumor spread. As malignant brain tumors rank among the deadliest of all neurologic diseases, the identification of new cellular targets may have profound implications in neuro-oncology. Novel drugs that target stem cell pathways active in brain tumors have been efficacious against cancer stem cells suggesting that anti-cancer stem cell therapies may advance brain tumor therapy. The cancer stem cell hypothesis may have several implications for other neurologic diseases as caution must be exercised in activating stem cell maintenance pathways in cellular therapies for neurodegenerative diseases. The ability for a small fraction of cells to determine the overall course of a disease may also inform new paradigms of disease that may translate into improved patient outcomes.

Keywords

Cancer stem cell; Stem cell; Cell-of-origin; Brain tumor; Glioblastoma; Glioma; Medulloblastoma; CD133; Prominin-1; Neurosphere

Evil enters like a needle and spreads like an oak tree

-- Ethiopian Proverb

The War on Cancer was declared by President Nixon in 1971. In the intervening decades, cancer treatment has improved such that the diagnosis of cancer is not a certain death sentence but victory remains out of grasp. In particular, patients diagnosed with malignant brain cancers continue to suffer high morbidity and mortality [1]. Multiple scientific advances – radiotherapy modalities, gene therapy, the Human Genome Project, and targeted molecular drugs – have been hailed as signs of a coming revolution in brain cancer care only to disappoint when the benefits of each have proven more modest. Now, excitement is building around the cancer stem cell field. Viewing cancer through the prism of the cancer stem cell hypothesis may fundamentally transform brain cancer biology and translate into improved diagnosis, prognosis, and treatment of brain tumor patients, but the reality is likely complex and

challenging. The brain cancer stem cell field is immature – we are only beginning to recognize the rules governing cancer stem cell biology – but common themes are emerging in studies of cancer stem cells derived from a diversity of tumor types suggesting that core mechanisms may underlie tumor formation and maintenance. However, controversies surround cancer stem cells with some researchers disputing the existence of cancer stem cells [2]. Cancer stem cell biology has been recently reviewed in several publications [1,3-6] so I will focus this review on the potential and challenges of cancer stem cells.

A common cancer stem cell terminology

The cancer stem cell field suffers from an identity crisis. The use of the term “stem cell” engenders confusion over the meaning of “cancer stem cell”. Frequently, the question is immediately posed if brain cancer stem cells are derived from normal neural stem cells; essentially, stem cells as the cell-of-origin in brain tumors. While it is possible (perhaps, even likely) that stem cells may be transformed to initiate tumor formation [7-10], studies using genetically engineered cancer models also support more differentiated progenitor cells as possible cells-of-origin in cancer [11-14]. As convergent evolutionary forces may drive phenotypic similarities in divergent species, so too might genetic and epigenetic processes promote a tumor initiating phenotype. Rather, a cancer stem cell more definitively identifies a tumor cell population with special properties that resemble stem cells – self-renewal and differentiation abilities – and potent generation of complex tumors that phenocopy parental tumors. To capture the essence of these features, many groups have employed a series of names: cancer stem cells, tumor stem cells, tumor initiating cells, stem cell-like cancer cells, tumor-propagating cells, etc. The lack of standardized terminology limits our ability to effectively communicate the precise meaning of these labels and inform literature searches. While imperfect, the term cancer stem cell appears to be the most common moniker but it should be distinguished from cell-of-origin.

Defining brain cancer stem cells

The use of established brain cancer cell lines has permitted great advances in brain tumor biology but provides artificially simplistic model of tumor biology. In reality, brain tumors consist of not only neoplastic cells but also supporting vasculature, inflammatory cells, and recruited astrocytes. Essentially, solid tumors mimic aberrantly developed organs or tissues [15]. Tumor cell populations are commonly heterogeneous with hierarchies of cellular populations that display a range of differentiation phenotypes, including specific morphologies and lineage markers. The majority of cells in bulk tumors may be non-tumorigenic cells derived from stem and progenitor cells. With clearly defined roles for normal stem cells in organ development and reaction to injury, it is of little surprise that cancers contain cells with stem cell phenotypes as cancers share features of normal developmental processes and wound responses. Therefore, advances in stem cell biology in development and injury response may provide insights into cancer stem cell biology.

