Cancer Stem Cells and Brain Cancer: Biology and Therapeutics

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Abstract— The existence of cancer stem cells was once a controversial theory but for at least some cancers has become a widely accepted and proven mechanism explaining the development of multiple tumor types. These stem cells are more resistant to therapy and may be the leading cause of resistance to current cancer directed therapy, which leads to increased rates of relapse. If effectively targeted, without other systemic toxicity, the elimination of cancer stem cells may lead to improved survival. Central nervous system tumors have some of the highest mortality and morbidity rates of any cancer type and new therapies are desperately needed. We describe several small molecule based therapeutic strategies aimed to target cancer stem cells in some of the most aggressive central nervous system tumors: glioblastoma multiforme, medulloblastoma and atypical teratoid rhabdoid tumor.

Keywords — Brain Cancer, Cancer stem cell, small molecule inhibitors, Glioblastoma, Medulloblastoma.

I. INTRODUCTION

Stem cells are undifferentiated cells that have the remarkable ability to divide indefinitely. Stem cells can be characterized in two ways. The first is self-renewal, resulting in the generation of daughter stem cells which are biologically identical to the parent stem cell.\(^1\) The second is differentiation into several lineages of specialized cell types.\(^1\) Consequently, stem cells provide the raw material necessary for human and tissue development by fulfilling various niches throughout the body.\(^2\)

There are three main classifications of stem cells: Embryonic stem cells (ESC), adult stem cells, and induced pluripotent stem cells (iPSC). Embryonic stem cells are derived from embryos (blastocysts) and can differentiate into almost every cell in the body (all somatic cells) as they possess the highest level of pluripotency.\(^3\) The enhanced telomerase activity in ESCs also allows unlimited replication.\(^4\) Various types of adult stem cells occupy more specific niches in the body, differentiating into the specialized cell types of the organ or tissue where they reside.\(^5\) Unlike ESCs, however, adult stem cells have a limited ability for self-renewal, which makes generating large quantities difficult.\(^6\) Finally, iPSCs are differentiated adult cells whose genetic makeup has been experimentally modified to induce an ESC-like state.\(^7\) Once reprogrammed, iPSCs express the genes and factors of ESCs, achieving a pluripotent state. While iPSCs are useful for testing drugs and studying disease development, clinical strategies for transplanting cells derived from them have not yet been developed.\(^8\) In fact, the first trial using iPSC-derived pigmented retinal epithelial cells was recently halted, current with the discovery of potentially oncogenic mutations.\(^9\)

Research has shown that a population of cells with stem cell properties within a tumor may be responsible for resistance to therapy and relapse of disease. These cells, termed cancer stem cells, are thought to be responsible for generation of all cancer cells within a specific tumor type. These cancer stem cells must be effectively targeted if a cure for these cancers is to be achieved.

II. CANCER STEM CELLS

Studies indicate that small populations of cancer cells have unique regenerative and proliferative capabilities leading to self-renewal and differentiation which is a feature shared by other stem cells. These cancer stem cells (CSCs) promote tumorigenesis and tumor growth due to their potential for self-renewal and differentiation.\(^10\)\(^11\) Unlike other stem cell types, which have pathways regulating the extent of self-renewal and differentiation, these pathways are deregulated in cancer stem cells allowing for relatively unrestricted growth.\(^12\) Furthermore, the upregulation of the stem cell factors such as BMI1 (B cell-specific Moloney murine leukemia virus integration site 1) in CSCs propagates cancer cell proliferation and metastases.\(^13\) Therefore, in order to accomplish lasting remission (or a permanent cure) of cancer and prevent metastasis, therapeutic strategies must target CSCs (Fig. 1).
Because cancer stem cells share many of the traits of normal stem cells, it is possible that CSCs result from mutations in normal stem cells. Research also shows another viable pathway of CSC formation through mutated progenitor cells which also have the ability to replicate (though not as readily as normal stem cells). The connection between cancer stem cells and normal stem/progenitor cells is therapeutically challenging as it is difficult to formulate strategies that do not adversely affect normal stem cells while eradicating malignant ones.

