# Reactive Oxygen Species in Glioma

# 166

## Mechanisms and Challenges

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## Contents



**Abstract**

Glioma represents the lethal neoplasm of the brain with dismal progression in its most aggressive form, that is, glioblastoma. Hypoxia is well appreciated to have a role in cellular migration and proliferation of a range of malignancies; however, its multifaceted impact on tumors of glial origin is not clear. The aim of present chapter is to present the integrated view of hypoxia involvement in the biology of gliomas. Hypoxia impacts the biology of glioma mainly through the transcriptional factors HIF1 alpha and exists in spatiotemporal heterogeneity in its expression in the tumor. The hypoxic microenvironment impacts the pathology of

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glioma on account of several mechanisms such as by altering the metabolic switch from oxidative to glycolytic metabolism, promoting the expression of tumor, promoting signaling in the form of VEGF, PI3k, and MAP kinase cascade. In addition, hypoxia is notorious to induce the refractiveness to the existing therapies such as radiation and temozolomide-mediated chemotherapy. Targeting hypoxia mediators in combination with the present therapeutic regime could potentially sensitize glioma cells to induce cell death and prevent the inevitable recurrence.

**Keywords**

Hypoxia · Glioblastoma · Immunotherapy · Temozolomide · Circadian rhythm

## Introduction

### Basic Biology of Glioma

Glioma comprises of the most frequent malignancies of the brain. WHO classification 2007 segregates the glial tumors on account of its histopathology under the four grades: pilocytic astrocytoma (GI), diffuse astrocytoma (GII), anaplastic astrocytoma (GIII), and glioblastoma (GIV). The GI and GII comes under the roof of low-grade tumors while the GIII and GIV as high-grade ones. Among the high grades, glioblastoma is most aggressive neoplasm and is characterized with high mitotic index, microvascular proliferation, and necrosis (Louis et al. 2007). Growing evidences, however, have indicated the need to consider molecular markers to define the spread of disease. On this note, the WHO 2016 classification considers the markers as IDH mutations, 1p/11q co deletions, p53, and ATRX to assign the prognostic value (Louis et al. 2016). However, at present, many hospitals and diagnostic centers over the world are compelled to refer to the old system of classification as they cannot follow the new system on technological and financial grounds. In addition, with the new system, there is scope for disparity in diagnosis when IDH mutations and 1p/11q co-deletions are considered in certain cases. Nevertheless, the new regime of glioma classification has paved a way towards understanding the disease in a better way and facilitated the improvement of therapeutic management (Wesseling and Capper 2016).

## Current Standard of Therapy

Surgery to remove the tumor lesions is most widely accepted as initial standard of care, which could be either gross total or subtotal resection, in most of the cases. The anatomic origin of tumor is often taken into consideration at the time of resection which could otherwise compromise the normal cognition and associated physiological functions (Giese et al. 2003). Radiation therapy also remains a choice after

surgery and in case of recurrence. The selection of radiotherapy is often decided by the age of patients as radiation may potentially cause long-term cognitive impairment (Giese et al. 2003). At present, there is need to undertake the assessment of neurocognitive functions upon radiotherapy in low- and high-grade tumors for better evaluation of its efficacy. Despite of this, the median survival of glioblastoma patients still remains poor even upon the most aggressive course of treatment (Stupp et al. 2005). The failure to respond to the existing therapy is mostly because of infiltrative nature of tumor which impedes surgical removal and inherent heterogeneity guided by a subpopulation of cells called as glioma stem cells (GSC) which remain dormant and are potentially responsible for recurrence and potentiate temozolomide resistance (Grek et al. 2018). The presence of MGMT methylation is often reported to hinder the effect of temozolomide, which works by promoting mutations in the DNA and promoting cellular apoptosis. The MGMT methylation promotes DNA repair activity and hence directly counters the toxic effect of temozolomide. Thus, it is pivotal to eliminate the population of cells which potentially initiate the recurrence as well as are refractory to the treatment.

