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Decoding Chemotherapy Resistance in Gliomas: Novel Mechanisms and Translational Opportunities for Precision Therapy

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Abstract

Glioblastoma (GBM) is the most prevalent and lethal diffuse glioma in adults and is still linked with fast functional deterioration and a median overall survival of ~1–1.5 years after maximal safe resection and radiotherapy with concomitant and adjuvant temozolomide (TMZ). Although TMZ induces O6-methylguanine lesions in DNA, chemotherapy resistance is very heterogeneous and clinically unsolved. However, radiotherapy is only effective because the disease can recur after treatment, which means that no single biomarker (like methylation of the MGMT promoter) can account for radiotherapy resistance. The goal of this narrative review was to understand and emerging clinical mechanisms of resistance to TMZ into a multi-dimensional context for supporting biomarker strategies, patient stratification and mechanism-driven precision therapies. The PubMed and Google Scholar databases were searched to find all relevant review articles, preclinical and clinical cohort studies of TMZ resistance in glioma and these were grouped into DNA repair (MGMT, mismatch repair [MMR], base excision repair [BER]), tumor microenvironment/blood–brain barrier (BBB), signaling pathways, non-coding RNAs/epigenetics, metabolism/redox biology and therapeutic approaches, and narratively synthesized. TMZ resistance seems to occur in a multifactorial and interactive manner: MGMT activity has been found to reverse TMZ damage and methylation of MGMT is not a dichotomous predictor, but a graded one, with intermediate methylation showing a modest benefit, and intensive therapy having an impact on the survival difference between methylated and unmethylated TMZ resistant tumors. The MMR defects that occur after birth (such as MSH6/MLH1) promote the selection of TMZ and hypermutator recurrence, and BER (including APNG upregulation) can confer resistance even in the

presence of MGMT-methylation. Common mechanisms of resistance to glioblastoma treatment include metabolic and epigenetic changes, stress response pathways, non-coding RNAs, intratumoral heterogeneity, and microenvironmental factors, such as BBB-related drug exclusion and immunosuppressive cytokines. The use of clinical strategies against MGMT or immune checkpoints has been ineffective, possibly because of drug toxicity and delivery. There is current evidence for the value of integrated multi-omics profiling, as well as better delivery systems and combination therapies aimed at different resistance pathways to prevent recurrence.
Keywords: Glioblastoma, Temozolomide, Chemoresistance, Glioma stem cells, Tumor microenvironment, Precision therapy, Biomarkers.

Background

Gliomas are primary brain tumors originating from the glial cells and can vary from low-grade diffuse gliomas to high-grade gliomas like glioblastoma (GBM). GBM is the most frequent and most aggressive type of adult diffuse glioma. It tends to spread quickly and into the nearby brain tissue and has a poor prognosis, with median overall survival still around one to one and a half years despite current standard treatment [1]. Patients often deteriorate due to early mortality and progressive functional decline. In general, the policy for treating high-grade gliomas is to use multimodal therapy, typically including surgery, radiation therapy, and chemotherapy, especially when the tumor is IDH-wildtype GBM. Treatment usually starts with maximal safe resection to remove mass effect, alleviate symptoms and acquire tissue for histopathological and molecular analysis [2]. Tumor cells, however, are not confined to the areas of contrast enhancement seen on MRI, however, and therefore surgery alone can't give long-term control. Today, this is the standard treatment, with fractionated radiotherapy together with temozolomide (TMZ), and then adjuvant TMZ. This treatment regime has been shown to prolong survival, but not to alter the near invariable pattern of relapse following radiotherapy. TMZ is an orally active alkylating agent that can pass the blood-brain barrier and causes DNA damage, specifically at the O6 position of guanine, resulting in cell-cycle arrest and apoptosis in TMZ-sensitive glioma cells. Other treatments such as tumor-treating fields, bevacizumab, targeted drugs and immunotherapies have been evaluated but have so far given only modest and temporary survival benefits, except when used in specific groups of patients [2].

This intensive first-line treatment will not stop the recurrence of high-grade glioma in most cases, and recurrence may occur within months of the end of first-line treatment. Poor long term disease control, especially early relapses and the absence of clearly effective second line therapy, is a major barrier to many. One of the main reasons for this failure is resistance to TMZ and other cytotoxic agents. These all have established mechanisms: high activity of O6-methylguanine-DNA methyltransferase (MGMT), mismatch-repair and base-excision-repair pathway alterations, poor penetration of drugs across the blood-brain barrier and high expression of ATP-binding cassette transporters, and significant cellular and molecular diversity and adaptability in the tumor. Such mechanisms do not account for the highly variable response to treatment and prognosis among patients with similar clinical and molecular

characteristics [3]. In particular, while the methylation of MGMT is a common predictive biomarker for TMZ benefit, it does not clearly distinguish responders from non-responders and both methylated and unmethylated tumors can exhibit early progression or sustained disease control [2,3].