Defining a cancer stem cell is far from trivial. The requirements for a cancer stem cell are currently functional: extensive self-renewal and tumor initiation [16]. Self-renewal can only be measured if we have a measure of “self”. Further, uncommon cancer stem cells must be isolated from a large mass of mixed tumor cell populations. Several reports delineating the derivation of brain tumor stem cells have identified stem cells based on the formation of neurospheres, a cell culture phenotype associated with neural stem cells [17], as a relevant phenotype [18-24]. The significance of the neurosphere phenotype is supported by the success of tumor derived neurospheres to give rise to multiple lineages (neural, astrocytic, and oligodendroglial) and potent tumor formation in immunocompromised rodents with as few as 100 cells. However, concerns have been raised about the true reflection of “stemness” with the

generation of neurospheres are cells may coalesce to form similar structures [25]. In addition, and the culturing of tumor cells until the formation of neurospheres precludes the direct comparison of cancer stem cells with the non-stem cell populations. Therefore, novel approaches have been developed to prospectively enrich for cancer stem cells through the use of cell surface markers permitting the segregation of populations enriched or depleted in cancer stem cells. The laboratories of John Dick and Michael Clarke laid the foundation for the cancer stem cell field by applying techniques developed in normal stem cell biology based on differentiation markers to derive tumor populations enriched for cancer stem cells in leukemias and solid tumors [26-28]. Essentially, viable cancer stem cells are selected through the presence of specific stem cell markers and the absence of lineage markers. Importantly, cancer stem cells do not phenocopy normal stem cells. Cancer stem cells may display aberrant differentiation marker signatures that are divergent from matched normal stem cells. There are also controversies as to which markers are truly definitive for isolation of brain cancer stem cells. The cell surface marker CD133 (Prominin 1) is expressed by embryonic neural stem cells [17,29] and has been used to select for brain cancer stem cells [18,19,30] but other reports suggest that cells without CD133 expression may generate brain tumors [31-33]. Another group has found that the IQGAP1 scaffold protein regulates neural stem cells and is a marker for amplifying cancer cells in glioblastomas [34, 35]. The diversity of markers that may be used to define cancer stem cells may be due to patient-to-patient differences in cancer stem cells or a lack of absolute marker fingerprints. Discrepancy in cancer stem cell markers from tumors with similar histopathology may reflect alternative cells-of-origin or oncogenic processes. It is clear that the expression of a cancer stem cell marker is not sufficient to claim a cancer stem cell phenotype. However, several brain cancer types share cancer stem cell signatures suggesting that we may be able to apply findings from one cancer type to others. The precise methodologies employed for marker selection (even for the same marker) and in functional assays may be dramatically different between laboratory groups, limiting the ability to generalize conclusions regarding the cell biology of the derived populations. Thus, every lab performing cancer stem cell studies must confirm critical cancer stem cell functional assays to permit comparisons across studies. The cancer stem cell field would greatly benefit from an agreed set of defining studies required for publication in the cancer stem cell literature.

Cancer stem cells in tumor biology

Hanahan and Weinberg proposed a restricted set of capabilities for cancer cells: 1) self-sufficiency in growth signals, 2) insensitivity to anti-growth signals, 3) evasion of apoptosis, 4) limitless replicative potential, 5) sustained angiogenesis, and 6) tumor invasion and metastasis [36]. Genomic instability may be considered intrinsic to tumor formation (they considered this an enabling characteristic). This collection of traits may be reinterpreted within the context of the cancer stem cell hypothesis. Not only may cancers originate from single cells but also relatively small numbers of cells may maintain tumors. Thus, not every tumor cell may need to be able to proliferate indefinitely. The definition of a cancer stem cell requires sustained proliferative potential and self-renewal suggesting that the Hanahan-Weinberg hypothesis may identify key traits intrinsic to the cancer stem cell population. In development, neural stem cells display aspects of these behaviors, albeit under control from the external environment. Further, key signaling pathways that regulate neural stem cell fate and differentiation – Olig2, sonic hedgehog, Notch, BMI-1, bone morphogenic proteins (BMPs), maternal embryonic leucine zipper kinase (MELK), etc. – may contribute to brain tumor malignancy through regulation of proliferation, apoptosis, and angiogenesis of brain tumor stem cells [10,14,37-44]. These stem cell regulatory pathways may function through different molecular mechanisms to regulate neural stem cell maintenance or differentiation but intervention in each pathway regulates brain tumor stem cells and tumor growth. However, a key unresolved point is the absolute restriction of tumor formation to the cancer stem cell compartment [2].