Recently, the synthetic lethality approaches are being practiced to enhance the sensitivity of commonly used drugs such as temozolomide. Gaspar J Kitange et al. have shown that retinoblastoma-binding protein 4 (RBBP4) modulates the sensitivity of temozolomide. They demonstrate that RBBP4 interacts with CBP/p300 and drives the expression of MGMT methyltransferase through histone acetylation. Therapies targeting this complex could sensitize glioma cells to temozolomide as MGMT overexpression is documented in over 70% of the pathologies (Kitange et al. 2016). Similarly, Tor-Christian Johannessen et al. have shown that dopamine antagonist thioridazine synergizes with the temozolomide chemotherapy. Mechanistically, glioblastoma cells are shown to have increased autophagy flux in response to temozolomide, and thioridazine impedes the autophagy by preventing the fusion of auto phagosomes with lysosomes thereby improving temozolomide toxicity and cell death (Johannessen et al. 2019). In addition, targeted therapies have become prevalent to treat glioblastoma. The most common targets are the drugs which disrupt the downstream activation of receptor tyrosine kinases and concomitant MAP kinase cascade which is a hallmark of these tumors (Brennan et al. 2013). At present, the therapies targeting VEGF (bevacizumab) and EGFR (Afatinib) are evaluated in the clinical trials to determine its efficacy (Touat et al. 2017).

Immunotherapies have gained significant momentum in treating the cancers. It works by unleashing the T cell inhibition and abrogating the inhibitory receptor interaction between tumor and T cells (Deshpande et al. 2020). It is now well known that brain is not immune-privileged area and well connected with the CNS lymphatic system (Hatterer et al. 2006). At present, randomized phase III trials are ongoing to check the efficiency of anti-PD-1 antibody nivolumab. In addition to the PD-1, other immune inhibitory receptors as CTLA-4, PD-L1, TIM3, LAG3, and TIGIT are also under investigation (Fig. 1) (Touat et al. 2017).

Glioblastoma is usually observed with low oxygenation. This often endorses increased angiogenesis in response to hypoxic tumor microenvironment, promoting