Significant evidence is emerging that there are also other biological layers involved in chemoresistance in gliomas. These are the glioma stem-like cell populations, redox imbalance and cellular metabolism, non-coding RNA epigenetic regulation, and various factors of the tumor microenvironment, including hypoxia, immunosuppressive myeloid cells and extracellular-vesicle-mediated signaling. These processes can also engage with the classical processes of DNA-damage and repair and facilitate the persistence of drug tolerant cells and the support of tumor evolution under therapy stress. However, they are frequently taught as distinct, and they are not systematically integrated into clinical applied resistance models used for the development of clinical biomarkers and patient stratification and treatment design [4,5]. Consequently, existing approaches to precision oncology in glioma are still largely based on single markers, such as MGMT promoter methylation, and do not consider multidimensional profiles that reflect the complexity of the biology of resistance. The goal of this clinically relevant narrative review, titled “Decoding Chemotherapy Resistance in Gliomas: Novel Mechanisms and Translational Opportunities for Precision Therapy,” is to present established and emerging mechanisms of chemotherapy resistance in gliomas in a clinically relevant framework which supports multidimensional biomarker strategies, the more successful patient selection and the design of mechanism-driven therapeutic approaches for precision therapy [4,5].

Methodology

Literature Search Strategy

The study was conducted as a structured literature search about temozolomide (TMZ) resistance in gliomas. The articles chosen for this review were those that were relevant to resistance mechanisms in TMZ and that were potentially translatable. The studies were chosen based on their relevance for their temozolomide resistance in gliomas, according to the academic search databases including PubMed and Google Scholar. Selected studies comprised a mix of review articles (both narrative and peer review), preclinical studies, and clinical observational studies (cohort-based) on glioma and chemotherapy resistance.The review was done by research focusing on temozolomide (TMZ) resistance in gliomas, which is one of the main limitations in glioblastoma treatment. Beyond the main mechanisms (MGMT mediated DNA repair or mismatch repair (MMR) deficiency), the selected studies provide clues to other areas that impact treatment response such as metabolic reprogramming, oxidative stress and ROS activity, non-coding RNAs, autophagy, immune suppression and tumor microenvironment interactions. Others examine drug delivery, drug resistance and targeted therapy as means of combating resistance. They also illustrate the occurrence of resistance in actual patients and the management of resistance. As the topic was so broad, only core mechanisms of temozolomide resistance were reviewed, and other topics like biomarkers and therapeutic strategies were incorporated where appropriate. As a

result of the broad nature of the issue, this review focuses primarily on the primary mechanisms involved in temozolomide resistance. Other issues, such as biomarkers and treatment methods, have been covered as required in order to substantiate the main results. Major search keywords used for conducting literature search were “Glioblastoma,” “Glioma,” “Temozolomide,” “Temozolomide Resistance,” “Chemoresistance,” “MGMT,” “Mismatch Repair,” “Base Excision Repair,” “Glioma Stem Cells,” “Tumor Microenvironment,” “Blood Brain Barrier,” “Signaling Pathways,” “Noncoding RNA,” “Metabolic Reprogramming,” and “Precision Therapy.”

Inclusion criteria

A selection of studies was chosen based on specific criteria associated with the research focus on temozolomide resistance in gliomas. More attention was paid to articles focusing on glioma or glioblastoma that are explanatory of temozolomide resistance and how it can occur. Studies were considered as eligible as they explained well the important molecular mechanisms like DNA repair pathways (MGMT, MMR, BER), signaling pathways and the interaction between the tumor environment and signaling pathways. Furthermore, Research related to selecting key biomarkers such as MGMT methylation and hypoxia markers was considered, where applicable, in terms of treatment response and patient outcomes. Wherever mentioned therapeutic approaches, such as immunotherapy or targeted therapy and metabolism-based approaches, were discussed, the review also covered such studies in the context of overcoming resistance. In order to cover various perspectives, evidence was gathered from both lab-based studies and clinical studies, including cohort analysis.

Exclusion criteria

If a study met the following criteria it was excluded: were not directly related to glioma or chemotherapy resistance, made general observations about conditions other than those involved in the study, increased focus on drug delivery or biological processes, rather than resistance mechanisms, not provided any useful information for the aims of the research. For instance, studies on different types of cancer that aren't related, like hepatocellular carcinoma, were not included in the analysis. In addition, studies primarily focused on drug delivery in non-glioma settings, but not on chemotherapy resistance were not included but were briefly summarized for basic understanding.