### A. Unique Characteristics of CSCs

CSCs differ significantly from other cancer cells in both biology and function but do possess protein markers found in normal stem cells. These surface markers, such as CD44, CD24, and CD133, when present, allow CSCs to be distinguished from other cells within some tumors. Another hallmark of CSCs is the aldehyde dehydrogenase (ALDH) activity. Studies show that increased expression of ALDH has protective qualities against increased levels of reactive oxygen species, thus preventing cancer cell death and apoptosis. CSCs are similar to stem cells, but often arise as dedifferentiated, more primitive forms of stem cells, with certain genetic mutations.

Because CSCs have stem-like characteristics, they possess nearly limitless self-renewal and differentiation potential, which normal cancer cells do not. Consequently, CSCs can create many different types of cell clones (varying in differentiation state, proliferation rate, migratory and invasive capacity, and therapeutic response), even within one tumor, often resulting in extremely heterogeneous cancers.

### B. Types of CSCs

By definition, a cancer stem cell has enhanced capacity to initiate tumor growth, compared to bulk tumor cells. For this reason, they are often referred to as “tumor initiating cells” but here we will refer to them as CSCs. Evidence shows the presence of CSCs in many different types of cancers. CSCs have been discovered in breast cancer, head and neck cancers, leukemia, pancreatic cancer, and brain cancer among others. Breast cancer stem cells share with normal stem cells unique proteins on their surface membranes. CD44 and CD24 are two of the protein markers that distinguish breast cancer stem cells from other cells in the tumor. Also linked to the formation of breast CSCs is the mutation of the BRCA1 gene, which is known normally to suppress tumor growth. In head and neck tumors, CSCs were also distinguished by the presence of the surface marker CD44. In the basal layer of skin where cell division occurs, the endothelial cells further promote survival of head and neck CSCs by secreting certain self-renewal factors. Although not identical in structure and function, pancreatic CSCs have also been identified by the protein surface markers CD44, CD24, and ESA. Finally, brain CSCs play an important role in the formation of brain tumors and have been isolated by the surface marker CD133 in several studies. Though there are some excellent review reported on cancer stem cell especially GBM and all endorse the statement that the development of CSC targeting therapeutics is important for further improvement of survival rate. This review will delve deeper into the biology and functions of brain CSCs and potential therapeutic methods.

### III. Brain Cancer Stem Cells

There are over 120 different types of brain tumors that can arise in all areas of the brain and spinal cord. Brain cancer stem cells (bCSCs) have been shown to drive growth and spread of cancer cells in many such tumors, including glioblastoma multiforme (GBM), medulloblastoma (MB) and atypical teratoid/rhabdoid tumor (AT/RT) (Fig. 2). bCSCs share many of the same characteristics of normal neural stem cells which may be the progenitor of a bCSCs after transformation to an uncontrolled proliferation state.

### A. Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is an aggressive malignant brain tumor that originates from glial cells, accounting for 17% of all brain tumors (primary and metastatic). GBM is the highest grade (WHO grade 4) glioma and is the most common malignant brain tumor to occur in adults. A variety of cell types with different biological features are found in GBM and this intra-tumor heterogeneity makes effective therapy difficult to achieve. Targeting one type of cell in the tumor will not result in the elimination of all cancer cells present. Despite its lethal, aggressive nature, however, GBM rarely metastasizes to locations outside the brain.

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**Fig.1 Basic Concept of Cancer stem cell treatment.**

**Fig.2. Different types of brain cancer stem cells.**

**A. Glioblastoma Multiforme**

Glioblastoma multiforme (GBM) is an aggressive malignant brain tumor that originates from glial cells, accounting for 17% of all brain tumors (primary and metastatic). GBM is the highest grade (WHO grade 4) glioma and is the most common malignant brain tumor to occur in adults. A variety of cell types with different biological features are found in GBM and this intra-tumor heterogeneity makes effective therapy difficult to achieve. Targeting one type of cell in the tumor will not result in the elimination of all cancer cells present. Despite its lethal, aggressive nature, however, GBM rarely metastasizes to locations outside the brain.
As noted above, bCSCs are present in GBM and have been identified by the presence of the stem cell surface protein marker CD133. The ability to isolate a population of cells with stem-like properties including formation of neurospheres from patient derived GBM tumor samples also indicates the presence of CSCs. Due to their self-renewal and virtually unlimited proliferation capabilities, along with resistance to chemotherapy and radiotherapy, bCSCs pose significant challenges to the design of therapeutic strategies targeting GBM.

