Glioma Stem Cells and their Therapy Resistance

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Abstract

Glioblastoma multiforme (GBM) is the most highly invasive and malignant primary brain tumor in humans with median survival after diagnosis as low as 12-15 months. The poor prognosis of GBM is attributable to its resistance to current therapeutic approaches, consisting of maximal debulking surgery, chemotherapy with temozolomide, and radiotherapy. Amongst the heterogeneous population of tumor cells found in GBM, a self-renewing and proliferating cell type known as glioma stem-like cells (GSC) has been identified as a potential source for glioma therapy resistance. It has been well documented that current therapies fail to effectively eliminate GSC from the tumor population. This contributes to the virtually inevitable tumor recurrence in GBM patients following treatment. Therefore, GSC provide a particularly attractive target for the development of future therapies. This review highlights several proposed mechanisms behind therapy resistant glioma cells including DNA repair mechanisms, cell cycle checkpoints, drug efflux processes, and the role of the tumor microenvironment. Furthermore, several therapeutic strategies to target genes or pathways specific to GSC survival and proliferation will be discussed.

Therapy Resistance of Glioblastoma

Glioblastoma multiforme (GBM) is widely recognized as one of the most devastatingly aggressive primary brain tumors in humans, with the median survival of affected patients being as short as 12-15 months. Extensive research is being dedicated to identify effective therapeutic targets for these malignant tumors but current therapies have shown only palliative effect. One of the major contributing factors to the high morbidity and mortality of GBM is its resistance to these therapies, as virtually all patients in developed countries die due to recurrent tumors following the current standard care of treatment, not because of primary tumors. Therapy resistance, therefore, refers to the inability of current therapeutic approaches to destroy or arrest growth of the entire tumor mass, either by de novo or acquired mechanisms.

De novo therapy resistance is characterized by the presence of tumor cells (possibly a subpopulation of tumor cells) that possess certain genetic and/or epigenetic mechanisms to overcome the actions of chemoradiotherapies for cancers and lead to re-growth of residual tumor cells following treatment. Thus, current therapies may result in accumulation of tumor cells that are selected in the course of cancer treatments. In turn, acquired resistance implicates phenotypic alterations of naive tumor cells to more aggressive tumors after chemoradiotherapies. This resistance emerges over time, possibly due to stimulated genetic instability, accumulated gene mutations, and/or epigenetic alterations of tumor cells in response to treatment. The principle of therapy resistance manifests as recurrent GBM tumors in spite of extensive surgical resection, chemotherapy, and/or radiotherapy (Figure 1). Subsequently, the efficacy of second rounds of chemo or radiotherapy for recurrent tumors end up substantially less appreciable after the initial short-term disease control for primary tumors.

One common feature of various malignant tumors, including GBM, is that they are composed of heterogeneous populations of tumor cells. Cancer formation is described as a constant evolutionary process characterized by a loss of organized histology and gain of complexity during malignant transformation (Figure 2). Until recently, the significance of the presence of multiple different types of tumor cells has been underestimated. In addition to contributing to the phenotypic manifestations of the specific tumor, this diversity of tumor cell populations also affects the tumor’s response to treatment measures. Recognition of the increased heterogeneity in GBM raises the question of which subset or subsets of cells within GBM is (are) responsible for this resistance.

Thus, an understanding of which cells in malignant tumors such as GBM are resistant, or gain resistance, to current therapies is imperative to elucidate the mechanisms responsible for therapy resistance.

What are cancer stem cells?

In the last decade, cancer stem cell populations have gained substantial attention by physicians and scientists. Lapidot et al. were amongst the first to document the idea of cancer stem cells in scientific literature. Through transplantation of acute myeloid leukemia cells into severe combined immune-deficient (SCID) mice, they identified a population of proliferating cells capable of generating morphologically similar cells to the original leukemia patient [1]. This concept was extended further by Reya et al. who proposed the possibility that these cancer stem cells play a role in the tumorigenesis of additional cancers beyond leukemia [2]. Subsequent studies by other labs provided experimental evidence for the existence of solid tissue tumor-stem-like cells in glioblastoma [3] as well as in tumorigenic breast cancer cells [4]. While substantial attention has been focused on the potential role of unlimited self-renewal and proliferation of stem cells as an initiating event for many cancers, Passegué et al. suggested it is important to also consider the possibility of more committed cancer stem cells that can give rise to tumor heterogeneity and resistance against therapies.

