Review

Mechanisms of Resistance to Current Glioblastoma Therapies

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Mechanisms of Resistance to Current Glioblastoma Therapies

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Abstract: Glioblastoma (GBM) poses a formidable challenge to patients for several reasons. Given its grim prognosis, understanding the various mechanisms GBM tumors utilize to resist therapy is essential to improve patient outcomes. Using PubMed, this focused review identifies and characterizes five critical elements of GBM tumors that contribute to their resistance to treatment: DNA repair enzymes, temozolomide (TMZ) and radiation mechanisms, anti-apoptosis mechanisms, GBM tumor heterogeneity and its effects on the cell cycle. This review explores various challenges associated with GBM tumors, such as their resistance against standard treatments such as TMZ and radiation therapy (RT). We explore the importance of epigenetic reprogramming, genetic mutations critical for cell proliferation and tumor suppression, and the role of mismatch repair (MMR) processes that influence RT and immune response interplay as contributors to GBM resistance. In addition, this review highlights vital DNA repair enzymes such as O6-methylguanine-DNA methyltransferase (MGMT) and Alkylpurine-DNA N-Glycosylase (APNG), which repair DNA damage introduced by alkylating agents such as TMZ. The involvement of the NuRD complex, particularly CHD4, in regulating access to DNA repair enzymes. Recent advancements in understanding the transcriptional regulation of MGMT through NF-κB activity are examined. Further, we explore novel approaches, including using anticancer neural stem cells and targeting hexokinase 2 (HK2) with antifungal drugs. Examining critical elements of the GBM cell cycle, such as the role of CDK's, cyclin(s) and proliferation markers such as *ki67*, can also give us a foundation for identifying possible target proteins and kinases for cancer drugs. While targeting DNA repair enzymes, proteins, and regulatory elements shows promise in enhancing GBM treatment efficacy, we acknowledge the challenges, including potential side effects and the risk of secondary cancers. Future research should focus on leveraging personalized medicine approaches and emerging biotechnologies, such as CRISPR gene editing, to develop targeted therapies that can overcome resistance mechanisms of GBM and improve patient outcomes.

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controlled trials (RCTs); Relative biological effectiveness (RBT); Secrete soluble TRAIL (sTRAIL); Temozolomide (TMZ); TUSC3-overexpressing (TUSC3-OE); Transforming growth factor α (TGFα); Tumor protein p53 (TP53); Wild type (WT)

Introduction

Glioblastoma (GBM) is the most aggressive and common type of malignant brain tumor in adults, accounting for half of all cancerous brain tumors (1). GBM originates from glial cells called astrocytes in the brain and spinal cord, rapidly invading surrounding healthy brain tissue (1). Radiotherapy (RT) is a critical component of standard GBM treatment, typically administered after surgical tumor resection. It uses high-energy beams to damage cancer cell DNA, causing single-strand and double-strand breaks, to kill tumor cells or prevent their growth and recurrence (1). Temozolomide (TMZ), an oral chemotherapy agent**,** directly attacks cancer cells by damaging their Deoxyribonucleic Acid (DNA), which helps reduce tumor growth. Currently, patients receive six weeks of daily RT along with concurrent oral TMZ chemotherapy, followed by six monthly cycles of adjuvant TMZ (1). Despite advances in treatment, GBM remains incurable, with a poor prognosis and a median survival of about twelve-fifteen months with standard treatment, which includes a combination of RT and chemotherapy (1). This review will cover factors currently believed to be responsible for resistance, including DNA repair enzymes, TMZ and radiation mechanisms, anti-apoptosis mechanisms, genetic heterogeneity of GBM cells, and cell cycle effects.