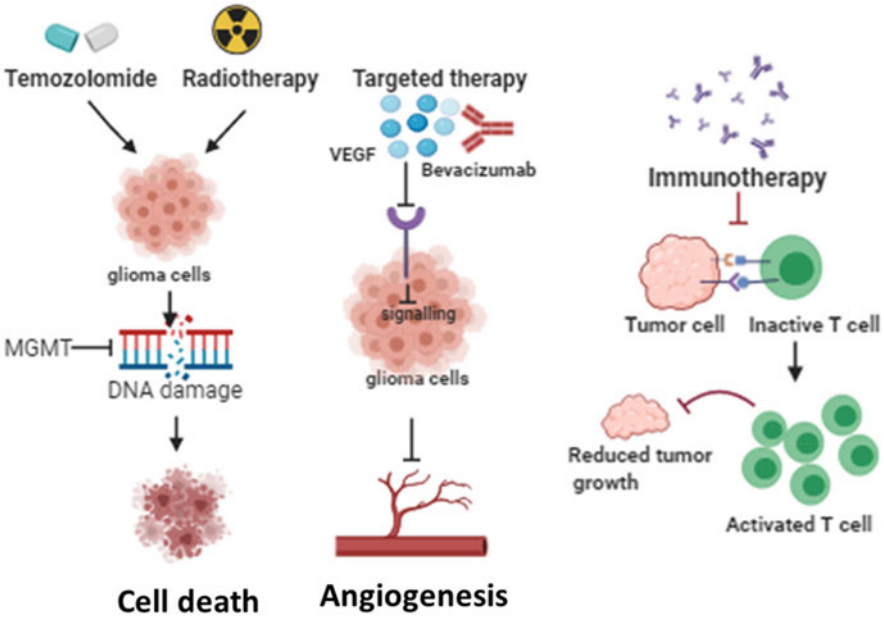


**Fig. 1** Standard of care for glioma patients

cancer stem cell formation which leads to potential resistance and recurrence. The nucleoside adenosine is shown to mediate the chemoresistance by interacting with the ABC transporter proteins in the hypoxic microenvironment (Uribe et al. 2017). Eroje M Ahmed et al. have shown that hypoxic glioma microenvironment induces the CD133 expression which is widely regarded as marker for glioma stem cells and promotes resistance to cisplatin-mediated chemotherapy. Mechanistically, HIF2 alpha was observed to drive the expression of CD133 (Ahmed et al. 2018). Additionally, hypoxia is shown to compromise DNA damage induced by radio and chemotherapy thereby reducing its efficacy and promoting resistance (Dalerba et al. 2007).

Thus, it is imperative that oxidative stress plays a pivotal role in shaping the tumor microenvironment (TME) over the course of tumor progression. The altered metabolic profile of cells of TME shares a bidirectional cross talk with the tumor and the immune cells leading to altered viability of the immune cells which thwarts its cancer cell-killing efficiency. In other way, tumor cells also uphold the intrinsic resistance to the external therapies resulting in recurrence and ultimately dismal prognosis to the combination of radio, chemo, and immunotherapy. Thus, it is imperative to know more about the mechanisms involved that lead to the alteration of metabolism and oxygen scarcity. So, presently, we have attempted to represent the hypoxia and oxidative stress-driven resistance mechanisms in glial tumors, paving a way towards its enhanced understanding for possible therapeutic interventions.

## Oxidative Stress in Brain and CNS

Oxidative phosphorylation occurring through mitochondria is the major energy production source in the brain. The reactive free radicals as reactive oxygen species (ROS), reactive nitrogen species (RNS) are generated as by-product of oxidative phosphorylation and are essential to normal neuronal functions (Pero et al. 1990). The excessive ROS are scavenged by the antioxidant system to maintain the normal homeostasis. The oxidative damage to brain is mitigated by antioxidant enzymes as glutathione peroxidase, catalase, and superoxide dismutase (Griendling et al. 2000). The free radicals are scavenged by superoxide dismutase to generate $H_2O_2$ which is further removed by the glutathione peroxidase and catalase enzymes (Saso and Firuzi 2014). However, when the antioxidant system compromises, it results in excessive lipid and protein peroxidation causing oxidative damage leading to weakened neuronal and concomitant physiological functions (Knapp and Klann 2002). The oxidative damage to brain is dependent on the anatomic origin. The vulnerability to succumb to the oxidative damage is guided by the set of neurons involved. For example, the neurons in the cerebellum and amygdala region are reported to be most susceptible to the oxidative damage (Wang and Michaelis 2010). Astrocytes and neurons in the CNS are equipped with the antioxidant system to mitigate the oxidative damage. In particular, the expression of enzymes such as catalase and superoxide dismutase is higher in astrocytes, thus making them vulnerable to the oxidative damage (Salazar-Ramiro et al. 2016).

## Oxidative Stress and Tumor-Associated Inflammations

Traditionally, the brain was considered to be immune-privileged area as it is covered by blood-brain barrier and lacks functional lymphatic system. But later, it was observed that immune cells and antigens in the brain interact with the cervical lymph nodes through the cerebrospinal fluid-filled channels (Louveau et al. 2015). The compromised blood-brain barrier in brain tumor further helps to enhance the migration of antigen-presenting cells in the CNS to the cells of immune system as T cells and return back (Roopenian and Akilesh 2007). Glial tumors, like other cancer cells in the body, activate the set of immune response. Tumor-associated macrophages (TAM) in the periphery and dendritic cells presents the antigen to the T cells (Sampson et al. 2017). The TAMs represent the major population of cells present in the TME and are involved in removal of debris, immune induction, and regulation of inflammatory response. Depending on the signals from the TME, the monocytes polarize the immune cells in two subtypes: M1 or pro-inflammatory and M2 or anti-inflammatory properties (Biswas and Mantovani 2010). The antigen-presenting cells near to the tumor create the immunosuppressed microenvironment through secretion of chemokines and cytokines. Thus, it is further aggravated by infiltration of antigen-presenting cells to the peripheral lymph nodes resulting in antigen-primed T cells (Reardon et al. 2014). To counterbalance the excessive immune reactivity, the tumor and immune cells also express a set of immune inhibitory receptors (PD-1, PD-L1,