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cells undergoing mutations or altered gene expression to reacquire stem cell characteristics and lead to cancer development [5].

As is the case with other cancers (e.g., breast cancers, colon cancers, prostate cancers, and some leukemias), cancer stem cells in glioma (glioma stem-like cells; GSC) appear to share many characteristics and functional properties with somatic neural stem cells (NSC). Both GSC and NSC are defined as cells with self-renewal capacity and multipotential differentiation capacity [6,7]. NSC in the subventricular zone of the adult brain give rise to three major cell types: neurons, astrocytes, and oligodendrocytes [8]. Likewise, in the experimental setting with an intracranial xenograft model using immunocompromised mice, GSC are defined based on their ability to give rise to tumors that recapitulate the original tumors from patients [9,10]. Therefore, GSC could play major roles in the initiation of de novo GBM [11]. More importantly, GSC may represent a therapeutic cellular target in recurrent tumors following failure of the current treatment regimens, as they may represent the origin of recurrence.

Hurdle to attack glioma stem cells

Despite improvement of surgical techniques and discovery of a new FDA-approved chemotherapeutic agent, temozolomide [12], recurrence of tumors is still inevitable in virtually all cases. Given that GBM is composed of multiple heterogeneous tumor cell populations, it is likely that current therapies are selecting for a relatively small fraction of therapy resistant cells among billions or trillions of tumor cells that continuously drive tumors more aggressive and life-threatening. If glioma cells that have properties of stem cells are more therapy resistant than the rest of tumor cells, one obvious challenge is how we target GSC by identifying mechanisms underlying self-renewal and proliferation specific for GSC [13].

A body of recent evidence supports the relative therapy resistance of GSC. Bao et al. described convincing evidence for resistance of GSC to radiation therapy in vitro and in vivo [14]. They used an antibody for a cell surface protein, CD133, to separate GSC from the rest of the tumor cells that possess no, or substantially less, tumorigenic potential. In this study, it was demonstrated that experimental radiation treatment on a mixed population of tumor cells preferentially kills CD133-negative cells, resulting in enrichment of CD133+ tumor cells. These data indicate that CD133-positive GSC is more resistant to radiation therapy than CD133-negative tumor cells [14]. While this study effectively demonstrates an important component of therapy resistance, it also highlights the lack of a definitive and universal method for isolating a pure population of GSC from the entire population of tumor cells. Improved methods are needed to determine if we can generalize the findings to GBM or if they are applicable to certain GBM patient subpopulations.

The ineffectiveness of temozolomide (TMZ) at blocking GSC self-renewal has been further demonstrated by Clement et al. in a study focusing on the regulation of GSC by the hedgehog signaling pathway [15]. This study highlights the effect of cyclopamine, a known hedgehog suppressant, on the activity of GSC. Cyclopamine was shown to reduce GSC proliferation, increase apoptosis, and prevent neurosphere self-renewal in vitro. Due to its general cytotoxic effects, TMZ was shown to reduce glioma cell proliferation and survival but was unable to prevent self-renewal, a characteristic unique to GSC. Therefore, while TMZ was shown to act synergistically with cyclopamine, this was not due to its effect on GSC, but rather its overall cytotoxic abilities [15]. The findings regarding the ability of cyclopamine to target GSC self-renewal suggest the hedgehog pathway could serve as a potential therapeutic target. In fact, this approach is currently being implemented in a clinical trial and is showing promising initial results in patients with medulloblastoma [16]. However, due to involvement of the hedgehog pathway in normal stem cell self-renewal, a major concern of this approach would be potential side effects on maintaining normal NSC.

There is, however, at least one study which provides evidence for TMZ targeting of GSC. By exposing both CD133+ and CD133- cells to varying concentrations of TMZ, Beier et al. have shown a dose- and time- dependent decline in the proliferation of these cell lines [17]. This study also demonstrated a reduction in the sphere-forming, clonogenic potential of CD133+ cells in response to TMZ treatment - further evidence suggesting TMZ targets GSC. While TMZ did not effectively induce cell death, data indicating its ability to potentially inhibit GSC proliferation has major implications for the future development of therapeutic measures. Additionally, this study shows increased efficacy of TMZ when used against tumors lacking specific DNA repair proteins [17], suggesting that, under optimized conditions, TMZ could serve as a therapeutic agent to target GSC. Despite these findings, TMZ is not believed to result in long-term survival of GBM patients. Further research and drug development is needed to understand why GSC-targeting agents continue to fail to provide a long-term cure.

