

# Advances in the targeting of HIF-1 $\alpha$ and future therapeutic strategies for glioblastoma multiforme (Review)

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**Abstract.** Cell metabolism can be reprogrammed by tissue hypoxia leading to cell transformation and glioblastoma multiforme (GBM) progression. In response to hypoxia, GBM cells are able to express a transcription factor called hypoxia inducible factor-1 (HIF-1). HIF-1 belongs to a family

of heterodimeric proteins that includes HIF-1 $\alpha$  and HIF-1 $\beta$  subunits. HIF-1 $\alpha$  has been reported to play a pivotal role in GBM development and progression. In the present review, we discuss the role of HIF-1 $\alpha$  in glucose uptake, cancer proliferation, cell mobility and chemoresistance in GBM. Evidence from previous studies indicates that HIF-1 $\alpha$  regulates angiogenesis, metabolic and transcriptional signaling pathways. Examples of such are the EGFR, PI3K/Akt and MAPK/ERK pathways. It affects cell migration and invasion by regulating glucose metabolism and growth in GBM cells. The present review focuses on the strategies through which to target HIF-1 $\alpha$  and the related downstream genes highlighting their regulatory roles in angiogenesis, apoptosis, migration and glucose metabolism for the development of future GBM therapeutics. Combined treatment with inhibitors of HIF-1 $\alpha$  and glycolysis may enhance antitumor effects in clinical settings.

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*Abbreviations:* ARC, apoptosis repressor with a CARD; CAIX, carbonic anhydrase IX; COX10, cytochrome *c* oxidase assembly protein; DCA, dichloroacetate; Drp1, dynamin-related protein 1; eIF4E, eukaryotic initiation factor 4E; FAT1, FAT atypical cadherin; GBM, glioblastoma multiforme; GSCs, glioma stem cells; GLUTs, glucose transporters; GSK3a/b, glycogen synthase kinase 3A and B; HDACs, histone deacetylase inhibitors; HER2, human epidermal growth factor receptor 2; HGG, high grade glioma; HH, Hedgehog; HIF, hypoxia-inducible factor; HIF-1 $\alpha$ , hypoxia-inducible factor-1 $\alpha$ ; HIF-1 $\beta$ , hypoxia-inducible factor-1 $\beta$ ; HRE, hypoxia response element; HSP90, heat shock protein 90; IRE1, inositol requiring enzyme 1; ISCU, iron-sulfur cluster assembly proteins; IMQ, imiquimod; ISL, isoliquiritigenin; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; 2ME, 2-methoxyestradiol; MGMT, O<sup>6</sup>-methylguanine-DNA methyltransferase; mTOR, mammalian target of rapamycin; MNT, Myc antagonist; MVD, microvessel density; MDR, multidrug resistance; PDGFR- $\alpha$ , platelet-derived growth factor receptor- $\alpha$ ; p70S6K, protein S6 kinase; PKM2, pyruvate kinase M2; PI3K/Akt, phosphatidylinositol-3 kinase/Akt; OXPHOS, oxidative phosphorylation; PDK1, pyruvate dehydrogenase kinase 1; ROS, reactive oxygen species; SAHA, suberoylanilide hydroxamic acid; STAT3, signal transducer and activator of transcription 3; TMZ, temozolomide; TLR, Toll-like receptor; TOP I, topoisomerase; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau; VPA, valproic acid

*Key words:* HIF-1 $\alpha$ , glioblastoma multiforme, mitochondria, glucose metabolism, oncogenes, signaling pathways