B. Medulloblastoma

Medulloblastoma (MB) is an aggressive tumor that is frequently metastatic throughout the neuraxis. Although rare in adults, MB is the most common malignant CNS tumor of childhood. MB is a tumor of the cerebellum, consisting of four principle distinct subtypes. The origins of MB depends on the subtype (WNT, SHH, group 3 or group 4) of the specific tumor. For instance, the subtype characterized by mutations of the WNT pathway, originates from cells of the dorsal brain stem, while the sonic hedgehog (SHH) subtype originates from granule neuron precursor cells. Group 3 and group 4 have more genetic heterogeneity. Metastasis often occurs in group 3 tumors, and this makes its prognosis very poor. There are currently few viable biomarkers for group 3 MB, other than natriuretic peptide receptor 3 (NPR3) positivity and amplification of v-myc avian myelocytomatosis viral oncogene homolog (MYC).

For group 4 a possible marker is KCNA1 and isochromosome 17q is also present on a large number of group 4 tumors. The intra-tumor and inter-tumor heterogeneity of MB indicates a role of bCSCs in tumorogenesis. Research also shows the presence of CD133 positive cells, a putative stem cell marker. Furthermore, the WNT and SHH pathways are closely related to CSCs, as both have been associated with a wide variety of tumor types.

C. Atypical Teratoid/Rhabdoid Tumor

Atypical Teratoid/Rhabdoid Tumor (AT/RT) is a rare (approximately 1-2% of all pediatric CNS tumors), aggressive brain cancer typically originating in the posterior fossa of very young children. AT/RT can be confused with a primitive neuroectodermal tumor (PNET), as 70% of its histologic fields are indistinguishable from PNETs. However, AT/RT is a distinct type of tumor consisting of rhabdoid tumor cells as well and are defined by mutations in the INI1 gene, which can occur spontaneously or be inherited. Like other aggressive tumors cellular necrosis if often present.

Cells positive for the stem cell surface marker CD133 have been isolated in AT/RT, indicating the presence of bCSCs. Therapy designed to target both the resultant effects of INI1 mutation as well as the CSC population will likely be more effective than simply targeting one or the other.

IV. THERAPEUTIC TARGETS

Standard therapeutic methods for cancers are generally ineffective due to cellular resistance to chemotherapy and radiotherapy. CSCs have been shown to have increased resistance to such therapy in the laboratory. Consequently, developing methods that target CSCs are of utmost importance. Specifically, in CSCs, there are three important pathways that may be targeted for cancer treatment: (i) the apoptosis pathway, (ii) autophagy pathway, and (iii) differentiation pathways.

A. Apoptosis-Targeted Therapy

Apoptosis, programmed cell death, is a key aspect of cell biology as a fail-safe mechanism. CSCs have a unique ability to evade apoptosis, allowing the onset of oncogenic events and resistance to the cytotoxicity caused by chemotherapy. Apoptosis in human cells is regulated via two main pathways: the extrinsic and intrinsic pathways. The extrinsic apoptotic pathway begins with the binding of proapoptotic ligands to surface death receptors (DRs), including CD95, nerve growth factor receptor (NGFR), and TNF-related apoptosis-inducing ligand (TRAIL) receptors. The resulting death-inducing signaling complex (DISC) initiates the cell death sequence. The intrinsic apoptotic pathway is initiated within the cell by stimuli such as oxidative stress, genetic damage, hypoxia, and ionizing radiation. Such stimuli result in excessive mitochondrial permeability and the release of pro-apoptotic molecules such as cytochrome-c into the cytoplasm. In both pathways, the activation of caspases is critical to cell death.