A potential reason for the mixed results concerning the effect of TMZ treatment on GSC is due to the lack of a definitive method to isolate pure populations of stem cells and non-stem cells in tumors. As described above, a number of recent studies rely on CD133 to enrich, but not to purify, GSC from the bulk of tumor cells. Studies by Phillips et al. have suggested that perhaps CD133+ is not the only marker which...
Recent subclassification of GBM into 3 (or 4) subtypes may deepen our understanding of therapy resistance of GBM, or it may make it complicated to characterize therapy resistance of GBM as a whole. Questions are raised as to whether GSC in different subtypes retain differences in their therapy resistance activity and whether or not targeting the various subtypes will have significant impact on GBM patient prognosis. Whole genome analysis has shed light on whole. Questions are raised as to whether GSC in different subtypes will have significant impact on GBM patient prognosis. Whole genome analysis has shed light on whole. Questions are raised as to whether GSC in different subtypes will have significant impact on GBM patient prognosis.

Whether GSC in the mesenchymal subtype are phenotypically and genetically similar to those in the proneural type remains to be fully elucidated. Also, whether using the same (or similar) experimental conditions reasonably enriches for individual GSC is another open question. Continued research for potential additional GSC subtypes that could contribute to GBM therapy resistance is also required. If each tumor has individual GSC with different genotypes and phenotypes, development of patient-specific GSC-targeting therapy is presumably mandatory. The goal is then to characterize how extensively we can identify shared therapy resistant features of GSC between tumor subtypes in order to effectively target the tumorigenic cells.

Various studies have demonstrated the existence of these multiple GSC subtypes and each one raises more questions on the implications for therapy resistance. For example, Yan et al. identified the presence of an especially malignant subtype of GBM with decreased survival rates in younger patients [25]. Likewise, another study showed that each subpopulation contains distinct molecular and functional characteristics that contribute to its tumorigenicity and therapy resistance [26]. An understanding of how many subtypes exist and what molecular components contribute to their unique functional characteristics is critical for the future development of GBM therapies. Furthermore, whether or not distinct therapies should be developed for each GBM subtype, or if GSC are the appropriate and the only therapeutic target in each of these GBM subtypes, remains to be fully elucidated.

**Distinct Molecular Mechanisms for Therapy Resistant Glioma Cells**

Recent studies about therapy resistance of GSC have proposed distinct cellular mechanisms that may actively resist the cytotoxic effects of current treatments. Several specific mechanisms that have been uncovered include activation or inactivation of cell cycle checkpoint proteins, the presence of specific drug transporters avoiding chemotherapeutic agents to reach therapeutically effective doses, secondary activation of alternative pathways following targeting of a certain pathway, and mutation of the actual drug targets. Additionally, molecular mechanisms including the role of the cell cycle status, the capacity for DNA repair, and elements of the physiologic tumor microenvironment have also been proposed to facilitate relative therapy resistance of GSC.

**DNA Repair**

The current standard treatment approach for GBM is the use of the DNA alkylating chemotherapeutic agent TMZ in conjunction with maximal surgical resection of the tumor mass and radiation therapy [12]. The resistance response of GBM tumors to TMZ treatment further exemplifies the role of DNA repair mechanisms in therapy resistance. TMZ acts partially by inducing methylation of DNA at its guanine residue, causing an overall cytotoxic effect. Some GBM tumor cells have developed resistance to this cytotoxic damage by increasing the expression of O-6 methylguanine-DNA methyltransferase (MGMT) [6], which helps to repair the TMZ-induced DNA lesions. Hegi et al. compared GBM patients receiving combined radiotherapy and temozolomide treatment with patients receiving only radiotherapy and found improved survival in patients with an epigenetically silenced MGMT promoter in their tumor [26]. Similarly, through an analysis of ten GBM tumor samples and neurosphere cell lines, Sciuscio et al. demonstrated an increased methylation status of the MGMT promoter in GBM-derived neurospheres [27]. The fact that this hypermethylation can be used to identify GSC. Using PTEN-deficient GBM tumors, they demonstrated that both CD133+ and CD133− subtypes are able to exhibit the GSC-like characteristics of self-renewal and tumor formation [18]. In fact, their findings suggest that GBM tumors may contain an entire lineage of cells with distinct tumor growth patterns. Further research is needed to investigate the efficacy of CD133− cells compared to CD133+ cells to identify self-renewing and tumor initiating cells from the tumor bulk. Other studies use different markers such as CD15 (also called LeX or ssea 1), aldehyde dehydrogenase [19], and side population isolated by Hoechst dye exclusion [20] to isolate GSC. Sphere formation in serum-free media is also considered to be one of the properties of GSC [21]. While all of these approaches have been shown to enrich for GSC in certain tumor samples, consensus is still lacking as to how we should generalize these methods to label GSC (Figure 3). Differences in experimental design and procedures in various labs make direct comparison unreasonable or even impossible. Furthermore, a definitive and universal marker or set of markers that label GSC may not exist, as recent studies demonstrated the difference of gene expression, activated signaling pathways, altered gene copy numbers, and phenotypes of GSC in several different subtypes of GBM [22,23].