Study Selection Approach

All the articles were read and categorized on their major content then classified into specific areas: the DNA repair mechanisms are also referred to as MGMT, MMR, BER., the interaction of tumor microenvironment and blood brain barrier, the molecular signaling pathways (PI3K/AKT, MAPK, Wnt, JAK/STAT) are covered, tumor is a mass of abnormal cells. A tumor is the growth of abnormal cells, on-coding RNAs and epigenetic regulation, metabolic changes, oxidative stress and activity of reactive oxygen species (ROS) and treatment and therapeutic strategies.

In addition to the above-mentioned themes, additional detail was provided in some studies. For instance, some gave explanations on metabolic changes in tumor cells, NAD+ related resistance

mechanisms and RNA based regulation of resistance. Other studies emphasized resistance related to immunity and treatment. The presence of reactive oxygen species (ROS) in chemotherapy resistance was also mentioned in some studies where it was observed that the tumor cells tolerate oxidative stress and become more resistant to chemotherapy. Finally, this enabled it to integrate both known and new ideas.

Data Synthesis Method

A narrative approach was used to combine the information from the selected studies. Since the articles included different types of research, such as review articles, laboratory-based studies, and clinical studies, statistical analysis was not suitable. The results were presented in major themes including chemotherapy resistance mechanism, biomarkers and clinical significance, and therapeutic approaches. The information from various studies followed by comparison to see the common themes, like DNA repair mechanisms, signaling pathways, and tumor microenvironment interactions; new concepts such as metabolic changes and non-coding RNA regulation. Overall, this helped collate findings and add known new ideas.

Discussion

Molecular Mechanisms of Temozolomide Resistance

Temozolomide (TMZ) works mainly by methylating the O6 position of guanine in DNA, which causes tumor cells to be unable to repair the methylation and thus causes them to become lethal during the process of DNA replication. So, on the clinical side, it is hoped that this damage will induce programmed cell death. In a large fraction of patients with glioblastoma, it does not and for 20 years [6]. The question of why has been unanswered, with no satisfying clinical answer. The best studied explanation relates to O6-methylguanine-DNA methyltransferase (MGMT): this is a DNA repair enzyme which essentially functions as an antidote to the damage done by TMZ before it can kill the cell. As a 'suicide enzyme', MGMT transfers the O6-methylguanine methyl group to one of its own cysteine residues to restore the DNA, and in the process, the MGMT is consumed. In the event that MGMT is active at adequate amounts, the chief mechanism that kills cells due to TMZ is neutralized at the site of injury [7].

Promoter methylation of the MGMT gene is important because it silences MGMT, resulting in its methylation status being the main predictive biomarker guiding clinical decisions in glioblastoma. This association has been well documented and is true for patients with MGMT-methylated tumors. The issue is that in clinical practice it has been considered as a binary, methylated or not. This is not supported by evidence from Kijima et al., who found that even after controlling the overall methylation level, patients with different levels of methylation had different survival benefits, and patients with intermediate methylation levels did not have the same benefit as those with high methylation [8]. There are also hints that the survival gap between the groups with and without methylation may

disappear in patients who receive the entire Stupp regimen, possibly due to the ability of this more intensive therapy to overcome intermediate resistance or because of the action of MGMT-independent mechanisms of cell death after prolonged exposure [7,8]. In either case the message is clear, we are making high stakes decisions about treatment based on a biomarker that we are not fully characterized. The mismatch repair (MMR) system adds to the complexity. MMR recognizes O6-methylguanine/thymine mismatches that form during replication and tries to repair them repeatedly leading to what are termed as futile repair cycles, ending in double-strand breaks and cell death in tumors without an intact MMR system. This dependence turns into a liability: a tumor with a mutation in MSH6 or MLH1 will be unable to participate in this whole cycle. Such mutations are more common in recurrent glioblastomas and have often been seen to come with hypermutator phenotype, indicating that the selective pressure applied by TMZ has a selective enrichment effect of MMR-deficient clones throughout the treatment course [9]. It is more than a curiosity and has a direct impact on re-treatment strategies at recurrence and it could be the reason why tumors that initially respond to TMZ, return in a form that no longer responds [9].

Base excision repair (BER) is in parallel. Although the O6-methylguanine lesion is the most lethal lesion that TMZ can form, it is not the most prevalent. The N7-methylguanine and N3-methyladenine lesions are, in number, more prevalent and BER efficiently removes them before they can be converted to cytotoxic intermediates, such as N7-methylguanine [10,11]. The upregulation of APNG in resistant cells is a factor that is not sufficiently addressed in the clinic since it makes the tumor resistant even if it is MGMT methylated. Some of the discrepancy in treatment response may be accounted for by BER status alone and not by MGMT.