Cancer stem cells in therapeutic resistance

Despite the development of molecularly targeted therapies, radiation and cytotoxic chemotherapies (particularly the oral methylator, temozolomide) remain the mainstay of brain cancer treatment [45]. While unusual cancers like testicular cancers can be cured at advanced stage with conventional therapy, therapeutic resistance is common among most advanced cancers. Many mechanisms may contribute to the development of therapeutic resistance, including the stochastic selection of resistant genetic subclones, microenvironmental factors (hypoxia, acidosis, etc.), and cell extrinsic factors. Several groups, including my own laboratory, have demonstrated that brain tumor stem cells or cells expressing stem cell markers from multiple cancer types exhibit resistance to conventional cancer therapies. We demonstrated that cancer stem cells derived from human glioblastoma surgical biopsy specimens and xenografts are resistant to the effects of ionizing radiation with proficient capacity to repair DNA damage due to preferential activation of the DNA damage checkpoint response [30]. Additional studies from other groups investigating brain tumor stem cell models confirmed that cells expressing cancer stem cell markers are resistant to radiation and chemotherapy [46-48]. If recurrent brain tumors contain greater numbers of brain tumor stem cells that are also selected for greater resistance to therapy, the lethality of high grade brain tumors might be better explained. However, these early studies raise as many questions as they answer. First, other proposed mechanisms of the treatment resistance may interact with brain tumor stem cells, e.g. cancer stem cells may reside in vascular or hypoxic niches [49] or secrete proteins that may act both in autocrine and paracrine manners [50]. Second, the role of cancer stem cells in tumor resistance must be validated in human subjects. This is not trivial as groups of patients will need to be followed during treatment with sequential characterization of cancer stem cells. Finally, it is unclear if mechanisms of cancer stem cell therapeutic resistance are shared across cancer types. Cancer stem cells with non-stem cell origins may respond differently to therapy than those derived from stem cells. The recognition of common resistance mechanisms that may be targeted in cancer stem cells may have broad utility.

Cancer stem cells in tumor spread and angiogenesis

Tumor invasion into normal brain tissue is a hallmark of the gliomas [1], but the mechanisms underlying this behavior is very poorly understood. Cell culture techniques are very useful for the study of cellular proliferation and apoptosis but are of very limited value for measuring invasion. Although animal models may be more helpful than cell culture systems for tumor dispersal studies, we remain largely ignorant of the relative contributions of cell autonomous vs. niche characteristics (seed-and-soil theory) in tumor spread. Gliomas are commonly invasive into distant sites at diagnosis even in areas without radiographic abnormalities and patients with some systemic cancers (e.g. breast cancer) have widespread micrometastases which are not clinically evident. Incorporating the concept of cancer stem cells as the initiator of tumor spread may inform the study of cancer invasion and metastasis. Quiescent cancer stem cells may be capable of surviving in distant locations for prolonged periods but may become activated in response to external signals. For example, numerous patients with systemic cancers suffer sudden growth of metastases after resection of primary tumors. This behavior has been linked to antiangiogenic effects of the primary tumor but it is also possible that loss of signals from the primary tumor mass instruct remote cancer stem cells to repopulate the tumor mass. Recent studies have suggested that micrometastases of breast cancers display a stem/progenitor cell signature consistent with cancer stem cells [51] and pancreatic stem cells may have greater propensity to undergo metastasis in animal models [52]. Similar studies in brain tumors have not been published yet. Many more studies will be required before we can primarily attribute tumor spread to cancer stem cells but the ability to identify the populations within cancer responsible for tumor spread would be a tremendous advance.

Tumor growth beyond 1 mm in size requires the formation of new blood vessels to supply the tumor, a process called neoangiogenesis [53]. The mechanisms of neoangiogenesis in tumor growth are complex, but one key mechanism is the elaboration of growth factors by tumor cells that support the proliferation and survival of the endothelial cells of the tumor vasculature. We recently demonstrated that brain tumor stem cells generate highly vascular tumors through the secretion of high levels of vascular endothelial growth factor (VEGF) [50]. This finding is important as VEGF is a validated therapeutic target in glioma therapy [54-56]. The relationship with the tumor vasculature is bidirectional as the Gilbertson group has demonstrated that the vasculature also supports brain tumor stem cell maintenance [57].