![Figure 3: GSC are capable of self-renewal and tumor initiation.](image)

A schematic diagram representing the proposed origin and activity of GSC. GSC are believed to originate from neural stem cells. Further research is needed to explore a potential origin for GSC from neural progenitor cells and terminally differentiated cells. GSC are capable of self-renewal through both symmetric and asymmetric cell division. It is this ability for self-renewal and proliferation that implicates GSC as a potential source for therapy resistance.
status represents an epigenetic modification resulting in decreased MGMT activity raises the question of whether or not methylation of MGMT is predominant in GSC and, if so, why does this occur. These open questions aside, these studies indicate that when the DNA repair capabilities of the GBM tumors are reduced through epigenetic silencing by CpG methylation, patients experience improved survival rates.

GSC may preferentially escape from radiation-induced cell death by activating proteins associated with DNA damage checkpoints and instigating repair of radiation-induced DNA damage [14]. These findings are further supported in a study by Ropolo et al. showing increased DNA repair levels of five cultures enriched in GSC compared to five non-GSC cultures [28]. In this study, CD133+ cells exhibited increased activation of Chk1 and Chk2 kinases, indicative of up-regulated DNA damage checkpoint activation in GSC. In addition to their effect on the DNA damage checkpoint, these kinases are recognized to affect tumor suppression and cell cycle regulation. The findings of this study suggest that this increased activation of Chk1 and Chk2 kinases may enhance radiosensitivity by delaying the cell cycle and providing more time for DNA repair [28]. Further analysis into the pathway by which radiation resistance of GSC is mediated implicates the L1CAM transmembrane protein as the key regulator of the DNA damage checkpoint response [29]. Yet another study measured levels of DNA repair proteins before and after four hours of irradiation and showed increased levels of the repair protein Rad51 in GBM cell lines compared to normal human astrocytes [30]. These findings have provided further evidence for the important role of DNA repair processes in GSC therapy resistance and indicate significance to establish targeted therapies for the DNA repair system in GSC.

Cell Cycle Checkpoints

Most cytotoxic chemotherapies for cancers target rapidly dividing tumor cells within the tumor bulk [7,31]. Studies have shown that while these actively cycling cells exhibit varying levels of response to available treatments, the GSC may remain largely unaffected. One possible explanation for why cancer stem cells escape therapy-associated cell death is that they exhibit a lower rate of proliferation and therefore are not targeted by those therapeutic agents [7]. It is, however, still an open question as to whether or not GSC truly reside in a more slowly-dividing stage of the cell cycle. Proponents of this idea suggest that because somatic neural stem cells in the adult brain are slowly dividing, GSC may also share this same phenotypic characteristic. If current therapies are just targeting the rapidly dividing cells in the tumor, cancer stem cells that are presumably in a slowly cycling state could potentially be less affected by the treatment. As a result, the stem cell population persists and could possibly once again repopulate the entire tumor cell population, leading to tumor recurrence. Further studies are required to more fully address this hypothesis and clarify the actual GSC cell cycle status.