In CSCs, these pathways are deregulated resulting in apoptotic evasion and resistance to standard therapy. One mutation in CSCs is reduced caspase activity. Because caspases such as caspase-8 and caspase-3, important to apoptosis initiation and execution, are not as highly expressed in CSCs, apoptosis is decreased. The Bcl-2 family genes, which largely decide the fate of apoptosis in cells, are also altered in CSCs. Overexpression of anti-apoptotic genes of the Bcl-2 family in CSCs is crucial to apoptosis evasion. Abnormalities in death signaling pathways, such as downregulation of DR expression, also contribute to apoptotic evasion in CSCs. Small molecules that can restore apoptosis in CSCs may result in successful cancer treatment.

B. Autophagy-Targeted Therapy

Autophagy is a self-digestion process in cells, which maintains energy homeostasis and protein synthesis during cellular stresses. Autophagy plays a critical role in cancer stem cells biology. Evidence clearly shows that autophagy can either function as a tumor suppressor or a tumor promoter. In tumor initiation and inflammation, autophagy can act as a tumor suppressor; however, once formed, tumor cells become more resilient due to autophagic processes. When stressed by factors such as oxidative stress, tumor cells have been shown to activate autophagy, allowing them to survive for long periods. For instance, when faced with hypoxia, tumor cells are able to trigger release of Beclin1, which induces autophagy.

This type of resistance to stresses, including treatments (e.g., chemotherapy), gives CSCs a distinct advantage over therapeutic methods. Autophagy in cancer cells results in stress tolerance, which limits damage, maintains viability even in dormancy, and facilitates their recovery. As a result, therapy
that directly inhibits autophagic capabilities in CSCs could be more effective than current practices.

C. Differentiation-Targeted Therapy

CSCs have the ability to quickly proliferate through self-renewal and even undergo metastasis. However, once CSCs transition into normal cancer cells, whether benign or malignant, they may be able to differentiate and then cannot further proliferate. Differentiation therapy targets this aspect by inducing CSC differentiation. Once the pool of CSCs is reduced, chemotherapy and radiotherapy may be more effective as resistant cells are eliminated from the population. In acute promyelocytic leukemia, this strategy has been effective, with a long-term survival rate of 75%. In many tumors, such as GBM, all-trans-retinoic acid (ATRA) has been shown to increase differentiation and decrease proliferation. Two transcription factors—retinoic acid receptors (RARs) and retinoic X receptors (RXRs)—mediate the actions of the RA pathway. ATRA acts by interacting with RARs and RXRs, which bind as homodimers or heterodimers to specific DNA response elements. This triggers the transcription of nearby, relevant genes, which can cause cell differentiation. ATRA has also been found to inhibit CSC proliferation promoters such as VEGF and EGFR.

When retinoic acid differentiation therapy is followed by chemotherapy, which kills the differentiated cancer cells, tumors can go into long-lasting or even permanent remission. The challenge lies in the fact that some CSCs do not differentiate, but rather remain in the cycling phase due to chromatin-modification enzymes. To this end our group is developing novel boron based retinoids to study the biology of stem cell and small molecule based CSC inhibitors.

V. SMALL MOLECULES AVAILABLE FOR THERAPY

Because of the presence of bCSCs in various forms of neurological tumors, therapeutic strategies using small molecules to target CSCs have potential to improve therapy. Specifically, differentiation/renewal pathways and transcription factors of bCSCs may be useful molecular targets for the small organic compounds.

A. Inhibitors of Signaling Pathways

Two major growth factor pathways in GBM CSCs are vascular endothelial growth factor (VEGF) and platelet-derived endothelial growth factor (PDGF). Targeting these pathways can block proliferation of tumor cells originating from CSCs, restricting progression of GBM. Bevacizumab, a humanized monoclonal antibody, has been shown to prevent tumor angiogenesis to a certain extent by inhibiting creation of normal tumor cells from progenitors, but not the generation of cancerous progenitor cells from stem cells. Other VEGF inhibitors, such as the small molecule Cediranib (AZD2171), and EGFR inhibitors, including gefitinib, have potential for therapy as well.