On the outside of the DNA repair axis, there are other ways to resist. The secondary cytotoxic effect of TMZ is oxidative stress and resistance involves activation of NRF2 that leads to upregulation of antioxidant programs such as superoxide dismutase 2 (SOD2) and glutathione reductase that result in maintenance of redox homeostasis under drug-induced stress [10,11]. The long non-coding RNA (lncRNA) PDIA3P1 has been identified to have the ability to stabilize the transcription factor C/EBPβ, which contributes to the proneural to mesenchymal transition (PMT) and the development of an aggressive and therapy-resistant phenotype [12]. Additional pathways that regulate apoptosis, stemness, and drug metabolism are further modulated by other microRNAs such as miR-21 and miR-195, but which are not represented by any single biomarker. Resistance-associated proteins in cell adhesion and downstream signaling have also been identified through proteomic analysis that further supports the notion that TMZ resistance is not a single event or a single protein, but a complex trait of the cell [12].

All this leads to the conclusion that the resistance against TMZ is not a consequence of a single defect, but rather a combination of intertwining systems of DNA repair, redox control, transcriptional reprogramming, and networks of non-coding RNAs, all of which reinforce one another. This hierarchical structure is the very reason why blocking any one part of the system has been shown to be ineffective in a variety of pre-clinical and clinical trials [11,12].

Table1: Mechanisms vs Drugs

Mechanism	Drug / Therapeutic Strategy	Summary
MGMT-mediated DNA repair	O6-benzylguanine, O6-(4-bromothenyl) guanine	MGMT repairs the DNA damage caused by TMZ and is the main reason for resistance; inhibitors can improve TMZ effect but may cause toxicity.
MMR deficiency	Lomustine, PAR/PARG-based combinations	Loss of MMR proteins stops proper DNA damage processing, leading to resistance and tumor recurrence.
BER activity / PARP-linked repair	PARP inhibitors, olaparib	BER fixes DNA damage caused by TMZ; blocking this pathway can make tumor cells more sensitive to treatment.
Glioma stem-like cells	CAR-T targeting stem cell markers, anti-stemness strategies	These cells survive treatment and help the tumor grow back after therapy
PI3K/AKT/mTOR activation	Paxalisib, temsirolimus, perifosine	This pathway helps tumor cells survive, grow, and resist cell death
MAPK signaling	Sorafenib, p38 inhibitors, BRAF/MEK inhibitors	This pathway helps tumor cells adapt, survive stress, and become more resistant
JAK/STAT / STAT3 signaling	STAT3 inhibitors, WP1066	This pathway increases resistance by supporting tumor survival and reducing immune response.
Hypoxia / HIF signaling	HIF inhibitors, hypoxia-targeted therapy	Low oxygen levels help tumor cells survive and reduce the effect of treatment.

Efflux transporter overexpression	P-gp/BCRP inhibitors	Tumor cells pump drugs out, reducing the amount of drug inside the cell
ncRNA / exosome-mediated resistance	ncRNA targeting, exosome-based therapies	Small RNAs control important processes that help tumor cells resist treatment.
Metabolic rewiring	DCA, ferroptosis strategies, metabolic inhibitors	Tumor cells change their metabolism to survive and resist therapy.
BBB-limited delivery	Nanoparticles, focused ultrasound, intranasal delivery	The blood–brain barrier prevents enough drug from reaching the tumor.

Tumor Heterogeneity and Clonal Evolution

To understand the mechanism of TMZ resistance, one must consider more than just what is happening in any one cell and realize that GBM is not a homogeneous disease. It is a group of sub clonal populations that can be broadly designated as proneural, classical, and mesenchymal subtypes having different molecular profiles, metabolic dependencies, and importantly, different sensitivities to therapy [13]. This heterogeneity is not a minor difficulty; it's a fundamental reason why even the best treatment won't achieve long-term control.

The main issue is that TMZ is a selective pressure in this heterogenous population. It selectively enriches resistant clones while removing the TMZ-sensitive ones and is enhanced by the genomic instability of glioblastoma. Long-term exposure to TMZ has been directly linked to the emergence of a hypermutator phenotype, mainly due to MMR deficiency that leads to a significantly higher mutational burden [13]. When the tumor reaches this state, it will accumulate new mutations at a rate greater than it did previously, and some percentage of these new mutations will have new adaptive benefits. This hypermutated signature is often observed in recurrent glioblastomas sequenced following TMZ treatment, suggesting that TMZ is causing a qualitative shift in tumor biology. However, genetic changes are not the only factors. Also, there is increasing recognition that tumor cells can change their behavior, without changing their DNA sequence a process known as phenotypic plasticity. Non-mutational adaptation is very much what is described by the proneural-to-mesenchymal transition outlined in the previous section. Under therapeutic pressure, tumor cells undergoing PDIA3P1 stabilization remodel themselves towards a gene expression program related to increased invasiveness, decreased apoptotic sensitivity, and increased stemness, which is associated with a mesenchymal gene expression state, as observed for C/EBPβ [13,14]. The clinical consequence in this situation is quite unexpected: that is, in certain circumstances, The clinical consequence in this situation is quite

unsettling: that is, in certain circumstances, the treatment itself may cause the development of a more aggressive tumor type compared to the original one that existed. This evolution can only be captured by performing molecular profiling longitudinally at diagnosis and at recurrence, and not just once which is challenging in current routine clinical practice [14].