Hjelmeland et al. identified a potential regulator of cell survival, A20, which is highly expressed in CD133+ GSC. Implementing multiple techniques to knockdown, inactivate, or decrease the activity of A20, they demonstrated subsequent drops in GSC growth and survival [32]. However, analysis of A20 in other cancer types has raised the question of whether A20 acts as a tumor repressor or a tumor enhancer. This was demonstrated by two independent studies focusing on the inhibitory effect of the NF-κB pathway on A20. In one study, NF-κB inhibition induced squamous cell carcinomas in mouse epidermis [33] while in the other it prevented the development of hepatocellular carcinoma in transformed hepatocytes [34]. Therefore, it is possible that A20 actions are cancer type-dependent [32]. Similarly, inhibition of an important cell survival and cell invasion protein kinase, AKT, has been shown to lead to a decrease in stem cell fraction and invasiveness while facilitating longer survival in mice [35]. Further understanding of each of these molecular pathways in GSC is essential for development of effective GBM therapies.

Tumor Cell Drug Efflux

There are several other mechanisms by which GBM tumor cells may effectively resist chemotherapeutic measures. These mechanisms include decreased drug influx into tumor cells, active drug efflux preventing attainment of therapeutically effective drug concentrations, increased drug inactivation due to secondary mutation of target molecules, and double stranded break repair [36]. The transporters responsible for protecting cancer cells from cytotoxic chemotherapeutic agents are the ATP-binding cassette (ABC) drug transporters [37]. ABC transporters utilize the energy derived from ATP hydrolysis to actively pump chemotherapeutic drugs out of the cell [38]. ABCG2, in particular, is a multidrug resistant gene found in malignant gliomas and plays a key role in drug efflux from the GSC population. Various cytotoxic chemotherapeutic agents fail to eradicate the side population (a subset of tumor cells that are capable of pumping out dye from the cells) [39]. On the other hand, treatment of cultured glioma cells with ABC inhibitor, such as miRNA-328, may eliminate the side population, providing evidence that the ABC transporter may play key roles in chemoresistance [40]. Using flow cytometric analysis, Bar et al. further demonstrated that targeting GSC with cyclosporine specifically depletes GSC, as indicated by a reduction in side population cells. The fact that this side population is known to express ABC transporters suggests that ABCG2 may not function merely as a marker for drug resistance, but rather as a GSC marker [19]. Thus, targeting the ABC drug transporters would be one means of establishing therapies towards resistant tumor cell populations.

Although the elevated “drug pump-out” mechanism is an attractive theory for therapy resistance of GSC, another recent study has presented contradictory evidence. Broadley et al. demonstrated that the action of ABC transporters may be insufficient or altogether unnecessary for the stem-like characteristics of GSC [21]. In this study, the fluorescent dye Hoechst 33342 was used to determine if the side population of cells with distinct efflux activity was present in either immortalized GBM cell lines or in cells derived from GBM tumors. Their data indicate that the side population could not be found in GBM spheres, suggesting the properties of self-renewal, tumor-initiation, and differentiation ability associated with GSC are possible completely apart from the ABC drug efflux theory [21]. Continued investigation will likely elucidate whether the ABC drug transporter remains one of the mechanisms for GSC therapy resistance.

Physiologic Microenvironment

Additional therapy resistant mechanisms beyond the intrinsic factors encompass the role of the physiologic tumor microenvironment. This concept was convincingly illustrated in a study that compared the growth characteristics of CD133+ GBM cells at 20% oxygen levels to the more physiologically relevant oxygen level of 7%. The data demonstrated that the depleted oxygen state resulted in enhanced stem cell behavior of the GBM cell lines [41,42], including increased self-renewal, retained multi-lineage differentiation potential, and reduced doubling time. The increased CD133+ expression in response to lower
oxygen levels reflects an increased fraction of GSC within the cultured tumor cells. Subsequently, it increases the radiosensitivity of the tumor by promoting self-renewal and proliferation following reduction of the tumor mass. Increased expression of protective autophagy proteins, secretion of tumor-promoting cytokines [43], and the cell-to-cell interaction of Notch signaling all participate in contributing to protect the cancer stem cells from radiation insult [44] and illustrate the importance of the tumor microenvironment in GBM therapy resistance (Figure 4).