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## 1. Introduction

Tissue hypoxia is an important element in tumor progression and therapeutic resistance as tumor cells can activate a range of adaptive molecular mechanisms that facilitate glioblastoma multiforme (GBM) development (1). Hypoxia inducible factor-1 (HIF-1) is a basic helix-loop-helix transcription factor that is expressed in most cells in response to hypoxia. It regulates the cellular response to oxygen deficit (hypoxia) in order to minimize tissue damage. At the gene level, HIF-1 is the primary oxygen-sensitive transcriptional activator that helps

cells to adapt to low oxygen tension (2). HIF-1 is a heterodimeric protein consisting of an  $\alpha$  (HIF-1 $\alpha$ ) and a  $\beta$  (HIF-1 $\beta$ ) subunit (3). Under normoxic conditions, HIF-1 $\alpha$  has a short half-life where it is degraded rapidly through the ubiquitin proteasome pathway (4-6). In hypoxic conditions, it is stabilized and complexes with the  $\beta$  subunit to form a functional transcription factor. The complete HIF-1 molecule translocates to the nucleus and activates the expression of downstream genes in response to hypoxia (7,8). Although HIF-1 $\alpha$  is primarily switched on during hypoxia, it is also frequently activated in cancer cells even under normoxic conditions through oncogene activation and/or tumor-suppressor gene inhibition (9,10). Furthermore, HIF-1 $\alpha$  also regulates a number of genes involved in major events in carcinogenesis including cell immortalization, angiogenesis, invasion and metastasis.

The following review focuses on the relationship between HIF-1 $\alpha$  and its target genes/pathways associated with glucose metabolic pathways, cancer proliferation, cell mobility and chemoresistance. The roles of HIF-1 $\alpha$  in the regulation of cell proliferation, angiogenesis, apoptosis, migration and invasion are explored. Methods of targeting HIF-1 $\alpha$  by regulating signaling pathways and subsequent modulation of the target genes involved in glioblastoma development are also discussed.

## 2. Metabolic pathways are linked to HIF-1 $\alpha$ in GBM

Numerous studies have shown that abnormal cellular metabolism is a prominent feature in tumorigenesis (11,12). Metabolic reprogramming is now recognized as a hallmark of tumors and abnormal energy metabolism is found in GBM (13). Glioblastoma cells obtain their energy primarily through glycolysis instead of mitochondrial oxidative phosphorylation (OXPHOS) (14). The upregulation of glycolysis that results in increased glucose consumption, first reported by Warburg (15), appears to be a universal feature in both primary and glioma stem cells (GSCs) (16).

It is generally recognized that ATP production from OXPHOS in mitochondria is much more efficient than glycolysis in the cytoplasm. To satisfy the energy needs of tumor cells, a large amount of glucose must be made to fulfil the ATP needed to sustain their growth and survival. During hypoxia, pyruvate dehydrogenase kinase 1 (PDK1) is induced in cells to minimize the production of reactive oxygen species (ROS) such as from glycolysis in glioblastoma cells (17). HIF-1 also induces the transcription of miR-210, a microRNA that partially rescues Myc antagonist (MNT) protein expression and increases the apoptotic rate and caspase-3/-7 activity and decreased invasive capacity, ROS and lactate production and radioresistance in hypoxic GSCs (18,19). Thus, HIF-1 is crucial during hypoxia to reduce mitochondrial respiration, leakage of electrons from the electron transport chain, and to prevent ROS production to ensure the survival of GBM cells (20).

On the contrary, increased levels of HIF-1 may induce glycolytic enzyme expression resulting in higher levels of lactate being produced (21). This biochemical shift from OXPHOS to anaerobic glycolysis is a major hallmark of malignant glioma cells. The lactate produced causes the acidification of the extracellular environment. Together with the HIF-1 $\alpha$ -induced expression of carbonic anhydrases, the pH ratio between the intracellular and extracellular compartments is significantly

changed (22,23). As a consequence, the passive absorption of many drugs may be decreased within the cell. Active efflux of drugs is also taking place due to the HIF-1 $\alpha$ -induced transporter overexpression (24,25). All of these actions are achieved by the hypoxia driven activation of HIF-1 via stabilization of HIF-1 $\alpha$  and its target genes (26,27).