DNA Repair Enzymes

DNA enzymes, deoxy ribozymes, are catalytic DNA molecules that can perform specific biochemical reactions. These molecules contribute to the resistance of GBM cells to the treatment of GBM because they have upregulated DNA repair enzymes and an enhanced ability to fix DNA damage induced by standard treatment of GBM. Scientists could recommend updating the pathology of central nervous system diseases such as GBM through their work with the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy - Not Official WHO, also known as cIMPACT-NOW (2). cIMPACT-NOW is an international group that provides accurate and timely updates on GBM and other central nervous system tumor classifications and subtypes, ensuring that treatment protocols are based on the latest molecular findings (2)**.** The molecular classifications provided by cIMPACT-NOW identify specific genetic alterations and subtypes, which clinicians can use to develop more targeted therapies that overcome resistance mechanisms related to DNA repair enzymes. This personalized approach may lead to more effective treatments tailored to the patient's GBM prognosis. cIMPACT-NOW has substantially advanced the molecular classification of GBM through its updates, mainly Update Three (2). This update introduced molecular criteria to diagnose IDH-wildtype GBM, even without typical histological features, based on specific genetic alterations such as TERT promoter mutations and EGFR amplification. This type of analysis allowed researchers (3) to examine the many resistance mechanisms of GBM. Various experimental and preclinical models were used to study DNA repair in GBM, including cellular models (established cell lines, patient-derived primary cultures, and glioma stemlike cells) for in vitro studies, as well as in vivo animal models such as the use of xenograft models in immunodeficient mice, as well as genetically engineered mouse models that recapitulate vital genetic alterations found in human GBM. Through their in vivo and xenograft models, they came across DNA repair enzymes, uncovering the ability of GBM cells to resist treatment methods such as the TMZ mentioned above. As researchers continue to explore each pathway and enzyme in detail, further scientific analysis will allow for treatment methods to overcome these DNA repair enzymes.

Two investigators (4) were among the first to explore DNA repair enzymes, exploring how DNA Damage Response (DDR), a sophisticated network of enzymes and proteins, repairs DNA damage in

 doi:10.20944/preprints202503.1011.v1

GBM cells. The DNA damage response (DDR) is a system of cellular pathways responsible for detecting and repairing DNA damage to maintain genomic stability. In GBM, cancer cells exploit the DDR to their advantage and survive despite aggressive treatments like radiation and TMZ. Targeting specific components of DDR could weaken the tumor cells' defenses, making them more vulnerable to existing treatments. Their work highlighted DDR's weaknesses, suggesting that disrupting specific DDR components might enhance treatments like radiation, TMZ, and chemotherapy (4). Two enzymes play key roles in repairing DNA damage caused by TMZ and other alkylating agents used to treat GBM: Alkylpurine-DNA N-Glycosylase (APNG) and O6-methylguanine-DNA methyltransferase (MGMT). APNG initiates base excision repair by removing alkylated DNA bases like N3-methyladenine and N7-methylguanine. MGMT directly repairs O6-methylguanine lesions by transferring the methyl group to itself in a suicide reaction. Experimenters (5) researched these two enzymes using photodynamic therapy (PDT). PDT uses light-activated compounds to create reactive oxygen species, causing DNA damage in tumor cells. This method allowed scientists to observe DDR activity in response to DNA damage. PDT helped define the functions of APNG and MGMT in tumor cells such as GBM. APNG repairs DNA lesions N7-methylguanine and N3-methyladenine, which arise from DNA damage caused by chemotherapy. MGMT repairs O6-methylguanine lesions, a specific DNA alteration induced by TMZ. Now that particular DNA repair enzymes have been identified, it is time to explore the mechanisms to target them.

To understand how to approach DNA Enzyme Repair in GBM, biochemists (6) developed a mathematical model to simulate the response of GBM cells to TMZ treatment, incorporating the effects of DNA damage and repair mechanisms. The model was a computational simulation that used mathematical equations to model the response of GBM cells to (TMZ) treatment, incorporating DNA damage and repair mechanisms. The mathematical model simulates GBM cells' response to TMZ treatment by integrating DNA damage and repair dynamics, including the repair actions of APNG and MGMT. Using Xenografts, researchers investigated resistance mediated by efficient repair of treatment-induced DNA damage in cancer cells. Strategies. The model is also a precursor for testing potential treatments in a controlled environment. Their work paved the way for future research to simulate response mechanisms and effective treatment to inhibit those mechanisms produced by APNG and MGMT.

Despite considerable progress (6), the relationship between specific enzymes and GBM must still be analyzed. Although APNG and MGMT have been identified as critical repair enzymes, specific targeted methods are still lacking. Researchers have identified two additional DDR complex components, Nucleosome Remodeling and Deacetylase Complex (NuRD) and Chromodomain Helicase DNA-binding Protein Four (CHD4) (7). The NuRD (nucleosome remodeling and deacetylase) complex, which includes CHD4, is a chromatin remodeling complex that controls gene expression by adjusting chromatin accessibility. In GBM, CHD4 within the NuRD complex regulates access to DNA repair enzymes, which can influence how effectively GBM cells repair damaged DNA. Investigators (8) found that CHD4 was a chromatin remodeling factor that plays a role in gene expression and indirectly influences DNA repair processes. Chromatin remodeling is when cells alter the structure of chromatin, making DNA accessible for transcription and repair.