Cancer stem cells in diagnosis

The greatest advances in modern oncology to decrease cancer mortality have involved cancer prevention and early detection and treatment which unfortunately do not impact Neuro-oncology as we are unable to modify risks for the overwhelming majority of these cancers. Even if the study of brain tumor stem cells elucidates the causes of tumor spread, high grade brain tumors are likely to remain therapeutic challenges. If cancer stem cell techniques provide tools to detect systemic cancers at earlier stages, we may be able to modify the impact of these cancers on society. Brochoalveolar, gastric, and colon carcinomas develop from dysplastic lesions that may be visualized and biopsied by endoscopy. No fully reliable method currently informs the management of patients with early lesions. The presence of cancer stem cells in dysplastic tissues may support aggressive early intervention. While this approach will not benefit brain tumor patients, detection of circulating cancer stem cells may provide a minimally invasive cancer diagnostic assay. Genetically engineered cancer models may provide ideal for the development of these techniques before translation into clinical trial.

Cancer stem cells in prognosis

Immediately after patients receive a brain tumor diagnosis, they want to know how long they have to survive. Neuro-Oncologists are often remarkably poor at predicting individual patient survival because apparently identical cancers can behave with strikingly different outcomes. Current prognosis (and thus clinical management) of brain tumor patients utilizes patient characteristics (age, performance status, etc.) and tumor characteristics (histology, grade, extent of resection and presence of metastasis in some tumor types). To date, molecular testing has only modestly contributed to patient management [1,58,59] as these markers are imperfect and require refinement as the status of the direct molecular target for any therapy does not solely determine the outcome of treatment. Numerous studies employ genomic signatures to predict patient prognosis and response to therapy. Generally, these studies have used whole tumor specimens as RNA sources. If brain tumor stem cells account for only a small fraction of the overall tumor (notably some investigators have found potential stem cells as the majority of medulloblastoma tumor cells), then the expression pattern from cancer stem cells may be lost in the larger signal from the non-stem cancer cells. However, a recent study found that a cancer stem gene expression profile derived from breast cancers did predict patient survival [60] perhaps indicating that stem cell prevalence can dictate both gene expression and tumor growth. While similar studies are not yet published in brain tumor patient populations, one study has found that the percentage of glioblastoma cells that express CD133, a potential glioma stem cell marker, correlates with patient survival and risk of tumor regrowth [61]. It remains possible that prognostic models may be strengthened if cancer stem cell populations are directly characterized. Cancer markers like CA-125 have been developed to follow the course of patients in response to therapy and predict recurrence. Many of these markers are expressed by the more differentiated cancer cell compartments. Characterizing cancer stem cells at diagnosis and during treatment may yield novel cancer markers that more closely predict the clinical course of cancer patients.

Imaging of Cancer Stem Cells

Current imaging techniques utilized in brain tumor patient management frequently quantify tumor burden indirectly through edema, vascular integrity (i.e. contrast enhancement), or metabolic activity. Small numbers of brain tumor cells present formidable challenges to detect in live subjects. As brain tumor stem cells may occur at low density and exhibit early propensity towards dissemination (a key behavior that underlies the need to identify these cells), imaging cancer stem cells is likely to present severe technical challenges. Potential methodologies under development for cancer stem cell identification are based on expression of specific channels/pumps (e.g. the side population [62-65]), enzymatic activity (aldehyde dehydrogenase, ALDH [66,67]), or marker expression. As these aspects may be shared with normal stem cells, interpretation of cancer stem cell imaging modalities will face anatomic restrictions near high density areas of normal stem cells. Cancer stem cell imaging may have implications in the evaluation of clinical outcome. The FDA has required improved survival as the endpoint for approval of most cancer therapies. Surrogate endpoints, such as radiographic tumor response, are attractive for clinical trials, but tumor response (i.e. shrinkage of tumor) may not correlate with survival. Non-stem tumor cells account for the bulk of the tumor and may display preferential sensitivity to some cancer therapies while cancer stem cells may represent restricted subsets of tumor populations but contribute to tumor progression and recurrence — and thus, tumor lethality. Based on this model, the majority of the tumor may respond to treatment but survival may be determined by the residual cancer stem cells. If correct, imaging of cancer stem cells becomes of even greater importance.