Glioma Stem Cells

Glioma stem cells (GSCs) give rise to recurrence, if tumor heterogeneity is the reason for the persistence of resistant populations after treatment. GSCs are not just resistant variants of regular tumor cells but are a distinct cell subpopulation that has self-renewal capacity, plasticity, and several features that render GSCs particularly resistant to TMZ [15]. The first problem is location. GSCs have been found to preferentially live in hypoxic and perivascular niches microenvironments that play a role in maintaining them and decreasing the drug exposure, especially in areas where the blood-brain barrier is not fully compromised [15]. Their relative quiescence exacerbates this: TMZ is replication-dependent, so slowly cycling or quiescent cells are less affected by TMZ, even if they have the same molecular profile. This leads to the presence of GSCs in protective niches in the bulk tumor, which is a source for seeding recurrence even after TMZ therapy. The second issue is that they have a greater ability to fix the damage that TMZ can inflict. The expression of both MGMT and BER components is higher in GSCs than in non-stem tumor cells, allowing them to more effectively repair the lesions in the TMZ and consequently survive. GSCs have higher expression of both MGMT and BER components that allow TMZ-induced lesions to be repaired more efficiently, and thus the GSCs survive. Importantly, therapeutic stress activates key stemness signaling pathways such as Wnt/β-catenin, the Notch pathway, and Hippo effectors YAP/TAZ, which all help the survival of the GSCs and the maintenance of stemness [15]. The relation between Wnt/β-catenin signaling and MGMT expression is particularly significant which establishes a direct mechanistic link between stemness and the ability of the cells to repair the damage caused by TMZ, and as the cells become more stem-like, they become more able to do so. This is not a coincidence because, to some extent, stemness and drug resistance exist in the same state of biology.

The third is phenotypic plasticity of GSC populations, and perhaps the most clinically frustrating. The stem-like reservoir can be eliminated by targeting cells that express canonical stem cell markers, but it is clear that GSCs can switch between stem-like and more differentiated states in response to microenvironmental signals, making this task difficult [16]. Tumor cells that are not stem cells can return to a stem cell-like state in response to stress, which means that they can regenerate the population being targeted by therapy. In these cells, there are various microRNAs and transcription factors that regulate mitochondria apoptosis pathways which further favor cell survival [16]. If not, then until the GSC-instructed strategies can be introduced into frontline therapies, they will continue to be the primary biological cause of GBM recurrence. The above sections explain what occurs in the tumor cell and in the stem-like derivatives of the tumor cell. The next section focuses on what occurs around those cells, the microenvironment that protects and sustains them.

Tumor Microenvironment

It is now recognized that the tumor microenvironment (TME) is not passive backdrop to the biology of glioblastoma but actively promotes resistance to therapy to the tumor cells, acting at all three levels: physical, metabolic and immune. Its contributions happen at multiple levels and are such that resistance mechanisms within the tumor cell itself may not ever need to be complete, if the surrounding environment is already limiting enough the delivery of the drugs and immune surveillance. The most immediate of the structural factors is the blood-brain barrier (BBB). TMZ is one of the handful of chemotherapeutic drugs that can penetrate the BBB reasonably well in normal situations, but not uniformly throughout the tumor mass. The BBB can be relatively intact, even in a disrupted tumor core, at the invasive edges where GSC and resistant subclones are overrepresented [17]. The regions are thus defined as 'pharmacological sanctuaries' where therapeutic concentrations of drugs are not achieved and where the biology most important to recurrence is least influenced by therapy. In the tumor, all of the TME cells contribute to the survival of them beyond the structural support. Glioma cells are linked to astrocytes via gap junctions, and it has been suggested that these junctions are able to transmit metabolites and mitochondria that enhance the metabolic resistance of the tumor cells to treatment-induced stress [17]. This cell-cell metabolic support is hard to target with therapeutics since it does not necessitate any change in those cells itself. The same goes for the immune microenvironment. Cytokines such as IL-10 and TGF-β are produced by glioma-associated macrophages (GAMs), regulatory T cells and myeloid-derived suppressor cells that create a very immunosuppressive microenvironment. In this context, any endogenous anti-tumor immune response triggered by immunogenic cell death following TMZ treatment is significantly reduced, potentially reducing the additional immune-mediated cytotoxicity that could be responsible for therapeutic benefits [18].