CHD4 and the NuRD complex can affect chromatin accessibility to DNA repair enzymes like APNG and MGMT, which are crucial in repairing DNA damage caused by alkylating agents such as TMZ. For instance, regulating CHD4 activity might indirectly affect the efficiency of DNA repair processes, potentially enhancing the sensitivity of GBM cells to TMZ or radiation therapy. To further highlight this ability, scientists (9) used western blotting, among other analyses, to see how CHD4 reacted to DNA damage caused by TMZ. The findings confirmed that targeting proteins in CHD4 and MGMT will effectively target GBM cells. Biochemists (10) investigated the transcriptional regulation of MGMT in GBM cells, finding that stemlike glioma-initiating cells express higher levels

 doi:10.20944/preprints202503.1011.v1

of MGMT due to increased NF- -activity. NF-κB is a family of transcription factors influencing cell survival and gene expression in cancer cells. In GBM, NF-κB regulates the expression of MGMT, an enzyme that repairs DNA lesions caused by TMZ. Higher NF-κB activity leads to increased MGMT levels, making these cells resistant to treatment. Targeting NF-κB could, therefore, reduce MGMT expression, decreasing the repair of DNA damage in GBM cells. This approach opens new avenues in GBM treatment by addressing the tumor's repair mechanisms at the molecular level.

Experimenters (11) discovered a connection between MGMT activity, treatment response, and promoter methylation in GBM. Promoter methylation adds methyl groups to the DNA sequence in a gene's promoter region. In GBM, methylation of the MGMT promoter region is a crucial factor, as it reduces MGMT levels, thereby decreasing the cancer cell's ability to repair DNA damage from treatment. This idea set the stage for analysts (12) who explored targeting the repair enzymes Rad51 and BRCA2 to sensitize GBM cells to alkylating anticancer drugs. Rad51 and BRCA2 are two enzymes that repair DNA double-strand breaks through homologous recombination (11). This repair pathway is essential for GBM cell survival. Targeting this could potentially enhance the effectiveness of treatments such as TMZ.

Scientists (13) explored a therapy for GBM that uses special anticancer neural stem cells. Anticancer neural stem cells are modified stem cells that deliver therapeutic agents directly to cancer cells. These specialized stem cells can go on GBM sites, reducing the tumor's repair and explicitly targeting cancer stem cells. This study primarily utilized in vitro cell culture experiments and in vivo mouse models to investigate DNA repair mechanisms in GBM. The in vitro work involved various human GBM cell lines, glioma stemlike cells, and engineered neural stem cells, employing coculture assays, migration assays, and flow cytometry. For in vivo studies, the researchers used an orthotopic xenograft model in nude female mice, where human GBM cells were implanted into mouse brains, followed by intravenous injection of engineered neural stem cells. The mouse experiments included bioluminescence imaging to track tumor growth and neural stem cell distribution, blood sampling, and histological analysis. The in vitro and in vivo studies showed the ability of these neural stem cells (NSCs) to migrate across the blood-brain barrier and target glioblastoma tumors, a finding further substantiated by fluorescent imaging showing their selective localization at tumor sites. Notably, the combination of NSCs engineered to secrete soluble TRAIL (sTRAIL) with the cardiac glycoside Lanatoside C (Lan C) not only reduced tumor burden but also significantly enhanced apoptosis in GBM stem-like cells, overcoming resistance to sTRAIL alone. Their findings suggest that focusing on cancer stem cells could be a potential treatment for GBM repair enzymes.

Meanwhile, scientists (14) investigated how targeting the DNA repair process in glioma-initiating cells could make them more vulnerable to RT. This research highlights the significance of zeroing in on cancer stem cells and their unique DNA repair enzymes to enhance treatment success. Researchers (15) took a closer look at a proteolytic enzyme called Hexokinase Two (HK2). HK2 is an enzyme involved in glucose metabolism and is often overexpressed in cancer cells by supplying energy. Research found that the antifungal drugs ketoconazole and posaconazole can specifically attack GBM cells with elevated levels of HK2. Antifungal drugs like ketoconazole and posaconazole inhibit HK2, disrupting the cancer cells' energy supply and potentially providing treatment options for GBM patients.