Hypoxia activates the expression of HIF-1 $\alpha$  which can result in the switching from OXPHOS to anaerobic glycolysis, angiogenesis, increased cell migration potential, and genetic alterations that prevent hypoxia-induced apoptosis (28-30). The activation of oncogenic signaling pathways, such as the phosphatidylinositol-3 kinase/Akt (PI3K/Akt), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), signal transducer and activator of transcription 3 (STAT3) signaling pathways, also promotes HIF-1 $\alpha$  expression at transcriptional levels and increases glucose utilization in GBM even when oxygen is abundant (31-33). Activation of HIF-1 $\alpha$  can increase the expression of anti-apoptotic proteins such as Bcl-2 to block glioblastoma cell apoptosis (34-36). Aerobic glycolysis can also prevent cells from undergoing apoptosis through the inhibition of mitochondrial respiration, a mechanism involving the release of cytochrome *c* and activation of the caspase cascade (37). Thus, HIF-1 $\alpha$  plays a master regulatory role in controlling aerobic glycolysis in glioblastoma cells to meet their high-energy consumption and at the same time preventing them from hypoxia-induced damage (38). The metabolic preference of glioblastoma cells for glycolysis as an energy source is also a potential therapeutic target. Thus, targeting HIF-1 $\alpha$ , its regulated pathways and metabolic enzymes may form a basis for the development of effective glioblastoma therapies (Fig. 1). Further identification of more detailed mechanisms on how HIF-1 $\alpha$  regulates apoptosis and pro-proliferative metabolic signaling pathways may be beneficial for GBM therapy. As HIF-1 $\alpha$  expression and aerobic glycolysis are essential for glioblastoma growth, inhibiting both may enhance the effects of antitumor agents. Furthermore, targeting HIF-1 $\alpha$  and/or glycolysis may be a potential option for selective glioblastoma therapy.

## 3. Angiogenesis and migration are linked to HIF-1 $\alpha$ in the development of GBM

Hypoxia, a common feature in cancers, is associated with poor response to treatment. As discussed above, HIF-1 not only facilitates glioblastoma cells to adapt to the hypoxic environment, but also regulates a number of genes involved in major events in carcinogenesis including cell immortalization, angiogenesis, invasion and metastasis (39). The subunit HIF-1 $\alpha$  is central to the response of mammalian cells to low oxygen tension. It triggers the expression of vascular endothelial growth factor (VEGF), which is central to angiogenesis by stimulating endothelial cell growth, migration and invasion into the extracellular matrix forming new blood vessels to support tumor development (40). Thus, HIF-1 $\alpha$  appears to be a promising therapeutic target in angiogenesis-related GBM.

BIX01294 (BIX), a G9a histone methyltransferase-specific inhibitor, has been shown to promote apoptosis and suppress glioma cell proliferation, migration and invasion. BIX01294 induced an Akt-dependent increase in HIF-1 $\alpha$  expression and