Increasing attention is being shifted towards investigating components of the unique microenvironment surrounding GSC as compared to that of somatic cells, or non-stem tumor cells, to identify potential GSC-specific pro-survival factors. Recent findings have focused on the role of tumor vessels and their endothelial cells supporting the tumor as niche for the maintenance of GSC within the tumor cell populations. A study by Wang et al. demonstrated the presence of a subset of cells within the GSC population that possess endothelial progenitor-like characteristics and are capable of developing into mature endothelial cells [45]. Similarly, using in vitro cell culture of GSC under endothelial differentiation conditions, Ricci-Vitiani et al. demonstrated the development of GSC into cells with morphologic and functional characteristics of endothelial cells [46]. If cancer stem cells indeed carry this vasculogenesis-initiating subpopulation of cells, it would serve as a critical source of nutrients for sustained GBM tumor growth as well as a mechanism for GSC survival following therapy that effectively kills the rest of the tumor bulk. This idea is supported by the fact that GSC secretes vascular endothelial growth factor (VEGF) to promote the highly vascularized environment necessary for tumor growth [46]. Secretion of VEGF from GSC may attract existing tumor vessels originating from normal vascular endothelial cells or “trans-differentiated” GSC. Regardless of origin, the unique capacity of GSC to activate tumor angiogenesis contributes, at least in part, to the entire tumor growth and therapy resistance.

Therapeutic approaches to target this mechanism of GSC-induced tumor angiogenesis have been highlighted by the development of bevacizumab (Avastin), a blocking antibody for VEGF. Physicians may notice, with surprise, that a given number of patients with GBM experience a shrink of contrast enhanced lesions with T1-weighted images on MRI following bevacizumab treatment. However, a caution was then raised as to whether a shrink of abnormal enhancement on MRI images indeed corresponds to the change of the actual tumor sizes, as blocking VEGF may decrease leakage of contrast agent into the tumor cavity and reduce peritumoral edema in the surrounding brain. In fact, a recent study indicated that Avastin treatment does not affect long-term survival of GBM patients [47]. In addition, creating a hypoxic environment with Avastin may accelerate GBM cells to migrate out of the original site of tumors into the adjacent normal brains, where the blood brain barrier is still intact and the contrast agent may not detect the new tumor lesions. These observations suggest that monotherapy to inhibit angiogenesis partially blocks the ability of GSC to promote tumor growth but does not altogether eliminate the GSC population. Another way in which GBM therapy resistance is influenced by the tumor microenvironment relates to the difference of the GSC in the tumor core versus the periphery of the lesion. Studies have shown that the core GSC are immature while the peripheral cells appear to be more differentiated [48]. Regional differences in genotype and phenotype of GBM cells require more attention in the future. This impacts therapeutic approaches to GBM because GSC in different stages of oncogenesis may require therapies with unique action.

**Blood-Brain Barrier**

The blood brain barrier (BBB) is a brain-specific structure composed of tight junctions between adjacent endothelial cells of capillaries in the central nervous system. Claudin and occludin proteins make up the main component of these tight junctions, which function to prevent large molecules and toxins from entering the brain. Loss of expression of certain proteins in the claudin family has been demonstrated in GBM, suggesting a potential tumor suppressor function [49]. Similarly, in vitro and in vivo data by Ishihara et al. have shown down-regulation of tight junction proteins such as claudin and occludin when exposed to cultured GBM cells as well as over-expression of these same proteins in GBM brain samples compared to non-neoplastic brain samples [50]. These findings suggest that reduced expression of these proteins may contribute to a loss of integrity of the BBB, resulting in increased invasion of tumor cells from the blood vessels into the adjacent brain tissue. Disruption of the BBB in tumor vessels of GBM is widely recognized. Gadolinium (Gd)-based contrast agent is used clinically to enhance the tumor sites, which suggest that tumor vessels are at least partially leaky [51]. However, recent studies suggest that tumor cells extend beyond the area of Gd enhancement, where FLAIR or diffusion images of MRI detect as radiographically abnormal brain lesions. These observations endorse potential regional difference of tumor vessels and further indicate that drug concentration within tumors may differ depending on microenvironment. It is likely that tumor vessels are not one and the same within a tumor lesion. Given the prominent infiltrative potential of GSC into adjacent normal brains, it is assumed that GSC may be preferentially located at the periphery of tumors with intact BBB, which can block penetration of various chemotherapeutic agents rather than the central core of tumors. However, thus far, no evidence has supported this assumption. Future studies have to further clarify the microenvironment of GSC in the context of interaction with vascular endothelial cells.