Moreover, this finding opens the possibility of using these existing drugs as a new treatment option for GBM, particularly for patients whose tumors have a lot of HK2. Experimenters (15) analyzed the role of DNA repair mechanisms in specific GBM treatments such as TMZ and its effect on p53 in clinical trials to expand this discussion. Their analysis detailed clinical trials testing DDR inhibitors in combination with standard therapies like radiation and TMZ for GBM. Specific Inhibitors were identified, such as AZD1390, which is a DNA damage response (DDR) inhibitor

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explicitly targeting the ATM kinase and is in phase one or two trials and is designed to cross the blood-brain barrier, specifically to improve treatment outcomes in GBM patients (16). Trials are exploring tailored approaches based on MGMT expression, assessing if combining DDR inhibitors with radiation or chemotherapy can increase effectiveness in patients with different resistance profiles (16).

Targeting DNA repair enzymes for advancing GBM treatment has much potential. Enzymes like MGMT, APNG, Rad51, BRCA2, CHD4, and HK2 are pivotal in repairing DNA damage (17) and helping GBM cells resist treatment. By disrupting these repair pathways through targeting the NuRD complex, NF-κB signaling, and metabolic regulators like HK2, researchers can enhance the tumor's sensitivity to treatments such as TMZ, radiation, and even repurposed antifungal drugs. There are clinical downsides and challenges associated with targeting these pathways. Many DNA repair enzymes, such as MGMT, BRCA2, and Rad51, maintain genomic stability in normal cells. Inhibiting these pathways could lead to off-target effects, especially in fast-dividing tissues like bone marrow, gut lining, and hair follicles, resulting in side effects such as immunosuppression (17). Disrupting DNA repair pathways can increase genomic instability in cancerous and healthy cells. Over time, this instability may elevate the risk of secondary cancers. Tumors are highly adaptable and may develop alternative repair pathways or mechanisms to survive despite inhibiting specific DNA repair enzymes. Drugs targeting these pathways must effectively cross the blood-brain barrier, as not all compounds achieve sufficient brain penetration (17). Since specific DNA-specific pathways are also implicated in maintaining neuronal function, their inhibition might exacerbate neurological symptoms or neurodegeneration in patients already affected by GBM. As ongoing studies identify new repair mechanisms and therapeutic targets, personalized approaches to inhibit these pathways could improve treatment effectiveness and patient outcomes in GBM.

Temozolomide And Radiation Mechanisms

GBM and glioblastoma stem cells (GSCs) face challenges in treatment due to resistance to therapies (18). Cancer treatment, especially for brain tumors like this condition, often involves RT (19). Because these cells divide quickly, TMZ is frequently utilized as a therapeutic agent (20). This medication is particularly effective for brain tumors since it can penetrate the blood-brain-barrier (BBB) (21).

The average survival duration for individuals diagnosed with this disease (GBM) is about 15 months (21). However, patients treated with RT and TMZ usually have better results when MGMT promoter methylation is present. In comparison, proton radiation therapy (PRT) stands out because it carefully targets the area needing treatment, which helps to reduce harm to nearby healthy brain cells (22). Additionally, carbon-ion radiation therapy (CIRT) proves advantages in targeting tumors characterized by oxygen depletion in the brain because of its high linear energy transfer (LET) and relative biological effectiveness (RBT). Moreover, the integration of TMZ and CIRT boosts the overall efficacy of the treatment. This strategy aims to attack the tumors more thoroughly while the benefits of each therapy, which could improve patient outcomes (22). It shows a rate of overall survival (OS) of 77.4 percent at 12 months and 61.0 percent at 18 months. The progression-free survival (PFS) also stands at 61.3 percent at 12 months and 42.7 percent at 18 months (22).

A systematic review and meta-analysis were conducted to assess patients with some methylation of the MGMT gene promoter who underwent adjuvant TMZ for six and twelve cycles. Data from two clinical categories, randomized controlled trials (RCTs) and non-randomized trials (NRTs), will be used to evaluate the effects of continued treatment beyond six cycles. There were 2,578 patients in 21 studies, five RCTs, and sixteen NRTs selected during the original collection of 294 records. The hazard ratio (HR) shows more than six cycles of reduction of disease and mortality rate of (HR 0.72 and HR 0.71). Furthermore, egger's test is a publication bias with a p-value (p). There was a lack of data on whether myelotoxicity was associated with TMZ after treatment. The test results

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(p=0.44 PFS and p=0.28 OS) show no bias because these studies individuals had no skew on the results (23).