A more recently recognized pathway of dissemination of resistance traits across the tumor is via extracellular vesicles (EVs). Proteins and non-coding RNAs are carried by EVs, which allows for the spread of resistance determinants horizontally between cells, so they do not need to be evolved in each subclone [18]. This conceptually alters the scale of the resistance problem: when a small minority of resistant cells exist, even these can, via EV-mediated communication, spread some level of resistance to a much larger population of tumor cells. Taken as a whole, these findings clearly illustrate the necessity to understand and treat resistance not just on the level of the individual tumor cell.

Metabolic Reprogramming and Adaptive Resistance

The metabolic characteristics of glioblastoma are not merely a consequence of rapid tumor proliferation. Actively they are increasingly recognized as a contributor in therapy resistance. Glioblastoma cells prefer aerobic glycolysis even when oxygen is available. This is called the Warburg effect, and it enables these cells to generate ATP quickly and produce biosynthetic materials that fuel their growth. They also become less dependent on mitochondria, making them more susceptible to triggers that cause cell death. Oxidative stress management is a parallel axis of metabolism with clear links to TMZ function. The other mechanism of TMZ toxicity is the formation of ROS. TMZ-resistant

tumor cells combat this by an NRF2-dependent antioxidant program with an increase in glutathione production and SOD2 expression to clear the detrimental effects of ROS before they can cause any further damage [19]. It is worth mentioning that the upregulation of NRF2 in glioblastoma cells is not a simple defense mechanism but plays a role in the resistance to TMZ, radiotherapy, and hypoxia in the tumor microenvironment.

One of the most intriguing aspects of resistance to metabolic therapies in terms of the underlying mechanisms might be related to NAD+ metabolism. NAD+ is an essential cofactor for PARP-mediated DNA repair, and the presence of NAD+ in the cancer cells regulates the ability of repair of TMZ-induced DNA strand breaks. In their study, Fakouri et al. showed that the combination of high levels of bioavailability of NAD+ along with PARG inhibition promotes the accumulation of PAR chains and unresolved DNA repair intermediates leading to the death of TMZ-resistant cells [19]. The strategy is promising since it focuses on the very process through which the cancer cells become resistant to TMZ. Interestingly, this method does not require the direct inhibition of MGMT, which was difficult due to systemic toxicity in patients. Another important aspect of lipid metabolism pertains to enhanced fatty acid oxidation and lipogenesis. Through these pathways, the cancer cells can obtain extra energy and have enough material to build their membranes [19].

Emerging and Novel Mechanisms

With the resistance pathways of MGMT, MMR, BER, and ROS management having been elucidated, interest in other forms of resistance has grown since they are potentially responsible for the remaining resistance seen in tumors that have intact classical pathways. Histone deacetylase (HDAC) mediated epigenetics regulation has been recognized as one pathway where glioblastoma cells can preserve their transcriptional profile favoring resistance without resorting to genetic modifications [20]. The use of HDAC inhibition has been shown to partially re-sensitize preclinical glioblastoma cells to TMZ treatment, most likely via de-repression of pro-apoptosis and differentiation genes that may have been turned off. Clinical trials involving HDAC inhibitors on glioblastomas have thus far proven to be discouraging, and it is currently believed that their efficacy would be in combination regimens rather than solo therapy.

POU-domain transcription factor POU3F1 (Oct-6) appears to be involved in the response to genotoxic stress independent of DNA damage repair processes [20]. Exposure to TMZ causes an increase in oxidative defense and cell cycle arrest under TMZ treatment, facilitating recovery from the initial toxic shock associated with therapy and subsequent re-entry into the proliferative phase after TMZ levels decrease. This approach is clinically important since it can't be inhibited by MGMT or MMR targeting approaches – the survival of cells relying on the Oct-6 pathway in response to TMZ will ensure survival despite defective DNA repair mechanisms.

Non-coding RNAs continue to exhibit unexpected and diverse regulatory activities related to cancer drug resistance. Apart from microRNAs, some circular RNAs and other microRNA species have been found to regulate cellular sensitivity to ferroptosis and autophagic flux, as well as cellular drug efflux,

in a cell-specific manner [20]. The importance of these discoveries does not only reside in their possible clinical applications; several circulating non-coding RNAs detectable in the bloodstream might serve as diagnostic markers for monitoring the development of resistance, which would prove highly useful in light of the difficulty of obtaining tissue samples from glioblastomas.

Resistance-associated molecular pathways uncovered by multi-omics as well as high-throughput proteomic approaches have already transcended beyond the classical candidate-gene approach traditionally used in resistance research [20]. Such studies provide new insight into protein-level factors responsible for the development of resistance but still present a huge problem of differentiating between causative factors and secondary changes accompanying resistance. It seems probable that the next decade will bring new targets for anti-resistance therapies based on such technologies if the interpretation tools of high-dimensional data become sufficiently developed.