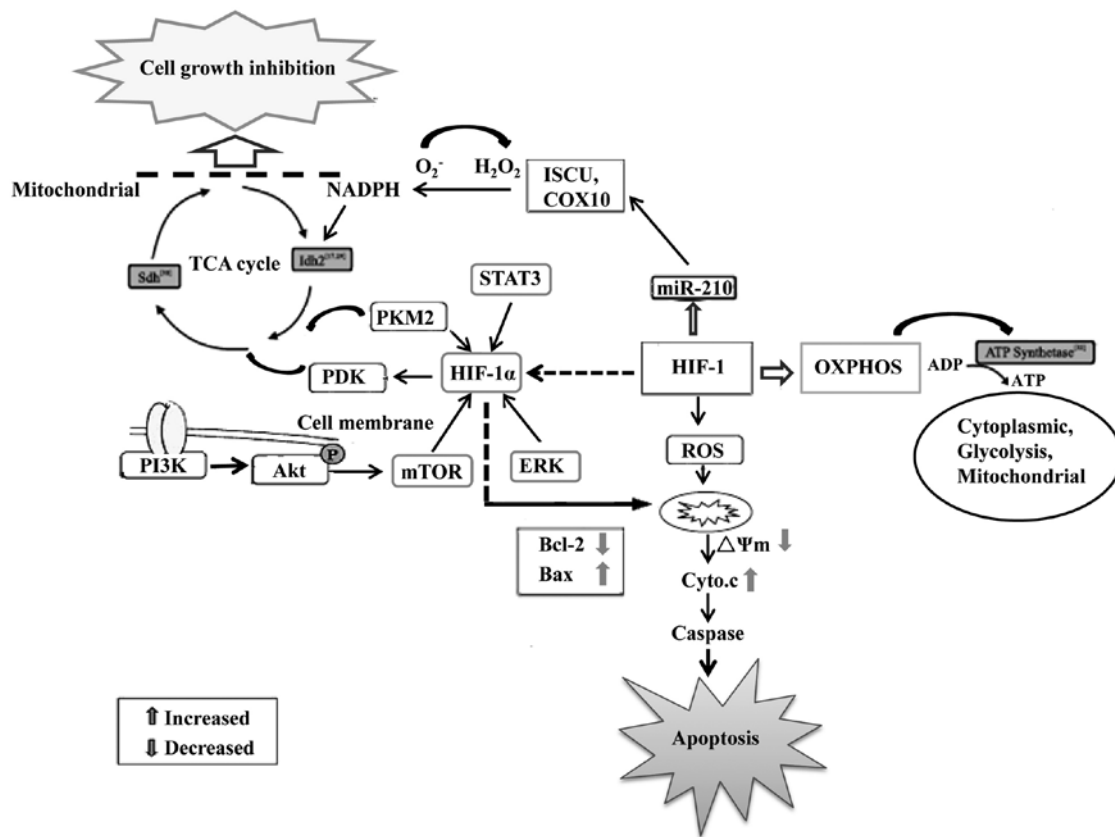


Figure 1. Targeting HIF-1 $\alpha$  through the HIF-1 pathway in glioblastoma multiforme (GBM). HIF-1 plays important roles in regulating aerobic glycolysis to meet the biosynthetic demands of GBM cells and to prevent cancer cells from damage of hypoxic stress. In this way, GBM cells shift from oxygen-dependent efficient ATP production via oxidative phosphorylation (OXPHOS) in mitochondria to the less efficient cytoplasmic glycolysis. The HIF-1 pathway protects cells from reactive oxygen species (ROS) damage through pyruvate dehydrogenase kinase-1 (PDK1) induction under hypoxic stress. Glycolytic enzyme pyruvate kinase M2 (PKM2) regulates HIF-1 $\alpha$  activity by enhancing its binding to hormone response element, eventually upregulating HIF-1 $\alpha$  target gene expression. The HIF-1 pathway also induces miR-210 transcription, which decreases the expression of iron-sulfur cluster assembly proteins (ISCU) and cytochrome *c* oxidase assembly protein (COX10), two important elements of the mitochondrial electron transport chain and the tricarboxylic acid cycle (TCA) cycle. Activation of HIF-1 $\alpha$  can increase the expression of anti-apoptotic Bcl-2 family members to prevent cell apoptosis. The activation of oncogenic signaling pathways, such as the phosphatidylinositol-3 kinase/Akt (PI3K/AKT), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), signal transducer and activator of transcription 3 (STAT3) signaling pathways, also promote HIF-1 $\alpha$  expression at the transcriptional levels to increase the rate of glucose utilization in tumors even in the presence of sufficient oxygen. Collectively, HIF-1 reduces mitochondrial respiration under hypoxia condition, which inhibits the aberrant electron leakages from mitochondrial electron transport chain, thereby serving as a safeguard for tumor survival by preventing ROS production under hypoxic stress. Targeting the HIF-1 $\alpha$  through the HIF-1 pathway by regulating metabolic enzymes or proteins of cancer may be the basis for the development of effective and selective GBM therapies.

