Glioblastoma Multiforme: A Review on Causes, Mechanisms, and Solutions

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ABSTRACT
Glioblastoma multiforme, or GBM, is one of the most invasive and destructive forms of brain cancer. One characteristic that makes GBM so destructive is its resistance to radiotherapy and other forms of therapeutic cancer treatment. Due to increased resistance, most modern therapeutic treatment protocols are not effective with median survival post-treatment of only from 9 to 14 months. Additionally, there are no known pharmaceutical agents that are effective in eliminating or limiting cancer stem cells. At this point, the most effective method of treatment is to target intracellular signaling pathways of cancer stem cells that have not undergone neoplastic transformation and use these pathways to arrest adhesion and proliferation of cancer stem cells. It is important to target these cells before they undergo neoplastic transformation because after that point they are tumorigenic. Many recent approaches of creating therapeutic drugs for treating these GBM cancer stem cells are based on modern cellular and post-genomic technology. At this point the most effective approach for therapeutic drug development is believed to be a combination of targeted therapeutic treatment procedures along with effective regulation of many of the cellular processes of cancer stem cells using their signaling pathways.

Keywords
Causes, Glioblastoma, Mechanisms, Multiforme, Treatment.

Abbreviations
CIMP+: CpG island methylator phenotype; CSC: Cancer stem cell; GBM: Glioblastoma Multiforme; GSC: Glioma stem cell; HGG: High grade glioma; MGMT: O\textsuperscript{6}-methylguanine-DNA methyltransferase; NSC: Neural stem cell; TMZ: Telozolomide.

Introduction
Cancer is among the most prominent and invasive diseases in today’s society [1]. In the majority of countries around the world, it is among the leading causes of death in both old and young people, behind infectious disease in working age people and cardiovascular disease in the elderly [1]. Cancer can occur in many varieties, with the most deadly contributors to increased mortality being lung, breast, colon, and prostate cancer, in that order [1,2]. In economically advanced countries, targeted therapeutic treatments have been used to treat and extend the lives of patients with solid tumor masses [1]. However, no such treatment has been found for glioblastoma multiforme, which is commonly referred to as GBM, or grade IV astrocytoma [1]. GBM accounts for over 50% of society’s primary brain tumors and roughly 20% of the world’s intracranial neoplasms [4]. GBM occurs with fairly high frequency, roughly 5.25 cases for every 100,000 people and is diagnosed in the United States with over 17,000 cases each year [4]. Treatment of GBM is fairly similar to that of other tumors and includes procedures such as surgical tumor removal and chemotheraphy, but overall success in patients with GBM is extremely poor. For patients following proper procedures of modern treatment methods, the median survival rate is 16.2 months in patients 20-44 years of age, 7.9 months for patients 45-69 years of age, and 3.2 months for patients over 70 years of age [4].

Cancer stem cells, or CSCs, are cells found within tumors that have characteristics associated with normal cells that do not express traits indicative of cancer cells. CSCs play a critical role in the increased resistance of GBM to known therapeutic treatments, as there are no known clinical means to effectively destroy these cells [5]. Studies have shown that genetic and metabolic pathways form complicated signaling networks to mediate crosstalk between oncometabolic (metabolic processes affected by cancer) and oncogenic (genome affected by cancer) pathways that lead to cancer.
initiation, propagation, and therapy resistance [5,6]. Experimental evidence has shown that targeted therapeutic treatments could be used to inhibit adhesion, proliferation, and migration of CSCs [6]. The main objective of treatment is to reach the membranous regions of intracellular signaling pathways of cancer cells that have not undergone neoplastic transformation to any extent [7].

Increased evidence has confirmed that GBM may arise from a collection of cells called glioblastoma stem-like cells, or GSCs [8]. These cells differ from CSCs in the sense that they possess both tumor-initiating and tumor-perpetuating properties and are capable of creating an identical phenocopy of the original tumor [8]. These cells have very high plasticity and can adapt to attain increased therapeutic resistance due to their immature differentiation state and strong-self renewal potential [8]. Evidence from various experiments performed in mice show that the most preferred cell type for glioma development is neural cells. Evidence has also been found indicating that GBM can arise from both progenitors or mature differentiated cells within the central nervous system [9-11]. This occurs through the addition of new neurons and remodeling of the neuronal circuiting in response to adult neurogenesis [11].

The centralized focus of this literature review is to combine and organize information regarding the creation and use of cell and post-genomic technology in the treatment of glioblastoma. Materials utilized include scientific and technical studies as well as current literature regarding this topic available through PubMed, Wiley, Spandidos, and ScienceDirect databases.