In many brain cancers, such as MB, the WNT pathway plays an important role in tumorigenesis and CSC development. Inhibiting the beta-catenin transcription factor of this pathway can, in turn, inhibit the WNT pathway and be effectively used in therapeutics. The small molecule XAV939 has been shown to stabilize axin (through tankyrase inhibition) and cause beta-catenin degradation. Other small molecules, such as ICG001, PKF118-310, and SEN461, also target the WNT pathway as a beta-catenin antagonist. This mechanism of WNT pathway modulation shows potential for targeting bCSCs in diseases like MB. The SHH and Notch pathways are also important signaling pathways in CSCs and drug resistance. Cycloamine, vismodegib, and LDE225 have all been shown to inhibit SHH, while RO4929097 and MK-0752 inhibit the Notch pathway.

Signal transducers and activators of transcription 3 (STAT3) signaling is common in many neural tumors. Inhibition of the STAT3 pathway has been shown to reduce cancer cell proliferation. A recent study has shown the cell-permeable small molecule called LY5 to effectively target STAT3 in MB cells. Application of this small molecule in other neural tumors like GBM will likely also have positive therapeutic effects and detail chemical structures are in (Fig. 3).
Small molecules are clearly effective at inhibiting pathways and factors important to bCSCs and tumorigenesis. The current challenges lie in synthesizing small molecules that do not have parallel adverse physiological effects and can achieve complete elimination of CSCs in tumors. Besides the small molecules themselves, the delivery systems are also critical components in therapy.

D. Small Molecule Delivery Systems

The primary challenge facing drug delivery to brain tumors is the blood-brain barrier (BBB). The brain is separated from peripheral circulation by the BBB, which serves to prevent harmful substances from entering the brain. Drug delivery can be achieved by targeting the four main endogenous mechanisms: (i) adsorptive-mediated transcytosis (AMT), (ii) transporter proteins, (iii) cell mediated transcytosis (CMT), and (iv) receptor-mediated transcytosis (RMT). Several strategies that target these mechanisms currently show promise. For instance, cell-penetrating peptide-based delivery can overcome the lipophilic barrier of cell membranes in AMT. Drug delivery systems modified for specific neural receptors such as the highly expressed transferrin receptor shows potential for targeting RMT.

Nanotechnology can be used for drug delivery as well. Nanoparticles (NPs) are small enough to pass through the BBB and can effectively carry and release the small molecular drugs. Proper modification of the NP will allow it to pass into the brain’s circulation by one of the above mechanisms. A common approach for NP modification is applying a coat of hydrophilic surfactants, including polysorbates and polyethylene glycol (PEG). Other methods used to increase availability of the drug in the brain include liposomes, microspheres, nonogels and nonobiocapsules.

VI. CONCLUSION AND FUTURE DIRECTION

Cancer stem cells play a crucial role in tumorigenesis of many different types of cancers. These cells share many properties of normal stem cells, including virtually unlimited self-renewal and differentiation. Surface protein markers, such as CD44 and CD133, have indicated their presence in tumors; additionally, the self-renewal capability and heterogeneity of many tumors points to the presence of a malignant stem cell driving tumor progression. Including above two cell surface marker other cell surface markers (laminin / integrin α6 and CD15) are also critical for cancer stem cell. While current methods of chemotherapy and radiotherapy attack normal tumor cells, cancers often relapse due to the presence of a self-renewing cancer stem cell. In order to effectively cure tumors, drugs that target CSCs (specifically the pathways and transcription factors critical to their development) must be developed and delivered to the tumor site. Although there are some small organic molecules and modifiable delivery systems available, further advances are necessary to eliminate or prevent the onset of cancers, such as GBM and MB. Future development in this field will open up new therapeutic avenues for a myriad of diseases.
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References