activity. Furthermore, Akt-HIF-1 $\alpha$  axis driven PKM2-YAP1 crosstalk activates autophagic responses in glioma cells by G9a inhibition (41). Similarly, ER-400583-00 targets HIF-1 $\alpha$  signal transduction causing it to become less stable and effectively decreased the expression of VEGF in U251 cells. As a result, it achieved sustained HIF-1 $\alpha$  suppression in xenograft gliomas in animal studies. Altogether, ER-400583-00 exhibited enhanced cytotoxicity against hypoxic glioma cells and enhanced antitumor activity in combination with radiation therapy (42). These findings indicate that inhibition of HIF-1 $\alpha$  could provide new insights into the discovery of drugs for cancer treatment.

Hypoxia-inducible factors, particularly HIF-1 $\alpha$  and STAT3 have important roles in angiogenesis and are associated with VEGF. Inhibitors of STAT3 and HIF-1 $\alpha$  downmodulated the hypoxia-induced immunosuppressive effects of GSCs. Thus, hypoxia further enhances GBM-mediated immunosuppression. Thus, immune therapeutic approaches can be used successfully in the vast majority of GBM patients by demonstrating

the importance of the tumor hypoxic environment (43). These findings suggest that GBM-mediated immunosuppression is mediated through HIF-1 $\alpha$  and STAT-3, and the inhibition of STAT3 and HIF-1 $\alpha$  is a promising anti-angiogenic strategy in GBM (Fig. 2).

#### 4. Transcriptional signaling pathways involved in HIF-1 $\alpha$ in GBM

**PI3K/Akt/HIF-1 $\alpha$  pathway.** The PI3K/Akt signaling pathway is involved in cell proliferation, apoptosis, angiogenesis, transformation and tumor growth. Increased expression of the PI3K/Akt/HIF-1 $\alpha$  pathway is therefore closely associated with tumor differentiation (44,45). Moreover, endothelial cells were programmed by GBM cell-derived hypoxic exosomes to secrete several potent growth factors and cytokines and to stimulate pericyte PI3K/AKT signaling activation and migration. Active hypoxia-related molecules including HIF-1 $\alpha$ , Glut-1, p27 (Kip1), and p-Akt were found to be significantly increased