Furthermore, epigenetic reprogramming can benefit efficacy and resistance to TMZ because MGMT can create obstacles to TMZ in two distinct approaches. One, patients with MGMT-hypermethylated (MGMT-M) reduced MGMT expression and increased sensitivity to TMZ and RT with a survival rate of 21.2 months. Secondly, MGMT-hypomethylated (MGMT-UM) has elevated MGMT levels, which decreases the survival rate at fourteen months with resistance in TMZ (24). For example, methods like western blot and qPCR in MGMT-M and MGMT-UM revealed epigenetic activation of tumor suppressor 3 (TUSC3) in GCSs with wild type (WT) and TUSC3-overexpressing (TUSC3-OE) resulted in a reduction of half maximal inhibitory concentration (IC-50) in these tumor cells elevated sensitivity to the drug after TMZ (24). The CRISPRoff, also known as epigenome editing, is a technique that increases methylation at lower TMZ concentrations, leading to cell death and DNA fragmentation. The MGMT gene can be epigenetically silenced and effective towards cytotoxic in cells (25).

In the absence of MGMT, MMR makes TMZ more effective. However, MSH2 and MSH6 genes can disrupt cell death. The malfunction with mutations can cause tumors to reappear and become more resistant (26). Also, mutations lead to mispaired O-6-methylguanine (O6-MeG) remains unrepaired, resulting in DNA strand breaks and tumor cells proliferating despite DNA damage. Additionally, genetic mutations or epigenetics enhance drug efflux and change drug metabolism. Moreover, inhibiting Src kinase can enhance the sensitivity of GBM and TMZ and reduce tumor cell migrations (27). Using TMZ with multi-drug resistance protein 1, MDR1 (ABCB1), increases drug levels in the brain and triggers cell apoptosis. In addition, drugs that target ATP-binding cassette subfamily G member 2 (ABCG2), such as KO143, reduce resistance and improve overall treatment outcomes (28).

Moreover, HK2, involved in glycolysis, contributes to how this tumor resists radiation, slows down tumor growth, and is more sensitive to RT. However, 2-deoxyglucose (2-DG) positively affects tumor and radiation treatment. RT can cause cell death, damage DNA, and cross the BBB and patient's response favorably (29). However, RT regulates the immune response in GBM, induces cell death in tumor cells, and activates dendritic cells through tumor antigens. Also, immune checkpoint inhibitors (ICIs) recognize and target cells and reduce evasion (30).

Furthermore, checkpoint kinases (CHK1 and CHK2) are the regulators of the cell cycle in response to DNA damage (30). Within GICs, activating these kinases in reaction to DNA damage delays the cell cycle, providing an opportunity for DNA repair and thereby contributing to their resistance against radiation therapy. Targeting and inhibiting these checkpoint pathways may increase the GICs vulnerability to radiation and chemotherapy treatments (31).

In GICs, when these kinases are activated due to DNA damage, it causes a pause in the cell cycle. Furthermore, this allows the cells to repair their DNA, which helps them resist radiation therapy. Blocking these checkpoint pathways could make GICs more sensitive to radiation and chemotherapy. Some early clinical studies indicate that taking TMZ in the morning might work better with the body's natural circadian rhythms, making it more effective and lessening resistance (32).

Anti-apoptosis Mechanisms

GBM is one of the most aggressive brain and spinal cord tumor to apoptosis types of brain cancer. Within GBM, anti-apoptotic mechanisms have become intricately balanced pathways that protect tumor cells from programmed cell death and are associated with poor prognosis and high recurrence (31). Numerous molecular mechanisms and possible therapeutic strategies have recently been studied to find new ways to defeat this devastating malady. In vitro and in vivo models were used to examine an innovative therapeutic strategy involving the combination of apoptotic inducers and inhibitors of key anti-apoptotic pathways (31). Chemotherapeutic agents, including TMZ, XPO1, Bcl-2, and Mcl-1 inhibitors, are integrated in the study. In tissue culture experiments, combination therapy decreased cell viability in GBM assay lines by 60%, compared to 30% with TMZ alone (31).

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Synergy of combining apoptosis inducers with anti-apoptotic inhibitors increased the survival rate from a standard 20 days to 35 days in rodent models (31). As a contributory factor in GBM pathogenesis, the interplay between cellular senescence, apoptosis, and autophagy was examined (32). In tissue culture assays, it was shown that senescent cells secrete inflammatory cytokines that support tumor growth and autophagy, which provides metabolic support for cancer cell survival under stress (32).