Translational Opportunities

Such deep understanding of the underlying mechanisms discussed in the previous parts of this review has produced a large number of well-reasoned therapeutic targets. Taking such theoretical advances from bench to bedside is precisely where the greatest difficulties have been encountered, and a critical assessment of why this is the case must come before opportunities are discussed. A simple and logical solution would be direct MGMT inhibition. The most thoroughly investigated compound of this class, O6-benzylguanine, proved to effectively deplete MGMT within the tumor tissue but was ultimately hindered by severe, dose-limiting haemato-toxicity when given in combination with TMZ, due to the inability to deliver sufficient TMZ concentrations alongside MGMT inhibition [18]. In order to bypass rather than overcome MGMT, research efforts have therefore focused on exploiting the synthetic lethality principle using compounds capable of selectively killing cells with compromised DNA repair machinery, including PARP inhibitors and, more recently, NAD+/PARG targets [12,18]. To date, neither compound has provided evidence of significant efficacy against glioblastomas, and both require improved patient stratification according to tumor characteristics.

Immunotherapy has attracted a lot of investment as an alternative to traditional chemotherapy for treatment-resistant glioblastoma. The reasoning behind this is that clearing tumors through the immune system does not depend on the DNA repair processes that make temozolomide ineffective. However, immune checkpoint inhibitors, like PD-1 and PD-L1 inhibitors, have repeatedly shown poor results in randomized trials for glioblastoma, including Checkmate 143 and others. These trials did not demonstrate any increase in overall survival for patients with recurrent tumors [16,18]. This is primarily due to the immunosuppressive nature of the TME, which has been elaborated on in Section 4. High-grade checkpoint inhibition is ineffective in an environment that is dominated by regulatory T cells, M2-polarized macrophages, and myeloid-derived suppressor cells that secrete IL-10 and TGF-β. Neoantigen vaccines and dendritic cell-based immunization therapies.

All of the above is constrained by drug delivery which still represents a practical limitation. The use of nanoparticle-delivered drugs as well as focused ultrasound for transient disruption of the BBB to

increase drug delivery are currently being investigated as methods to improve drug delivery to invasive tumor margins where current methods fail [15,18,20]. The application of liquid biopsy techniques for the detection of tumor circulating DNA and other extracellular components, such as EVs, and non-coding RNA molecules can facilitate monitoring of resistance development in a way that will allow for changes in therapy before radiological evidence of progressive disease becomes available. Radiomics can also add complementary information regarding subregional tumor heterogeneity without the need for further tissue biopsies.

Table2: Biomarkers vs Clinical Relevance.

Biomarker	Clinical Relevance	Summary
MGMT promoter methylation	Better TMZ response	When MGMT is methylated, the tumor responds better to TMZ treatment
MGMT expression	Associated with resistance	High MGMT levels are linked to poor response to treatment and more resistance
MSH6 / MLH1 / PMS2	MMR-related resistance and hypermutation	Loss of these repair proteins leads to resistance and tumor recurrence.
CD133 / stem cell markers	Stemness associated resistance	These markers show the presence of resistant stem like tumor cells
Survivin	GSC maintenance / therapy resistance	Helps tumor cells survive and is linked to treatment resistance
ABCB1 / ABCG2 / P-gp / BCRP	Drug efflux mediated resistance	These proteins pump drugs out of cells, reducing treatment effectiveness
HIF-1α / HIF-2α	Hypoxia related resistance	These markers show low oxygen conditions, which make tumors more resistant
YAP / TAZ	Hippo pathway resistance markers	These are linked to poor outcomes and help tumor cells avoid cell death (Apoptosis)

IDH mutation	Prognostic and subtype relevance	Helps classify tumor types and can affect how patients respond to treatment
ADAMTSL4	Emerging immune related prognostic marker	Linked to immune related changes and poorer patient survival
ACSS3	Emerging immune metabolic biomarker	Related to metabolism and immune suppression, linked to worse outcomes
Exosomal miR-21	Non-invasive monitoring biomarker	Can be used to monitor tumor progression and treatment response

Critical Analysis

Although there have been considerable advances made toward understanding the TMZ resistance mechanisms in all areas discussed above, it is worthwhile asking why, after decades of steadily advancing knowledge of the biology of glioblastoma, there have been no improvements in the survival rates associated with the disease. Survival with the Stupp protocol is around 14 to 16 months, and no significant improvement has been achieved even after hundreds of clinical trials [16,17]. While the resistance mechanisms outlined above are indeed valid, it is crucial to question why there is such a huge disconnect between science and medicine. The limitations associated with preclinical models play a key role in answering this question. Most of the resistance mechanisms discussed in this review paper have been studied using well-established cell line or PDX models. Though these models have been useful in forming hypotheses, they do not appropriately mimic human glioblastoma due to several limitations such as the absence of immune and stromal cells within the tumor microenvironment, a complete representation of the clonal diversity of patients' tumors, and experiments being conducted on immune-deficient animals to rule out immune-mediated effects [15,18]. Drugs that demonstrated significant efficacy in these models failed to show any positive results in randomized clinical trials, and there has been a lag in developing more clinically relevant models such as organoids and co-culture systems including TME elements and syngeneic models with an intact immune system.