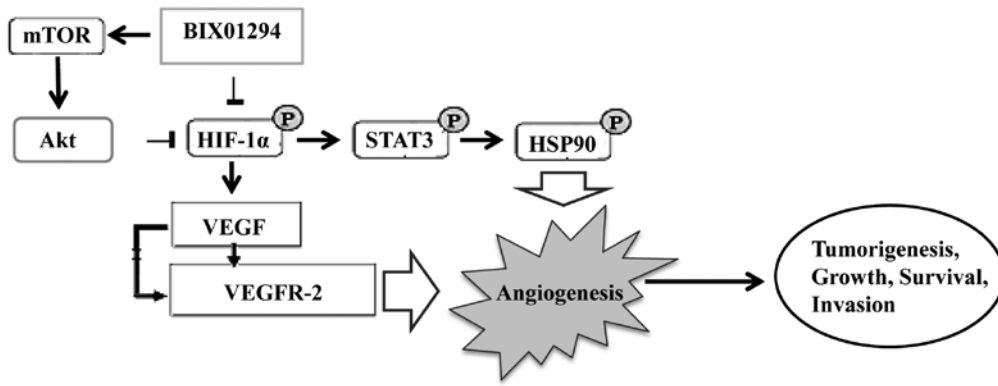


Figure 2. Angiogenesis, migration and invasion are linked to HIF-1 $\alpha$  in the development of GBM. Inhibition of HIF-1 $\alpha$  stability and vascular endothelial growth factor (VEGF)-induced angiogenesis through the VEGFR-2 signaling pathway. HIF-1 $\alpha$  activates the transcription of VEGF, which in turn promotes angiogenesis through its ability to stimulate the growth, migration and invasion of endothelial cells to form new blood vessels, contributing to tumor progression. BIX01294 suppresses hypoxia-induced VEGF expression and reduces HIF-1 $\alpha$  protein translation and global protein synthesis. In addition, BIX01294 suppresses mTOR signaling and increases the phosphorylation of eIF2 $\alpha$ , which is known to be related to the translational regulation of HIF-1 $\alpha$  expression. Hypoxia-inducible factors and STAT-3 play essential roles in angiogenesis, and HIF-1 $\alpha$  and STAT-3 are clients of the heat shock protein 90 (HSP90). HIF-1 $\alpha$  overexpression results in the increase of VEGF and STAT-3 expression. HIF-1 $\alpha$  knockdown inhibits VEGF and STAT-3 expression.

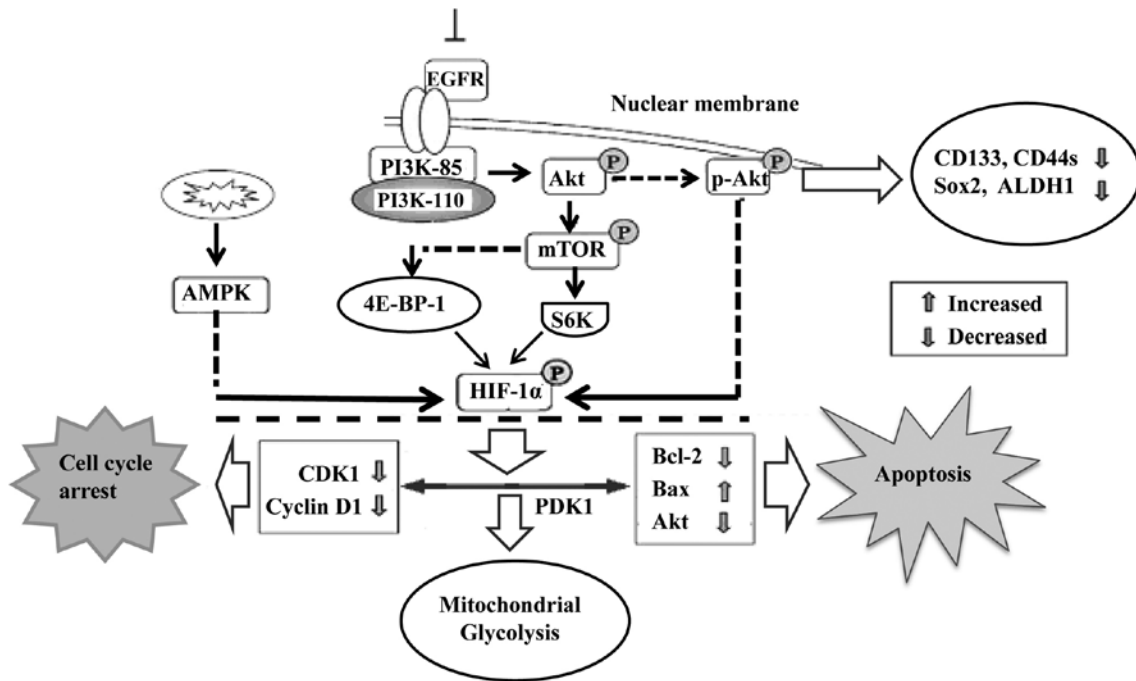


Figure 3. The transcriptional signaling pathways involved in HIF-1 $\alpha$  in GBM. Inhibition of the PI3K/Akt pathway enhances the therapeutic efficacy by decreased expression of HIF-1 $\alpha$  and markedly reduces the proliferative activity and induces cell apoptosis in GBM cells. The mitochondrial dysfunction-repressed HIF-1 $\alpha$  protein synthesis is associated with decreased phosphorylation of p70 (S6K) and 4E-BP-1, and decreases intracellular ATP content and elevates the phosphorylation of AMPK. AMPK inhibitor or knockdown of AMPK partially rescues mitochondrial dysfunction-repressed HIF-1 $\alpha$  expression.

in glioma stem cells, particularly under hypoxic conditions. Inhibition of endogenous Akt by LY294002 resulted in decreased expression of several CSC-related markers (46), including CD133, Sox2, CD44s and aldehyde dehydrogenase 1 (ALDH1) (Fig. 3).