Conversely, in in vivo models of tumor cells, suppression of autophagy sensitized tumor cells to apoptotic killing and increased chemotherapeutic efficacy by 25% (32). A dissection of the role of apoptotic signaling pathways in human glioma samples and in rodent models was made (33). It was found that upregulating anti-apoptotic proteins such as Bcl-2 and Mcl-1 are often used by GBM cells to escape apoptosis (34). Patient-derived tumors were immunohistochemically analyzed for elevated protein expression of these proteins and exhibiting lower survival rates (33). Bcl-2 inhibitors reduced tumor volume by 40% when tested with human glioma cells in vivo experiments, indicating possible clinical translation. A major therapeutic challenge is the resistance of GBM cancer stem cells to Fas-induced apoptosis (34). Researchers cultured human glioma cell lines, noting that stemlike cells expressed lower levels of Fas receptors and were less sensitive to apoptotic signals (34). It was also shown that chemotherapeutic agents can induce DR5 expression on GBM cells, rendering them sensitive to stimulations of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (37). In vitro assays and in vivo models were used to show how DR5 upregulation by TMZ enhanced TRAIL-induced apoptosis and confers synergistic cytotoxicity (37). This approach extended survival by approximately 30% in rodent models and holds therapeutic potential (37). It was identified as the PI3K/Akt/mTOR signaling axis as an essential contributor to GBM cell survival (41). Inhibitors targeting this pathway were shown in tissue culture studies to reduce GBM cell proliferation by 50% (41). A proteasome addiction is created in GBM tumors due to PTEN loss (42). It was also shown in tissue culture experiments that proteasome inhibitors killed only PTEN-deficient GBM cells and did not kill normal astrocytes (42, 43). The tumor regression rate of 40% seen in the preclinical rodent study indicated that proteasome dependency could overcome resistance mechanisms (42). A recent study used the role of TRAIL in the field of apoptosis and cancer immunosurveillance (44). TRAIL agonists selectively triggered apoptosis in GBM cells and spared normal tissues in tissue culture and animal models (45). However, trials with TRAIL-based therapies have shown mixed results, with checkpoint inhibitors' response rates improved from 20–35% (44). GBM anti-apoptotic mechanisms are intricate and serve to both challenge and provide opportunities for therapeutic intervention. The pathways have been better studied with studies utilizing varying models—from pure tissue culture to rodent and human clinical trials—and their therapeutic implications have been clarified (42,43). Researchers are homing in on key molecules and pathways that can be targeted, such as Bcl-2, PI3K/Akt/mTOR, and TRAIL, to create more effective treatments that will make a material difference to survival and life for GBM patients (42,43).

Genetic Heterogeneity of GBM Cells

The genetic heterogeneity of GBM tumors is a chief contributor to its resistance to treatment. Three genetic mutations, specifically those in the epidermal growth factor receptor (EGFR), phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and tumor protein p53 (TP53) genes, contribute to GBMs' high mitogenic potential, invasiveness, and resistance to treatment (48).

Identified through analysis of human GBM tissues, specifically in small-cell GBMs, mutant and wild-type epidermal growth factor receptors (EGFR) are overexpressed and contribute to GBM pathogenesis. The most common mutant form of EGFR (commonly named ΔEGFR) contains a deletion spanning from exon2 to exon7, which removes a portion of the EGFR extracellular ligand binding domain, leading to its inability to properly bind growth factor ligands such as transforming growth factor α (TGFα) or heparin-binding epidermal growth factor (HB-EGF) (49). Further, the exon2-exon7 deletion in ΔEGFR results in the receptor's reduced or slowed ability to be adequately

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endocytosed and removed from the membrane surface. As a result of ΔEGFR being unable to bind its ligand and its slowed internalization from the membrane surface, the downregulation of its activity is reduced, and ΔEGFR remains present on the cell membrane in a constant low-level "ON" state (50).

This constitutively active ΔEGFR favors cell growth signaling pathways, resulting in uncontrolled GBM cell growth (51). In addition, previous reports suggest that ΔEGFR upregulates the gene expression of wild-type EGFR ligands (TGFα and HB-EGF). Since ΔEGFR cannot bind these ligands due to its deleted extracellular binding domain, it has been observed that ΔEGFR stimulates the production of ligands to which wild-type EGFR would normally bind (51). Together, wild-type EGFR and ΔEGFR form an autocrine/paracrine loop. As ΔEGFR induces the expression of various growth factors, wild-type EGFR binds these growth factors and facilitates the unregulated proliferation of GBM cells (51). The ΔEGFR-induced unregulated proliferation and survival of GBM cells make them a difficult target for radiation and chemotherapy.