CTLA-4, Lag-3, TIM3, TIGIT) resulting in decreased immune reactivity of these cells forming immunosuppressive milieu (Razavi et al. 2016). This microenvironment further compromises the antigen presentation by downregulating the MHC expression (Schartner et al. 2005). The presence of regulatory T (Treg) cells in the GBM TME is well appreciated and postulated to comprise 25% of tumor-infiltrating lymphocytes and often correlate with dismal survival in clinical cases (Ebert et al. 2008). Tregs are often observed to be recruited near to tumor site partly because of binding with CCR4 as blocking this receptor cannot mitigate its infiltration completely leaving scope for further investigation (Crane et al. 2012).

The in vivo study inducted in murine model shows that B cells are involved in antigen presentation and stimulate the proliferation of T cells in vitro (Candolfi et al. 2011). Zhuo-peng Ye et al. show that tumor-derived ADAM10 (A Disintegrin and metalloproteinase domain-containing protein 10) promotes differentiation of B cells to B regulatory cells which ultimately inhibits the CD8 T cell proliferation and promotes Treg cell generation (Eissa et al. 1989).

## Oxidative Stress and Immune Cell Functionality

Oxidative stress created in the tumor microenvironment is often detrimental to the immune cells in the vicinity. For instance, the enhanced oxidative stress can induce apoptosis in T cells, causing hyporesponsiveness in cancer patients (Cemerski et al. 2002). Marcin M Kaminski et al. show that oxidative stress generated from mitochondrial respiratory complex I is essential for IL-2 and IL-4 secretion from the T cells. Inhibition of complex I result in decreased secretion of IL4, thus providing a link between T cell functionality and mitochondria (Kaminski et al. 2010). Glutathione peroxidase (GP) is a major enzyme involved in scavenging the activation-induced oxidative stress in T cells. Mai Matsushita et al. show that T cells GP knockout mouse has intrinsic defect in maintaining the CD4 and CD8 T cell homeostasis. GP-deficient T cells accumulate lipid peroxidase and are observed to undergo cell death in response to activation (Matsushita et al. 2015). The lipid peroxidase are reported to be upregulated in brain tumors as glioma and meningiomas (Yilmaz et al. 2006). The increased lipid droplet accumulation is observed in GBM tissues as compared with normal brain. Monounsaturated fatty acids are observed to promote glioblastoma proliferation by promoting triglyceride metabolism (Taïb et al. 2019). However, it is yet unknown if lipid metabolism in the tumor cells affect the proliferation and functionality of immune cells in the microenvironment. Oxidative stress is known to directly impact the homeostasis of Treg cells. Tomasz Maj et al. demonstrate that enhanced oxidative stress boosts the suppressive function of Treg cells in the TME. Mechanistically, the ROS promotes mitochondrial apoptosis in the T cells and amplifies its suppressive effect, potentially promoting resistance to anti PD-L1 therapy efficacy in metastatic ovarian cancer (Maj et al. 2017). However, the metabolic balances among T cells and tumor cells in brain and how it alters the efficacy of immunotherapy is yet unknown. Dendritic cells are essential to maintain the T cell-dependent anticancer immunity. Juan R