**Current Standard of Care**

Today’s standard of care for newly diagnosed GBM cases primarily consists of several different common procedures used together, such as removal of the tumor, chemotherapy, and radiotherapy [12]. Many surgical procedures used for tumor removal can be extremely destructive to tissues of the body, especially if the tumor is located deep within the brain matter or near vital centers of the brain stem [12]. At that point, precise surgical equipment and/or modern radiosurgical technology is required for the procedure to be successful and produce favorable patient outcomes [12].

Standard delivery of radiotherapy to the brain typically consists of 25-30 rounds of X-ray treatment spaced out over 5-6 weeks, equating to roughly five weekly treatments with a boost dose of roughly 1.8-2.0 Gy [13]. The maximum boost dose has been determined to be 60 Gy [13]. Exceeding this dose can lead to additional complications, most commonly brain necrosis [4,13]. Theories have been formed suggesting that a total brain irradiation of 50 Gy administered immediately will help to prevent relapse of glioblastoma, but a systematic course of 50 Gy irradiation over a span of 3-5 years will result in irreversible mental disability as a result of significant radiation damage to the central nervous system [4,14]. If this treatment were to produce successful results, the patient could potentially be seriously disabled and have severe post-radiation intellectual-mnestic and neurological disorders [13,14].

Another treatment that has been utilized in today’s standard of care for malignant glioblastomas is temozolomide (TMZ) coupled with radiation therapy, which is the basis of chemotherapy. Phase III clinical studies have shown that adding TMZ to postoperative radiation treatment has produces a significant survival benefit [15]. TMZ has shown to be an alkylating agent within the brain and is typically biologically counteracted by upregulation of O6-methylguanine-DNA methyltransferase (MGMT) expression [16]. Studies have been conducted that exhibit strong positive correlations between MGMT expression and resistance of malignant glioblastomas to TMZ treatment [16]. Results suggest that essentially all patients receiving TMZ treatment will have relapsed with a progression free survival rate of zero for patients five years post-treatment [16-18].

However, initial stages of standard treatment for glioblastoma multiforme have proven to work fairly effectively [18]. This initial treatment is usually followed by a period of no glioblastoma relapse and complete absence of tumor mass within the area of treatment known as a “clean slate”. After this “clean slate” period, relapse is prone to occur in the form of neurological deficits or growth of new tumor masses [19]. These tumors are usually located in the tumor bed of the preexisting tumor mass and occurs in 95% of patients 7-20 months post-treatment [19]. This relapse requires additional surgical tumor removal and X-ray treatment or additional chemotherapy if radiotherapy cannot feasibly be performed, due to tumor location or other characteristics [19]. Targeted therapeutic procedures for glioblastoma have not been proven to have significant effects on the survival rate of patients who have relapsed [20,21]. The survival rate of patients have proven to be almost no different following therapy [21].

Given this information, we can theorize that even though targeted procedures used to treat tumors of the central nervous system are based primarily on the prior principles of tumor treatment, these procedures are effectively useless on the cancer stem cells that are believed to be the primary cause of glioblastoma’s high therapeutic resistance.

**Glioma Stem Cell Subtypes**

Evidence has suggested glioblastoma and other high-grade gliomas (HGGs) have conferred their immense therapeutic resistance through both genetic and metabolic pathways [22]. Modern genomic expression profiling and DNA methylation analysis with clinical evaluation have allowed us to categorize high-grade gliomas into three different subtypes: classical, proneural, and mesenchymal [22]. Proneural gliomas comprise roughly 26% of all high-grade glioma cells, mesenchymal comprise 35%, and classical comprise 39% [22-24]. Classical HGGs typically target the oldest group of individuals, with their target group having an average age of 57.7 years compared to 53.4 years for mesenchymal HGGs and 47.8 years for proneural HGGs [24].

Extensive experimentation has shown classical and mesenchymal HGGs to have conferred resistance to modern therapeutic agents, whereas these agents have proven effective in killing mature proneural HGGs [25,26]. Proneural HGGs can be divided into
two subcategories: roughly half of proneural HGGs are associated with CpG island methylator phenotype (CIMP+), mutations in IDH1, a common metabolic enzyme, and the oncometabolite 2-hydroxyglutarate [26]. Clinical evidence has shown that this group of tumors begin as a low-grade glioma and slowly progresses into glioblastoma [27]. This subclass of glioblastoma has shown to be treated with much more success than other forms of glioblastoma, thus different therapeutic treatment plans should be created for this class of GBM [27]. The other half of proneural HGGs are associated with amplification of the gene PDGFRα. These two subclasses show various overlapping gene signatures but their responses to therapeutic treatments and their respective clinical outcomes have proven to be very different from one another [28]. On the other hand, mesenchymal gliomas are non-CIMP, retain wild-type IDH1, and are typically associated with deregulation in the NF1 gene and its signaling pathway as well as various somatic mutations [27-29].