Another related issue in translational design involves patient selection and the use of biomarkers. Many clinical trials involving glioblastomas tend to enroll unselected patients, thereby making it difficult to demonstrate the effectiveness of therapy for those patients who will respond positively. Methylation of O6-methylguanine-DNA methyltransferase (MGMT) is the only biomarker for which validation has been done in routine clinical practice; however, even this does not provide a perfect prediction.

Mechanisms related to mismatch repair (MMR), glioblastoma tumor subtype, glioblastoma stem cell (GSC) markers, metabolism, and immunology are not commonly used in clinical trials despite their relevance to the mechanisms of resistance highlighted in this review. There are also aspects in which the scientific understanding of the phenomena is inconclusive due to inconsistencies in the data gathered. In terms of BER and MMR dysfunction in the development of clinical TMZ resistance, for example, the roles played by each have yet to be conclusively established due to results garnered from research conducted in different backgrounds and patients that are not entirely congruent. NRF2-dependent activation of antioxidants, meanwhile, while linked to resistance in vitro experiments [15,17], has not been studied extensively in terms of its clinical implications and whether it can be exploited without harming the brain. Contradictory data on these mechanisms does not negate their existence instead, it only indicates that there is much left to learn about TMZ resistance.

Possibly more complex still is the problem of the changing and evolving glioblastoma tumor in response to therapeutic pressures. Indeed, the recurrent tumor that is now being treated is biologically different from the initial diagnosis due to mechanisms of selection, therapy-mediated mutagenesis, phenotypic plasticity, and other environmental modifications, which have occurred during the period of treatment [19,20]. Treatments based on targets that were present at the time of diagnosis might very well be treating biology that no longer exists. To address this, a paradigm change may be required, including not just new treatments and drug delivery mechanisms, but new ways of testing therapies altogether through adaptive design and the capacity to monitor changing biology and adapt the treatment accordingly [19,20].

Conclusion

Treatment of Glioma has traditionally been through the administration of TMZ; however, its resistance towards the drug has caused limitations in management of Glioma. In order to adapt to the situation, the mechanism of resistance has been considered. The main mechanism is considered to be due to expression of MGMT; (although it has been proven to not be an absolute biomarker). ROS, genomic instability, phenotypic plasticity and transcriptional regulation as well as the activity of MicroRNA and IncRNA are a handful that depict the multifactorial resistance of TMZ. An important concept of recurrence can be demonstrated through heterogeneity and stemness of tumor cells. Elucidation of which can be done through GSC's characteristic features which includes enhanced DNA repair, Quiescence, drug efflux pump activity and microenvironment protection. The rise in bioavailability of NAD+ caused by the metabolic reprogramming would result in increased cell survival levels. This is due to the optimization of DNA repair.

As the resistance is Mult-causative, LC-MS proteomics can be of (1) Prognostic value as biomarkers (2) Determination of additional resistance pathways, and (3) As drug target of action. As of now, administration of drugs according to the resistance mechanisms are encouraged. This includes combination therapies that affect multiple of mentioned mechanisms, rendering them futile. Targeting

GSC in order to prevent recurrence has proven to have long term effects. Although cellular sources of the tumor is eliminated in this method, as they share similar characteristics as normal cells, protected by the microenvironment and are able to adapt and change their phenotype, this method is met with restrictions. Patient specific resistant mechanisms have been studied where patency towards integrated molecular profiling including genomic, transcriptomic and proteomic analyses is given in order to tackle the heterogeneity of the tumor. Henceforth it overcomes recurrence. Vaccines and immune-modulating therapies targeting the immunosuppressive biome of the glioma are found to improve response and overcome resistance, however this method has its downsides as it is impenetrable to the blood brain barrier and thereby targeting of the antigens may have complications due to the heterogeneity of tumor cells. Although MGMT has prognostic value, it cannot be considered an absolute biomarker considering recent research's data (proving MGMT-non methylated tumors showing sensitivity to full drug therapy). Hence further studies on MGMT-independent drug therapies will be of great importance. Furthermore, a focus on overcoming heterogeneity which might further enhance previously mentioned drugs (i.e. Vaccines and immune modulating therapies) and decrease rate of recurrence.

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