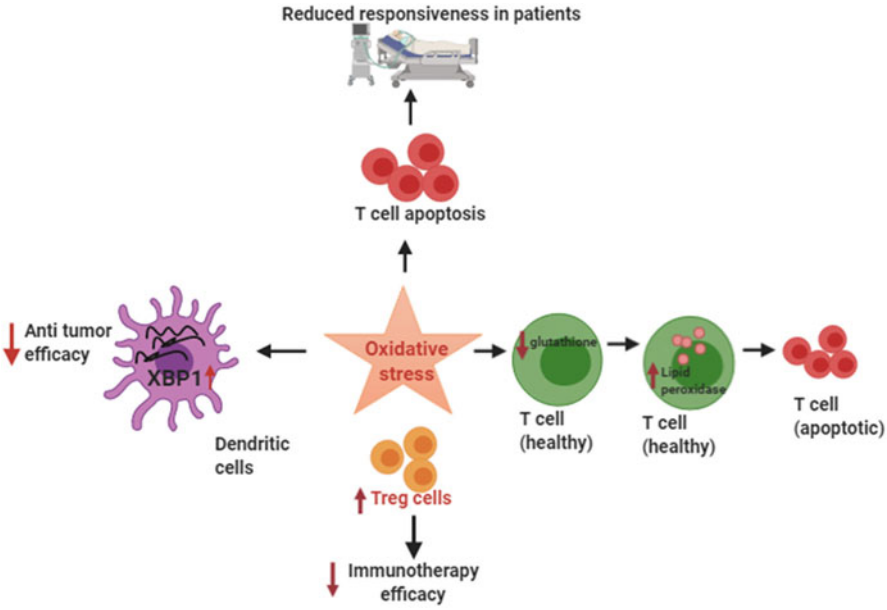


**Fig. 2** Impact of oxidative stress on immune cell functionality and therapeutic responsiveness

Cubillos-Ruiz et al. have examined the dendritic cell response to oxidative stress. They demonstrate that activation of ER stress factor XBP1 in dendritic cells blunts its antitumor immunity in ovarian cancer. Mechanistically, the increased lipid synthesis blunts the ability of dendritic cells to prime the T cells for antitumor activity. Conversely, XBP-1 inhibition leads to restoration of immunostimulatory activity and type I antitumor response (Cubillos-Ruiz et al. 2015). It would be interesting to examine if similar mechanisms exist in the glial tumors-T cell interaction (Fig. 2).

## ROS and Energy Homeostasis in Cancer

Mitochondrial metabolism is the main source of cellular energy production. Cancer cells produce higher ROS due to compromised mitochondria, oncogene activation which together lead to disturbed energy homeostasis (Chance et al. 1979). The glycolysis is the main channel whereby glucose is consumed resulting in energy production. The pyruvate is generally converted to lactate instead of channeling it to mitochondrial citric acid cycle. The intermediates reducing equivalents of the pathway are carried to the pentose phosphate pathway to generate building blocks for DNA and protein synthesis (Zhao et al. 2017). Oxidative stress plays a dual role in cancer progression. Moderate or low stress helps in cancer progression by inactivating tumor suppresser genes like PTEN and activates oncogenic signaling as PI3K, MAP kinase cascade (Locasale and Cantley 2011). Excess oxidative stress damages rapidly dividing cancer cells by inducing mutations through DNA repair

pathways leading to compromised genomic integrity (Gorrini et al. 2013). To overcome this damage, cancer cells activate the scavenging system composed of a set of antioxidant enzymes as SOD, catalase, and glutathione peroxidase, as discussed in previous section (Griendling et al. 2000).

The rampantly dysregulated signaling pathways as EGFR amplification, p53 loss, and PTEN mutation are also known to affect the redox balance of the cells. For instance, the signaling mediated by EGFR upregulates the intracellular production of ROS by downstream tyrosine kinase activity of EGF. Further, the EGFR signaling promotes the MAP kinase cascade leading to increased synthesis of DNA (Bae et al. 1997). PTEN is a known tumor suppressor gene and its mutated version does not respond to the anti-EGFR targeted therapy. Shilin Luo et al. have demonstrated dual role of PTEN-induced flavoenzyme NQO1 glioblastoma proliferation (Luo et al. 2018). The downstream signaling mediated by PTEN is also known to be affected by the redox status of cell. Jaeyul Kwon et al. have shown that oxidation of essential cysteine residue renders it inactive as a result of oxidative stress in presence of EGFR stimulation. Its inactivation resulted in increased intracellular concentration of PIP3 and triggered the downstream signaling including the AKT pathway which is known to promote cancer cell survival and cellular proliferation (Kwon et al. 2004). P53 is well documented to work as tumor suppressor in glioma. It has been shown that the p53-ARF-MDM2 pathway is deregulated in 94% glioblastoma cell lines and also in 84% of GBM patients. Alteration of this pathway is implicated in GBM proliferation, migration, and invasion (Zhang et al. 2018). P53 is also known to be involved in regulating the oxidative stress through mitochondrial superoxide dismutase 2 (SOD2) and glutathione peroxidase (Tan et al. 1999).