Another histopathological feature of vascular endothelial cells unique to GBM is endothelial proliferation, one of the criteria indicative of higher malignancy glial tumors. It appears tumor vessels in the core have endothelial cells piled up to create glomerular patterns of vascular walls [52]. These tumor vessels are leaky, thus providing an environment conducive to spread of tumor cells. In contrast, at the periphery of the tumors, the BBB appears to still be intact and the vascular structure maintains a normal histological appearance (Figure 5). Yet, it remains unclear if the endothelial proliferation contributes to therapy resistance of GBM. Bar has described the role of this endothelial proliferation and associated hypoxic necrosis of GBM as a

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**Figure 4: Distinguishing cellular features of GBM.** One of the distinguishing features of GBM compared to lower grade gliomas is the extent of tissue necrosis within the tumor mass. H&E stains of tumor tissue at 20X (A) and 40X (B). Pseudopalsading cells surrounding the necrotic foci, a feature suggestive of the poor prognostic nature of GBM, can be observed.
likely be insufficient to prevent the GSC self-renewal and proliferation associated with GSC therapy resistance and many more undiscovered pathways, specific to GSC survival and proliferation have been proposed. In one such study, Piccirillo et al. described the pro-differentiation activity of bone morphogenetic proteins (BMPs) on CD133+ GBM cells. They demonstrated that treatment of CD133+ cells with BMP4 led to reduced proliferation of GBM cells and differentiation of CD133+ cells within the total GBM cell population. Additionally, BMP4 administration was shown to prevent formation of invasive tumors and significantly increase in vivo survival rates in immunodeficient mice [54,10]. In support of these findings, Lee et al. showed that a subset of GSC responded to BMP-induced inhibition [55]. More specifically, they showed that, within this subset, epigenetic silencing of the BMP receptor 1B by methylation of its promoter led to reduced astroglial differentiation. While this supports the idea that BMPs may effectively target GSC, it is important to note that only a subset of GSC was affected. There may still be persistent subtypes of GSC in the tumor population that could be resistant to the BMP treatment. BMP4 is currently being investigated as a new clinical trial for GBM in Europe.

Another approach currently being implemented to target GSC therapy resistance is the development of anti-angiogenic factors to prevent GBM tumor growth. The aforementioned Avastin works along this mechanism by blocking vascular endothelial growth factor. An additional anti-angiogenic agent currently under clinical trial is ABT-510. This trial is investigating cohorts of recently diagnosed GBM patients receiving subcutaneous administration of varying doses of ABT-510 along with concurrent temozolomide and radiotherapy treatment. Initial data indicate that doses up to 200 mg/d have been well tolerated [56]. These findings are promising in light of current in vivo data which demonstrate decreased growth of human gliomas established in the brain of athymic nude mice upon daily ABT-510 exposure [57]. Further investigation is necessary to determine the efficacy of this anti-glioma therapy candidate at targeting GSC within the glioma cell population.

**Future Direction**

This review covered some of the mechanisms believed to be associated with GSC therapy resistance and many more undiscovered mechanisms are believed to exist. Targeting single GSC genes will likely be insufficient to prevent the GSC self-renewal and proliferation necessary to effectively deliver anti-cancer agents across the blood brain barrier into the central nervous system while simultaneously improving the treatment quality and reducing negative side effects. Convection enhanced delivery [61] is another potential delivery method geared towards depositing therapeutic anti-glioma agents at their desired target.

**Development for glioma stem cell-directed therapy**

Several therapeutic strategies targeting genes or pathways specific to GSC survival and proliferation have been proposed. In one such study, Piccirillo et al. described the pro-differentiation activity of bone morphogenetic proteins (BMPs) on CD133+ GBM cells. They demonstrated that treatment of CD133+ cells with BMP4 led to reduced proliferation of GBM cells and differentiation of CD133+ cells within the total GBM cell population. Additionally, BMP4 administration was shown to prevent formation of invasive tumors and significantly increase in vivo survival rates in immunodeficient mice [54,10]. In support of these findings, Lee et al. showed that a subset of GSC responded to BMP-induced inhibition [55]. More specifically, they showed that, within this subset, epigenetic silencing of the BMP receptor 1B by methylation of its promoter led to reduced astroglial differentiation. While this supports the idea that BMPs may effectively target GSC, it is important to note that only a subset of GSC was affected. There may still be persistent subtypes of GSC in the tumor population that could be resistant to the BMP treatment. BMP4 is currently being investigated as a new clinical trial for GBM in Europe.