Unlike the significant differences in the mutated cell signaling and gene expression patterns of both proneural and mesenchymal gliomas, classical gliomas show much more delicate epigenetic and genetic changes, other than the sharp changes in the EGF signaling pathway. Despite each subclass’s distinct collection of genetic changes, it has been shown that responses to subsequent therapeutic treatments for each of the three subclasses are extremely poor, with the exception of CIMP+ proneural gliomas [30,31]. Given that each subclass of HGG is based on a different collection of alterations to their cell signaling pathway, different therapeutic treatment methods should be developed for each individual subclass rather than applying the same treatment procedures to all cases of glioblastoma [31].

Specific Markers for Each GSC Type within Tumors
A significant characteristic of cancer stem cells is their ability to regenerate and reform tumors from the remaining tumor mass following surgical removal by common therapeutic procedures, directly indicating how effective these cells remain stable following treatment as well as the consequent risk of recurrence or relapse for individual patients [32]. Initially the definitive marker for glioma stem cells is the CD133 gene, but results revealing other possible markers have led to additional studies being conducted. Additional research has uncovered multiple other genes to be tumor-initiating markers, such as CD15, L1CAM, and integrin α6 [32,33]. These cells have shown to not only enrich the cells that possess tumor-initiating potential, but also play a significant role in the development and expression of the glioma stem cell phenotype. While many of these antigens have correlated with GSC expression, none of them have proven to be definitive markers for GSC expression within human-grade gliomas, or HGGs [34]. Many studies have shown proneural HGGs to contain CD133 and CD15 antigens in their GSCs, whereas expression of these two antigens is almost completely absent in mesenchymal HGGs [34-36]. Instead, mesenchymal HGGs have shown significant expression of CD44, another surface marker that have been shown to be correlated with GSC expression [34]. Experimental evidence has also shown that expression of any of these three antigens show a statistically significant negative correlation with survival rates of patients with GBM [34]. Based on these results, one could conclude that these genes have value in terms of predicting clinical outcomes for patients, however additional research must be conducted to determine the correlations between each of the individual markers with GSC expression [34].

While relating antigen presence to GSC expression can prove to be a valuable tool, it may be even more valuable to determine the expression of a lineage-specific antigen in specific subsets of GSCs. For example, CD15 is commonly expressed in both embryonic and mature neural stem cells, demonstrating the original cell of proneural HGGs is a neural stem cell in either embryonic or mature brains [34]. Another prime example of this is CD44, which is commonly present in astrocyte-restricted precursor cells that have lost the immature, differentiable state of neural stem cells and are committed to development as astrocytes [34]. Experimental evidence has shown that mature astrocytes have been successfully transformed in mouse models of glioblastoma, which supports the notion of lineage-specific antigens being present in specific subsets of GSCs [34]. Additionally, expression of cell surface markers such as PDGFRα and a NG2, both of which are characteristic of oligodendritic precursor cells, have led to the proposition of a potential non-stem cell source of oligodendrocytomas [34-36]. Based on the findings stated above, it can be theorized that different subtypes of gliomas likely originate from distinct cell types.

Cancer Stem Cells
Cancer stem cells, or CSCs, are distinguished by their ability to trigger the initiation of tumor formation when inserted into tissue [37]. These cells have been found in glioblastoma as well as breast, prostate, and pancreatic cancers. The origin of CSCs is not definite for any type of cancer [1,37]. They are likely to arise upon disruption of tissue mechanisms that control proliferation of clonogenic cells that cause phenotypic transitions to altered states causing subsequent tumor formation [37]. These cells also cause genetic changes, promoting activation of oncogenes, such as SOX2, in addition to slowing the action of tumor-suppressor genes and undermining the epigenetic control involved in gene expression [37]. These cells have a greatly improved survival mechanism that is largely based on telomere-shortening prevention, ultimately immortalizing the cell [38].