Muh *et al* demonstrated that PI3K/PTEN regulates HIF-1 $\alpha$  in glioma models (47). They utilized PTEN-deficient and PTEN-reconstituted isogenic-pairs of glioma cells to determine the role of PTEN, and a pan-PI3K inhibitor was combined with 2-methoxyestradiol, a microtubule inhibitor that potently inhibited HIF-1 $\alpha$ . This was correlated with

a synergistic suppression of HIF1- $\alpha$  accumulation under hypoxic conditions. These findings favor the notion that PTEN status predicts treatment efficacy. Thus, the combination of HIF1- $\alpha$  and pan-PI3K inhibitors may be a plausible regime for the treatment of this incurable brain cancer. Overall, these findings suggest that targeting the PI3K/Akt/HIF-1 $\alpha$  pathway may be an important target for treating GBM.

*Mitochondrial pathway.* Mitochondrial dysfunction is one of the hallmarks of cancer progression. It represses HIF-1 $\alpha$  expression through phosphorylation of p70S6K and

4E-BP-1 (48). Mitochondrial dysfunction was also found to decrease intracellular ATP levels and elevate AMPK phosphorylation. Reducing AMPK activity by inhibitors or gene-knockdown was found to partially rescue the mitochondrial dysfunction-repressed HIF-1 $\alpha$  expression (Fig. 3). Interfering with mitochondrial function and reducing HIF-1 $\alpha$  activity may be a novel approach to treat hypoxic tumors that are resistant to both chemotherapy and radiotherapy. Furthermore, Akt accumulates in the mitochondria during hypoxia and phosphorylates PDK1 to inactivate the pyruvate dehydrogenase complex, which switches tumor metabolism toward glycolysis, antagonizes apoptosis and autophagy, hampers oxidative stress and maintains tumor cell proliferation in the face of severe hypoxia. Mitochondrial Akt-PDK1 signaling may provide an 'actionable' therapeutic target for GBM (49).

**Oncogenes.** An adaptation of glioma cells to low-oxygen environment is the induction of HIFs. In many types of cancer cells, HIF-1 is primarily responsible for the expression of the apoptosis inhibitor apoptosis repressor with a CARD domain (ARC). It acts through direct binding to a region, known as the hypoxia response element (HRE), 190 bp upstream of the transcription site (50). ARC is not expressed in the normal brain but is expressed in GBM and HIFs have been implicated in GBM pathogenesis even under normoxic conditions. In GBM, HIF-1 $\alpha$  activity-dependent protein carbonic anhydrase IX (CAIX) expression was identified as a translatable non-invasive biomarker with potential clinical significance. This suggests that increased HIF mediates ARC expression and along with CAIX promote GBM cell survival (51).

In high grade glioma (HGG), HIF-1 $\alpha$  and FAT atypical cadherin (FAT1) are two key tumor-promoting factors in the hypoxic microenvironment. FAT1 with HIF1 $\alpha$  and its target genes such as CA9, GLUT1, VEGFA, MCT4, HK2, BNIP3 and REDD1 in GBM specimens, reveals the significance of the FAT1-HIF1 $\alpha$  axis in controlling the invasiveness of glioblastoma. Furthermore, FAT1 depletion-associated reduction in the level of HIF1 $\alpha$  was due to a compromised EGFR-Akt signaling as well as increased VHL-dependent proteasomal degradation of HIF1 $\alpha$ . These results indicate that FAT1 represents a novel potential therapeutic target for glioblastomas (52). Similarly, inhibition of inositol requiring enzyme 1 (IRE1) modified the effect of hypoxia on the expression of genes. IRE1 eliminated the sensitivity to hypoxia of IGFBP7 and IGFBP9/NOV genes, suppressed the effect of hypoxia on IGFBP6, IGFBP10/CYR61 and WISP2 genes, and slightly enhanced hypoxic regulation of WISP1 gene expression in glioma cells (53). Moreover, inhibition of IRE1, which correlates with suppression of cell proliferation and glioma growth, downregulated expression of pro-proliferative IGFBP genes. Taken together, HIF-1 $\alpha$  plays a crucial role in mediating the interactions between oncogenes and GBM.