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**Future Direction**

This review covered some of the mechanisms believed to be associated with GSC therapy resistance and many more undiscovered mechanisms are believed to exist. Targeting single GSC genes will likely be insufficient to prevent the GSC self-renewal and proliferation associated with tumor recurrence. Therefore, the future of glioma therapeutic development will most likely require a polypharmacologic approach and will combine various therapies to simultaneously target multiple GSC survival-promoting factors [58]. Due to an increasing body of evidence demonstrating the stem-cell like properties of the therapy resistant cells leading to recurrence of GBM, further elucidation of tumor stem cell-specific markers, survival pathways, and self-renewal signaling is essential for the development of effective treatment measures and drugs to specifically target these therapy resistant cells. Also, understanding of the intrinsic and extrinsic factors of GBM tumor cell populations will enable manipulation of cell cycle checkpoints in order to sensitize the cancer stem cells so they can be acted upon by chemotherapeutic agents and irradiation. To address the impact of the tumor microenvironment, or niche, on GSC survival pathways, Zhou et al. [59] suggest implementation of various imaging strategies to track GSC through the tumor compartments during in vivo treatment. Identification of GSC biomarkers, labeled GSC antibodies, and magnetically labeled GSC are just a few of the methods suggested. With the ability to monitor GSC and their niche, drugs could be developed to specify their targets and attack them safely and effectively.

The future direction for treatment of therapy resistant glioblastoma certainly includes the implementation of molecular-targeted therapy. With continued technological advances, treatment approaches will become increasingly tailored to the molecular identity of individual patients and individual tumors. In depth analysis of the molecular phenotype of GSC will bring to light the specific mechanisms by which they evade current treatment measures and carry out their pathogenic effect [60]. Identifying the specific molecular signature of the patient’s tumor makes it possible to select the most appropriate regimen of chemotherapy and radiotherapy [36]. Furthermore, genome-wide expression profiling of each tumor will further individualize treatment by providing the ability to predict the patient outcome to a particular therapy based on their genomic profile [36]. As our understanding of the mechanism behind GSC therapy resistance continues to expand, it becomes increasingly important to develop effective drug delivery systems to reliably deliver newly developed drugs to their proper cellular target. One drug delivery approach receiving attention is nanoparticle technology. Carsus et al. describe nanotechnology as a means to effectively deliver anti-cancer agents across the blood brain barrier into the central nervous system while simultaneously improving the treatment quality and reducing negative side effects. Convexion enhanced delivery [61] is another potential delivery method geared towards depositing therapeutic anti-glioma agents at their desired target.

**Figure 5:** Glomeruloid structures and endothelial proliferation in GBM. Comparison of normal brain at 20X (A) with GBM tissue at 10X (B), and 40X (C) demonstrates the presence of glomerular-shaped vascular walls within GBM. The leaky nature of these vessels may contribute to spread of the tumor cells.
As has been mentioned throughout this review, one of the qualities used to classify stem-like cells is their ability to self-renew. Through this process, stem cells are able to maintain an undifferentiated state until the tumor kills the patient. Self-renewal is maintained by interactions with and balance between proto-oncogenes, various tumor suppressor genes, and supporting factors from the microenvironment [62]. The recent discovery of induced pluripotent cells suggests some terminally differentiated somatic cells can reacquire stem cell characteristics, at least in an experimental environment [63]. These findings may have potentially serious implications for the treatment of GSC. For example, following eradication of GSC as part of the treatment regimen for a GBM patient, non-stem tumor cells could reprogram themselves to acquire stem cell properties and thus lead to therapy resistance and tumor recurrence. The concept of induced pluripotent stem cells remains an important area of focus for future research and has serious implications for the stem cell cancer theory.

Cancer stem cell-directed therapy is still in the early stages of development. As the field advances, more questions have continued to arise than answers, and the issue has become increasingly complex. This review has highlighted just some of the significant scientific advances contributing to our current understanding of GSC therapy resistance. In addition, the need for further investigation has been well-documented. GBM remains the most malignant and invasive primary brain tumor. Its resistance to the current best available treatment has caused most GBM patients to die within two years of diagnosis. GSC provide an attractive target for future therapeutic approaches. As new discoveries and new technologies continue to be applied to GSC therapy, there is increasing hope for patients with GBM.

References


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