When considering drug resistance in GBM, PTEN signaling is another important pathway to consider. PTEN is a tumor suppressor gene that, under normal, non-mutant conditions, will stunt the growth of GBM and reduce its metastasis primarily by arresting the cell cycle and suppressing its glycolysis pathway (52). PTEN performs this tumor suppressor function as a negative regulator for the PI3K/AKT pathway, a vital cell signal transduction pathway for cell growth, metabolism, and survival (53).

Oncogenic machinery in GBM will downregulate or inactivate PTEN signaling, ultimately promoting cell cycle progression and reducing DNA damage repair in GBM cells (52). Also, the inhibition of PTEN signaling in GBM cells plays a primary role in their morphology and invasive characteristics. Recall that PTEN usually acts as a negative regulator for the PI3K/AKT pathway; therefore, in cancerous cells, this transduction pathway required for cell survival is upregulated. As a result, the overexpression of Akt proteins results in the increased colony formation of GBM cells (54). The microtubule-associated protein Tau usually functions in cell-cell interactions, cytoskeleton maintenance, and cell migration. Under mutant PTEN conditions, the Tau protein can stimulate the PI3/AKT pathway, resulting in migration of GBM cells and increased invasion of surrounding brain tissue (55). Notably, the outcomes of inhibited PTEN signaling in GBM cells, such as cell cycle progression, reduced DNA damage repair, boosted metabolism, and improved invasiveness, collectively reduce GBM cell sensitivity to chemotherapy and radiotherapy agents (52). Further analysis of the PTEN and PI3/AKT pathway is required to counteract the oncogenic mechanisms GBM employs for cell survival.

The third genetic mutation essential to note in this discussion is tumor protein p53 (TP53). A highly conserved region in the human genome, the TP53 gene encodes p53, an inducible tumor suppressor protein that maintains chromosomal integrity and protects against cancer formation in the human body. TP53 responds to various stressors in the cellular environment. Depending on the intensity of these stress signals, it can trigger DNA repair mechanisms, alter cell metabolism and the differentiation state of cells, arrest the cell cycle, or prompt apoptosis (56).

An essential function of p53 is its transcriptional activity. To perform the stress responses listed above, p53 functions as a transcription factor, binding to specific DNA elements that regulate tumor suppressor functions in the cell. Mutated p53 (mut-p53) contains a loss-of-function (LOF) mutation in which its DNA-binding domain is compromised. This mutation causes p53 to lose its critical transcriptional activity and inhibits its tumor suppressor ability. This LOF mutation is central to GBM cells' increased proliferation, invasiveness, and cell migration.

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Further, mut-p53 increases resistance to DNA-damaging mechanisms presented by radiotherapy and chemotherapy drugs. Since mut-p53 tumor suppressor function is inhibited, it will not arrest the cell cycle or trigger apoptosis in response to DNA damage. Instead, GBM cells continue dividing, strengthening their metastasis and malignancy (57). Notably, this LOF in mut-p53 is not exclusive to GBM but is present in various cancers and oncogenic pathways (57).

Identified explicitly in GBM is a novel gain-of-function (GOF) mutation in p53 that increases inflammation in the tumor microenvironment. This GOF mutation in p53 is due to a point mutation resulting in a single amino acid substitution (missense) that replaces Arginine (R) with Leucine (L) at position 248 ($TP53^{R248L}$). This missense mutation promotes an augmented inflammatory tumor microenvironment via the nuclear factor-kappa B (NF-κB) pathway, a core signaling pathway controlling immune responses and inflammation. The $TP53^{R248L}$ mutation transitions GBM cells to a pro-inflammatory or "tumor-promoting state, "ultimately suppressing antitumor immune responses (58).

This pro-inflammatory and immunosuppressed state of GBM cells can also promote vascularization in GBM tumor cells (angiogenesis) and make GBM cells more elusive to endogenous immune responses. These malignant characteristics encourage drug resistance in GBM cells as it becomes increasingly difficult for immunotherapy and chemotherapy agents to target the tumor specifically (59).

Aside from the oncogenic mutations in p53, the accumulation of mut-p53 in GBM cells also contributes to their malignancy and resistance to treatment. Usually, cellular levels of p53 are regulated by murine double minute 2 (Mdm2), a dual-action protein that functions as a negative regulator of p53 and as an E3 ubiquitin ligase (59). Mdm2 negatively regulates p53 by suppressing its transcriptional activity through interactions with its N-terminal domain. Further, Mdm2 tags p53 for degradation via proteasomes (60). In GBM cells containing mut-p53, Mdm2 function is compromised, resulting in the accumulation of mut-p53 protein in the cytosol with the potential to form amyloid oligomers. Although the exact mechanism of formation of these mut-p53 amyloid oligomers is not fully understood, their presence indicates a GOF mutation in p53 and dramatically contributes to the malignancy and chemoresistance of GBM cells (61).