Most glioblastoma cancer stem cells arise in either the neural stem or progenitor cells, which are made evident by the similar immunocytochemical markers that are present on their respective surfaces [38,39]. Additionally, roughly 64% of neural stem cells and cancer stem cells are identical in nature [39]. On a smaller scale, microRNAs have shown to have a significant role in stem cells of both normal and cancer cells [40]. A microRNA is a small non-coding RNA molecule and deregulation of microRNA expression is a significant contributor to GBM development [40]. Various studies have produced substantial evidence showing aberrant expression of microRNAs in GBM cancer stem cells [40,41]. Expression profiling analyses have shown that miRNA-21 is amongst the most frequently upregulated microRNAs present in GBM, as this
aberration has shown to be present in 44-100% of GBM cases studied [40,41]. Northern blot and RT-PCR tests have also shown that miRNA-21 is frequently upregulated in glioblastoma tumor samples [40,41]. Functional analyses of miRNA-21 suppression in GBM cells have shown that this suppression usually leads to decreased cell growth, increased apoptosis rates, and reduced tumorigenicity, and overall invasiveness [40]. Experimental evidence has shown that miRNA-21 upregulation was linked to three major cancer pathways: p53, TGF-β, and mitochondrial-initiated apoptosis. The subsequent knockout of miRNA-21 in glioblastoma cell lines led to activation of several different tumor suppressor proteins, including p53, Bax, DAXX, APAF1, p21, Tap63, and TGFB2 [40]. These results suggest that miRNA-21 functions as an oncogene for GBM. Given this notion, further studies are being conducted in order to determine the effects of miRNA-21 in the realm of therapeutic treatment [40,41].

The most obvious mechanism by which GBM occurs is through the accumulation of mutations through consistent proliferation throughout the life of the individual, which eventually serves as the starting point for development of tumor mass [22,42]. Another mechanistic theory is that the products of oncogene expression can cause NSCs to undergo a transformation and subsequent development into cancer stem cells [42]. The oncogenes can produce byproducts as in response to interactions with pathologically altered cells, cell matrix elements, or during the cell fusion process [22]. CSCs are able to provide gliomas with considerably increased resistance by streamlining the processes involved in invasive growth, secure formation of timorous blood networks as well as lymph and nerve networks, and create a barrier between the developing cancer cells and the chemical agents of medications through interactions with fibroblasts and cells of the vascular endothelium [22].

CSC division is predicated on several signaling pathways, such as Notch, bone morphogenetic protein (BMP), Wnt/β-catenin, sonic hedgehog (Shh) and STAT3 [43]. Notch receptors are very diverse and are involved in cell differentiation, survival, and tumorigenesis [43]. Notch signaling occurs through direct intercellular contact, as Notch receptors typically serve as transmembrane receptors for the Jagged (Jag1-2) and Delta-like (DII, 3, 4) ligands [43]. These receptors are cleaved upon activation and their intracellular components traverse to the nucleus of the cell and act as transmembrane factors together with the CBF-1 protein [43]. This causes transcriptional repressor genes to slow the expression of proneural genes thus stopping neuronal differentiation [43]. Experimental evidence has shown Notch pathways to incur increased resistance to therapeutic treatment of GBM, leading to increased hardness of the tumor cells [43].

BMPs are a family of cytokines that moderate NSC proliferation, apoptosis, and differentiation [44]. More importantly to the process of tumor formation, BMP signaling is known to be an effective factor in the inhibition of neuron formation [44]. BMP signaling also serves to disrupt cellular proliferation in CSCs, promoting astrocyte-like differentiation [44]. The Wnt/β-catenin pathway is also believed to be critical in the modulation of mature NSC differentiation [44]. Within this pathway, β-catenin serves to transmit signals from the cell surface to the nucleus [44]. Alternatively, β-catenin is phosphorylated by a protein complex containing glycogen synthase kinase 3β in the absence of surface signals and is then degraded by proteasomes [44]. β-catenin buildup occurs upon GSK3β inhibition, which occurs in the presence of Wnt signal activation [44]. This accumulated β-catenin then traverses to the nucleus and upregulates the expression of growth-related genes. Alteration in this Wnt signal pathway in glioblastoma cells leads to a negative prognosis and inhibition of this pathway in CSCs decreases cell proliferation and therapeutic resistance [44].

Shh signaling pathway alteration has also been known to result in development of several forms of cancer and is associated with tumor development and metastasis [45]. Shh is secreted by the body at various points in development [45]. Shh then binds to the receptor Ptc (patched) in order to relieve Smo (smoothened) inhibition, which would allow Gli proteins to be transcribed [45]. The Shh/Gli signaling pathway is necessary for CSC survival [45]. STAT3 has also been found to be crucial in NSC development as well as GBM formation [46]. STAT3 can be activated by many different cytokines and growth receptor factors [46]. Upon activation, STAT3 acts as a transcription factor, entering the nucleus and triggering expression of several different precancerous proteins associated with cell cycle progression [46]. Experimental evidence has shown that treatment of CSCs with STAT3 DNA-binding inhibitors can inhibit cell proliferation as well as neurosphere formation. It was also shown to lead to apoptotic cell death in CSCs [46].