The mechanistic target of rapamycin (mTOR) pathway has emerged as a key player in glioma pathogenesis. Mechanistically, mTOR is serine/threonine kinase composed of two subunits mTORC1 and mTORC2. Upon activation, mTORC1 phosphorylates the eukaryotic translation initiation factor 4E (eiF4E) causing increased cellular proliferation (Laplante and Sabatini 2012). In addition, the mTOR1 is also implicated in protein, nucleoside, and lipid synthesis, which is aberrantly upregulated in GBM. Irrespective of this, targeting mTOR1 has not yielded clinically significant results (Mecca et al. 2018). M Dermit et al. have shown that ROS generated in mTORC1-dependent cells enhance the glycolytic activity to support the cellular homeostasis via hypoxia inducible factor. This metabolic imbalance could potentially create conductive environment for tumor progression and be utilized for possible therapeutic intervention (Dermit et al. 2017).

Cellular signaling mediated by FOXO is well implicated to control damage produced by stress. Under normal physiological conditions, FOXO exists in cytoplasm but translocate to nucleus to promote the activity of its target genes which includes antioxidant enzymes such as SOD, catalase, and sestrin (Chiacchiera and Simone 2010). FOXO also governs the metabolism of glucose through regulating the key enzymes as Glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), and peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α). PEPCK is a mitochondrial enzyme that connects the TCA cycle to glycolysis by regulating the conversion of oxaloacetate to

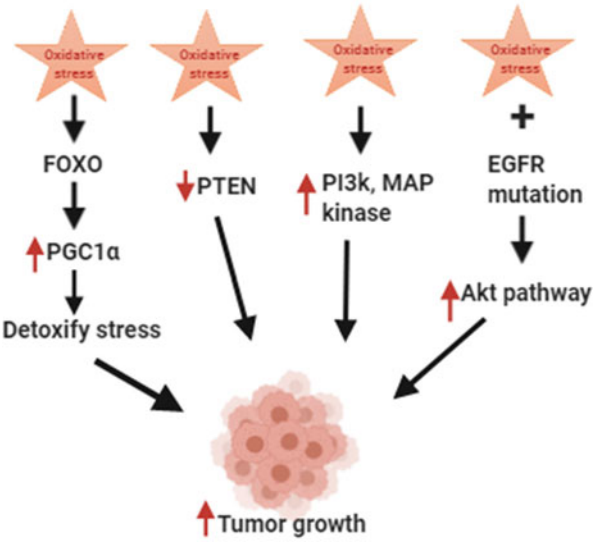


**Fig. 3** Impact of oxidative stress and energy homeostasis on tumor growth

phosphoenolpyruvate during gluconeogenesis (Méndez-Lucas et al. 2014). PGC1α is a transcriptional coactivator regulating oxidative phosphorylation. Francisca Vazquez et al. have shown that upregulated PGC1α helps to detoxify the ROS and helps cancer cells to survive under the conditions of oxidative stress while PGC1α negative cells were more glycolytic and sensitive to ROS inducing drug therapy (Vazquez et al. 2013). Daniel P Brucker et al. have examined the role of FOXO in gliomas. They observe that expression of FOXO increases with the WHO pathological grade of glioma and colocalizes with hypoxia-inducible factor 1α (HIF1α) in hypoxic microenvironment. In addition, its translocation to the nucleus was enhanced under the starvation condition and knockdown of FOXO3a sensitizes glioma cells to $H_2O_2$ under normoxic conditions (Brucker et al. 2016) (Fig. 3).