It is fundamental to note how mut-p53 and mutant forms of EGFR and PTEN discussed above contribute to chemoresistance in GBM cells. There is increased MGMT expression in GBM cells with mut-p53 (and in GBM cells containing mutant EGFR and PTEN) (62). The MGMT enzyme is responsible for repairing DNA damage introduced by alkylation. As mentioned, TMZ is an alkylating agent that introduces DNA damage to GBM cells to induce their apoptosis. MGMT upregulation in GBM cells bolsters their DNA damage repair mechanisms, enhancing their ability to repair the effects of alkylation introduced by TMZ and evade apoptosis, resulting in a chemo-resistant phenotype (62).

Cell Cycle Effects

One of the hallmark events of cancers is disrupting the normal cell cycle functioning (63). When the regulatory mechanisms for proliferation are mutated, such as the cell cycle checkpoints, disorderly growth is allowed regardless of cell contraindications (63). GBMs are remarkably resistant to most current forms of treatment, and the recurrence of tumors post-treatment can be explained by various molecular mechanisms related to the cell cycle (42).

GSCs are not all the same in terms of classifications. There are two suggested behavioral classifications of GSCs: quiescent and proliferative, or qGSCs and pGSCs, respectively (64). These cells have been found to exist at different locations when cultured within organoids, specifically with quiescent-type cells existing in the center and those more proliferative at the edges, concurrent with the highly aggressive spreading of these tumors (65). One proposed hypothesis is that a portion of

 doi:10.20944/preprints202503.1011.v1

aggressive GBM tumors contain quiescent cancer cells (66). The quiescent nature of these cells (also a key feature of stem cells) allows them to exist in a semi-dormant state, capable of being reactivated at any time, matching the tumor's recurrence patterns in human patients (67). Molecularly, qGSCs have inhibited CDKN1A and Cyclin B1 levels in the cell cycle, which are typically carefully activated to allow for cell cycle progression (64, 67). There are also methods like accumulating p27 (usually degraded), which aids the qGSCs to maintain their dormancy in the G0 phase (64, 68).

However, we see a much different cell behavior when looking at pGSC's. It is known that the more proliferative GSCs tend to order themselves at the periphery of tumors (65), and studies have been conducted to examine precisely how these cells work along with aggressiveness (69). The GSC cells are highly migrative and cause degeneration in surrounding tissue (70). A key marker of proliferation is that of *Ki67,* a cell cycle marker that indicates the cell is actively dividing (71). *Ki67* has been identified as appearing at specific times and different concentrations, indicating that the presence (positive marker) or absence (negative marker) would require cells at all different phases [of the cell cycle] to be present in the sample (71). Many other genetic markers exist, including the well-studied protein family of cyclin-dependent kinases (CDKs). Cdc2 (cell division cycle 2) is a notable marker for proliferating cells, as it is a stimulating mechanism for mitosis (72). There are also up to five different CDKs responsible for regulating the cell cycle of GBM cells, which emphasizes how difficult it is to treat these aggressive cells (73).

Novel treatments like various *azoles* have been found to target the G2 and M pathways of the GBM cell cycle (74). Specifically, *Flubendazole*, *Mebendazole*, and *Fenbendazole* were shown to have positive suppression against GBM cells, shutting down interphase via the P53, P21, and Cyclin B1 pathways (74). In addition, *oncolytic viruses* with simultaneous antibody therapy have been shown to act as checkpoint inhibitors (75). This finding is particularly important for future widespread clinical applications, as it was tested on humans in a clinical trial resulting in minimal adverse effects (75). Recent (2023) breakthroughs in the use of interferons have also shown promising evidence about the downregulation of GBM cell cycle gene expression (76). *HerberFERON* (consisting of multiple interferons), was found to be successful in treating cultured GBM cell lines within a laboratory setting (77), also indicating that novel co-formulations of proteins may be effective in specifically treating pGSC’s (76, 77). With knowledge of the molecular mechanisms controlling the cell cycle in qGSCs and pGSCs, new therapies may provide longer-lasting, efficacious patient results. Not only would a highly aggressive form of cancer like GBM indicate cell cycle mis regulation (63, 76), but it can also allow for a target of further GBM therapeutic research.

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