Phenotypic Expression of Cancer Stem Cells

In addition to their genotypic differences, each subtype of GBM has markedly different phenotypic effects as well [47]. Mesenchymal CSCs are notably more invasive, destructive, and resistant to therapeutic treatment than the other CSC subtypes both in vivo and in vitro [26,47]. Mesenchymal GBM is also derived primarily from de novo primary GSCs, as opposed to proneural GBMs that arise from both GSCs as well as Grade II gliomas [26]. However, more invasive growth in vitro does not necessarily make a GBM subtype more clinically destructive than others [47]. Cancer growth is fairly dependent on a patient’s immune response to invasive tumor cells [47]. Mesenchymal GBM growth appears to be reliant on an individual’s compromised immune responses as demonstrated by several specifically upregulated immune-related genes within mesenchymal CSCs [26,48]. This theory is affirmed by the significantly different clinical responses of mesenchymal GBM development in comparison to other GBM subtypes [26, 48]. Translational research laboratories have addressed this response by genetically engineering GBM models in mice to include different GBM subtypes in order to develop an understanding of the pathophysiology of each individual GBM subtype within an organism with an intact immune system [48].

Experimental evidence has shown that GSCs expressing
CD133 upregulation have demonstrated increased resistance to therapeutic radiation treatment compared to non-GSCs in the same tumor masses [32,33]. This is due to a better activation of DNA damage checkpoint responses in non-GSCs [32]. Proneural and mesenchymal GSCs also express significantly different levels of sensitivity to radiation [33]. Mesenchymal GSCs are notably more resistant to radiotherapy than proneural GSCs and exhibit significantly upregulated DNA repair genes [32]. Mesenchymal tumors have also demonstrated upregulated YKL40 and CD44 expression, both of which have correlated with increased resistance to radiation and decreased overall rate of survival [49]. Data has shown that mesenchymal GSC-derived mouse GBM tumors can undergo DNA damage repair much more quickly and efficiently in vivo than proneural GSC tumors, as demonstrated by the reduced γ-H2AX foci in mesenchymal tumor cells [49].

**Plasticity of Glioma Stem Cells**

Although the exact number of GBM subtypes is still unknown, proneural and mesenchymal GBM subtypes exhibit robust difference and are essentially mutually exclusive GBM subtypes [26]. It has been shown that in almost all cases of GBM tumor recurrence following failure of standard therapeutic treatment the tumor recurrence is accompanied by a phenotypic shift from proneural to mesenchymal GBM cells [50]. Studies have shown elevation of mesenchymal tumor markers in patients treated with bevacizumab, an anti-angiogenic agent [50]. The believed mechanism behind this elevation involves activation of oncogenic receptor tyrosine kinases induce EMT-like programs and enhance mesenchymal characteristics within GBM cells [50]. Additionally, radiotherapy of proneural GBMs led to a downregulation of proneural markers and overexpression of mesenchymal markers, showing that EMT-like phenotypic changes in both GSC and their progeny can occur as a result of radiotherapy [50]. It has been observed that more proneural GSCs are derived from Grade III gliomas and more mesenchymal GSCs are derived from Grade IV gliomas, furthering the point that a mesenchymal signature is of more significance in GSCs [26,50].

**Current Limitations/Conclusion**

Current studies have raised many questions that allow for further investigation. The effects of microRNAs and their effects on increased expression levels in GBMs and their effects on therapeutic resistance requires additional research. The clinical significance of both proneural and mesenchymal GBM subtypes also requires further investigation. While mesenchymal signatures are much more aggressive, lead to much worse complications, and have much more overall clinical significance, it is imperative to gain a greater understanding of the exact mechanisms and prognoses of both proneural and mesenchymal GBMs. The concept of cancer stem cells is one of the most recent advances in experimental oncology. Cancer stem cells allow us to observe the processes of dynamic modification of tumor masses in the use of traditional procedures of cancer therapy. This along with knowledge of malignant tumor growth, proliferation, differentiation, and self-renewal allows us to better understand the signaling mechanisms of malignant tumors and develop better therapeutic procedures to both destroy them and prevent their recurrence.

**References**