## Oxidative Stress and Circadian Rhythms

Circadian clock is a biological mechanism to synchronize the physiological functions to the environmental cues as sunlight (Sancar and Brunner 2014). It is regulated by central and peripheral clocks. The central clock is located at superchiasmatic nucleus in the hypothalamus while the peripheral clocks are located in respective body organs. SCN clock monitors the fine balance between peripheral clocks by neuronal and humoral signals (Stevens et al. 2007). At mechanistic levels, circadian clock is controlled by the positive and negative feedback loops handling transcription and translation. The central regulatory factors include CLOCK, BIMAL1, Period (Per 1–3), and cryptochromes (CRY 1 and 2) (Schibler and Sassone-Corsi 2002). The coordination of these genes is controlled by positive and negative

feedback loops. In general, BIMAL1/CLOCK1 forms a heterodimer and promotes the synthesis of PER/CRY genes by binding to its enhancer. In a feedback circle, when the concentration of PER/CRY increases, it inhibits the expression of BIMAL1/CLOCK complex. This cyclic process of expression is termed as circadian oscillations (Buhr and Takahashi 2013).

Circadian clock is reported to play an important role in pathogenesis of glioma. Z Chen et al. have evaluated the expression of core circadian gene Clock in 67 glioma tissues. They observe that Clock is significantly upregulated in glioblastoma as compared with low-grade glioma and nontumor tissue (Chen et al. 2013). Similarly, Per2 was also identified with high glioma risk (Madden et al. 2014). He-chun Xia et al., have examined the expression of per1 and per2 in 33 glioma tissues. They observe the expression of these genes is significantly lower in high-grade glioma tissues as compared with the surrounding non-glioma tissues thus confirming the tumor suppressor nature of these genes (Xia et al. 2010).

Circadian clock genes are also documented to share a bidirectional talk with the hypoxia response genes. Chao Yu et al.. have observed that hypoxic microenvironment upregulates the core circadian genes CLOCK, BIMAL1, and CRY2 while decreasing the expression of Per 1-3 and CRY 1. The expression of these genes was observed to regulate by HIF1 and HIF2 thus providing a rationale for hypoxia-circadian connection (Yu et al. 2015). Wai Hoong Chang et al. have studied the copy number and transcriptomic profile of 32 circadian genes to identify the loss of or gain of function in a range of malignancies including glioma. The expression of circadian genes was associated with poor prognosis in glioma. The Clock genes with putative tumor-suppressive properties were negatively correlated with the HIF alpha target genes (CA9, VEGFA, and LDHA) in hypoxic microenvironment. The hypoxic microenvironment of glioma was found to aggravate the expression of circadian genes. This study provides a rationale that hypoxia and the circadian genes could be co-regulated and poses an attractive therapeutic target (Chang and Lai 2019).

## Oxidative Stress and Therapy Resistance

Glioma is treated by surgical resection followed by radio and chemotherapy. The radiotherapy and the most commonly followed temozolomide chemotherapy work by inducing the DNA damage and generate the oxidative stress (Lo Dico et al. 2019). Alessia Lo Dico et al. have studied the cross talk between temozolomide-associated HIF alpha expression in human GBM cell lines and observe that HIF1 alpha silencing sensitizes the resistant cells to temozolomide therapy. Mechanistically, HIF1 alpha was found to influence the chaperone mediated autophagy. It induces the degradation of HIF alpha which mediates TMZ resistance in sensitive cells. Thus, they approach to target HIF alpha in combination with temozolomide as a potential way to treat glial tumors (Lo Dico et al. 2018).

Hypoxia is a well-known factor involved in maintaining the stem cell population in tumor microenvironment. Sascha Seidel et al. have provided an evidence that HIF2 alpha is known to regulate the glioblastoma stem cell maintenance in the

hypoxic niche. Mechanistically, HIF2 alpha is observed to induce the expression of stem cell signature genes including mastermind-like protein 3 of Notch pathway and nuclear factor of activated T cells 2 which play an important role in the calcineurin pathway (Seidel et al. 2010). Thus, it is logical to target the hypoxic microenvironment to eliminate the stem cells which can potentially induce reactiveness to the therapy and induce aggressive cellular migration leading to worse prognosis.