Anti-Cancer Stem Cell Therapies

Cancer cures require control of 100% of tumor cells. Obviously, brain tumor stem cells must be addressed for therapeutic success but cancer stem cells may present special challenges and opportunities. Pharmaceutical drug discovery platforms commonly employ cancer models including established cancer cell lines that poorly replicate cancer stem cell phenotypes. Therefore, most high throughput screening efforts may fail to identify brain tumor stem cell targets. A recent report suggests that these techniques may be extended to normal neural stem cells and brain cancer stem cells [68] although *in vivo* validation of efficacy remains to be addressed. Further, cancer stem cell maintenance mechanisms in culture may differ *in vivo* adding to the complexity of screening for anti-cancer stem cell therapies. With the poor capacity of current preclinical cancer models to predict therapeutic efficacy in human subject clinical trials, it would be of profound significance if cancer stem cell models demonstrated improved outcome predictions.

The first generation of anti-cancer stem cell therapies has focused on signal transduction pathways that regulate cell differentiation: Notch, BMP, hedgehog, etc. [37-39,41]. Growth factor pathways may function in stem cell maintenance (e.g. epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) [23]) and the relationship with the cancer stem cell niche may be targeted [57,69]. A cautionary note may be struck with potential toxicities to normal stem cells. The role of neural stem cells remains to be defined but neural stem cells clearly important in the pediatric population. Hematopoietic stem cells facilitate recovery from chemotherapy-induced myelosuppression, but the essential role of normal stem cells in other organs in adults is less clear. Normal organ-specific stem cells may promote tissue responses to injury but may be otherwise quiescent or dispensable (e.g. in the breast or prostate). Therefore, anti-stem cell therapies may display significant therapeutic indices. Target identification studies may identify novel molecular cancer stem cell targets that are differentially expressed or regulated relative to normal stem cells. Additionally, other modalities are being adapted towards brain tumor stem cell ablation, particularly vaccine

approaches [70] and viral therapy [71]. Cancer stem cell targeting may be used in isolation or, more likely, in combination with conventional therapies to improve patient outcomes.

Implications for Neurology

The importance of the cancer stem cell hypothesis may have broader implications for other neurologic diseases. Cellular therapies are in development for neurodegenerative diseases but a recent study suggested that modified embryonic stem cells can generate undifferentiated mitotic neuroepithelial cells that could be potentially tumorigenic [72]. The presence of a critical tumor population that initiates tumors and contributes to tumor malignancy suggests that constitutive activation of stem cell pathways may lead to lethal side effects. The concept of cancer stem cells may also inform new paradigms in neurology as most disease states are considered as a condition affecting the full diseased tissue but it is possible that restricted cellular compartments within diseased tissues may determine the course of the condition and the response to therapy.

Future Perspectives

The complexity of cancer parallels that of evolution with our ability to recognize the end product without fully understanding the rules governing the underlying process. Cancer stem cells may be considered the correlate of evolutionary nodes from which the diversity of progeny may be derived. The cancer stem cell hypothesis dates back decades [73] but has gained momentum in the last decade due to novel techniques that has enabled the characterization of the tumor cells. The cancer stem cell hypothesis remains controversial and the significance of cancer stem cells in clinical practice is poorly understood, however the study of cancer stem cell biology has the potential to inform the field of tumor biology but there may not be immediate translation into better oncologic care. The use of cancer stem cells could improve preclinical cancer models and therapeutic development but will require much greater understanding of normal stem cell biology. We may be entering a new phase in cancer research based on the cancer stem cell paradigm in which the ability of oncologists to provide improved prognosis and therapy may be at hand but setbacks and false leads are almost certain to occur.

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Figure 1. Neural stem cells and brain tumor initiating/stem cells

Both neural stem cells and brain tumor stem cells can undergo extensive self-renewal and can give rise to more differentiated progeny. However, brain tumor stem cells display aberrant differentiation and can develop into tumors that phenotypically resemble the parental tumors from which they are derived. Both neural stem cells and brain tumor stem cells may be enriched in cellular populations that express the CD133 (Prominin-1) cell surface marker, although some brain tumor stem cells may not express CD133. Several regulatory pathways appear to be shared in brain development and brain tumor formation through regulation of the stem cell. Targeting these pathways may have therapeutic benefit for brain cancer patients as brain tumor stem cells and the developed brain display differential dependence on the activities of these regulatory pathways.