HIF1 alpha is also known to regulate the expression of VEGF, which helps the angiogenesis in malignant gliomas. Randy L Jensen et al.. have studied the expression of HIF1 alpha downstream targets in 175 human glioma tissues and HIF1 alpha-VEGF cross talk in glioblastoma cell lines. Mechanistically, it was noted that HIF1 alpha inhibition also inhibits the secretion of VEGF and ultimately affects the tumor growth (Jensen et al. 2006). On similar note, Barbara Blouw et al. have provided evidence for the HIF1 alpha and VEGF function in GMB model created using genetically engineered astrocytes with loss of VEGF or HIF alpha. Subcutaneous injection of such deficient cells were observed to form tumors with reduced angiogenesis and growth (Leder et al. 1992). In view of this, therapeutic strategies aimed at knocking down the expression of HIF alpha could accelerate the effect of chemotherapy to mitigate the tumor growth (Tan et al. 2005) (Fig. 4).

There is scarcity of literature on radiotherapy and oxidative stress in glial tumors. Partially, it is known that oxidative stress increases with radiation and is associated with upregulation of HIF alpha. This enhanced expression of HIF alpha further promotes the production of VEGF and anaerobic metabolism which is helpful in protecting cancer cells from radiation damage (Cohen et al. 1992;

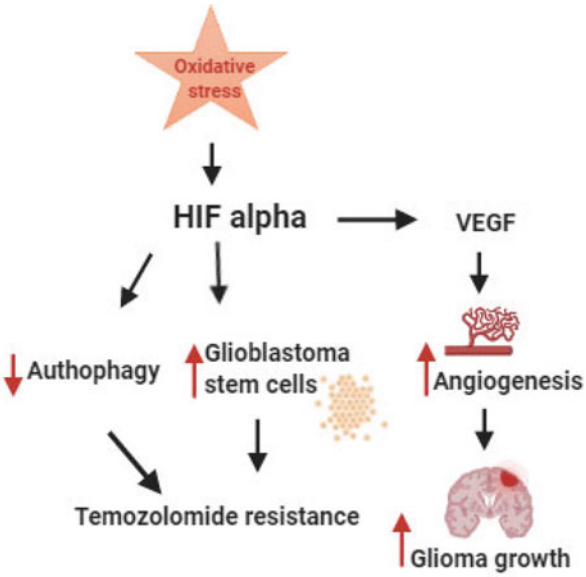


**Fig. 4** Impact of oxidative stress on temozolomide resistance and glioma growth

Dewhirst et al. 2008). Thus, there is urgent need to understand the molecular crosstalk involved in hypoxia response on radiation and chemotherapy to take best therapeutic measures.

## Conclusion and Future Direction

Hypoxia is known to share extensive cross talk with glioma cells severely influencing its pathology. It is known to impact the viability of tumor and the immediate immune cells present in the microenvironment through modulating the several signaling pathways as VEGF, PI3K/AKT, and MAP kinase cascade. On metabolic scale, hypoxia promotes the anaerobic glycolysis resulting in elevated lactic acid production which drives the multifaceted effects including drug resistance and promotion of cellular migration. Hypoxia induces the switch in the metabolism which results in increased tolerance to the cytotoxic chemo and radiotherapy. In addition, hypoxia also abrogates effectiveness of immune cells in the TME and ceases the functionality of immunotherapy. Supportive nature of hypoxia to the cancer stem cells is regarded as seminal mechanism to induce recurrence even after surgery, radio and chemotherapy. Circadian rhythms represent the less explored target in the context of glioma pathology, and here is the scarcity of studies on evaluating the cross talk between them and its cumulative impact on the aggressiveness of glioma. Together, it would be worth interesting to understand the mechanisms of hypoxia-induced molecular changes for better therapeutic targeting of therapy refractive glial tumors.

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