**MicroRNAs.** The hypoxic tumor microenvironment serves as a niche for maintaining glioma-initiating cells (GICs) that are critical for GBM occurrence and recurrence. Hypoxia-induced miR-215 is vital for reprogramming GICs to fit the hypoxic microenvironment via suppressing the expression of epigenetic regulator KDM1B and modulating activities of

multiple pathways (54). Notably, biogenesis of miR-215 and several miRNAs is post-transcriptionally accelerated by hypoxia-inducible factors (HIFs) through HIF-Drosha interaction. Moreover, miR-215 expression is inversely correlated with KDM1B while is positively correlated with HIF1 $\alpha$  and GBM progression in patients. It appears that the HIF regulates miRNA biogenesis and consequently activates the miR-215-KDM1B-mediated signaling required for GIC adaptation to hypoxia (55). Additionally, miR-497 is overexpressed in glioma and hypoxia can induce the expression of miR-497 at the transcriptional level by binding with the hypoxia response element in the promoter (56).

Recent research also indicates that microRNA-584-3p (miR-584-3p) is upregulated in hypoxic glioma cells and in high-grade human glioma tumors (WHO grades III-IV). High-grade glioma patients with high miR-584-3p expression had significantly prolonged postoperative survival time. Mechanically, miR-584-3p suppressed the migration and invasion of glioma cells by disrupting hypoxia-induced stress fiber formation. Altogether, miR-584-3p may function as a potent tumor suppressor and as a prognostic biomarker for malignant glioma (57). Furthermore, miR-584-3p is a potential therapeutic target for malignant glioma, particularly for patients with WHO III-IV GBMs.

Most researchers agree that the highly aggressive GBM subtype with its necrotic tissues, are affected similarly by hypoxia. The extent of the influence of hypoxia on these processes makes it an attractive therapeutic strategy for glioma (58). Considering that miRNA research has advanced from the identification of an initial association with glioma to the commercial development of miRNA-based therapeutics in less than a decade, the anticipation of significant developments in this field with the ultimate improvement of patient outcomes is reasonable (59).

## 5. HIF-1 $\alpha$ is linked to drug resistance in GBM

Hypoxia is an essential condition in tumor development and glioblastoma cells can utilize a range of adaptive molecular mechanisms leading to their subsequent therapeutic resistance (60). These mechanisms include switching from OXPHOS to anaerobic glycolysis, angiogenesis, increased cell migration potential and genetic alterations that help avoid hypoxia-induced apoptosis. These hypoxic areas can either promote cell death or provoke an adaptive response leading to death resistance (61-63). Once GBM cells become adaptive to hypoxia, they are more resistant to apoptosis and less responsive to targeted therapy. Tumor hypoxia is thought to play a crucial role in pathologic characteristics of GBM, including invasiveness, necrosis and microvascular hyperplasia. It also contributes to resistance to chemotherapy, immunotherapy and radiotherapy due to the possibility of dysregulation of apoptotic pathway or other mechanisms (64). Using *in vitro* models of glioblastoma, rhabdomyosarcoma and Ewing's sarcoma, it has been shown that the resistance to chemotherapy is dependent on HIF-1. Inhibition of HIF-1 $\alpha$  sensitizes glioma cells to temozolomide (TMZ) through the downregulation of the DNA repair enzyme O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT). HIF-1 $\alpha$  downregulation sensitized U251 cells to TMZ treatment and enhanced the proliferation-inhibiting,

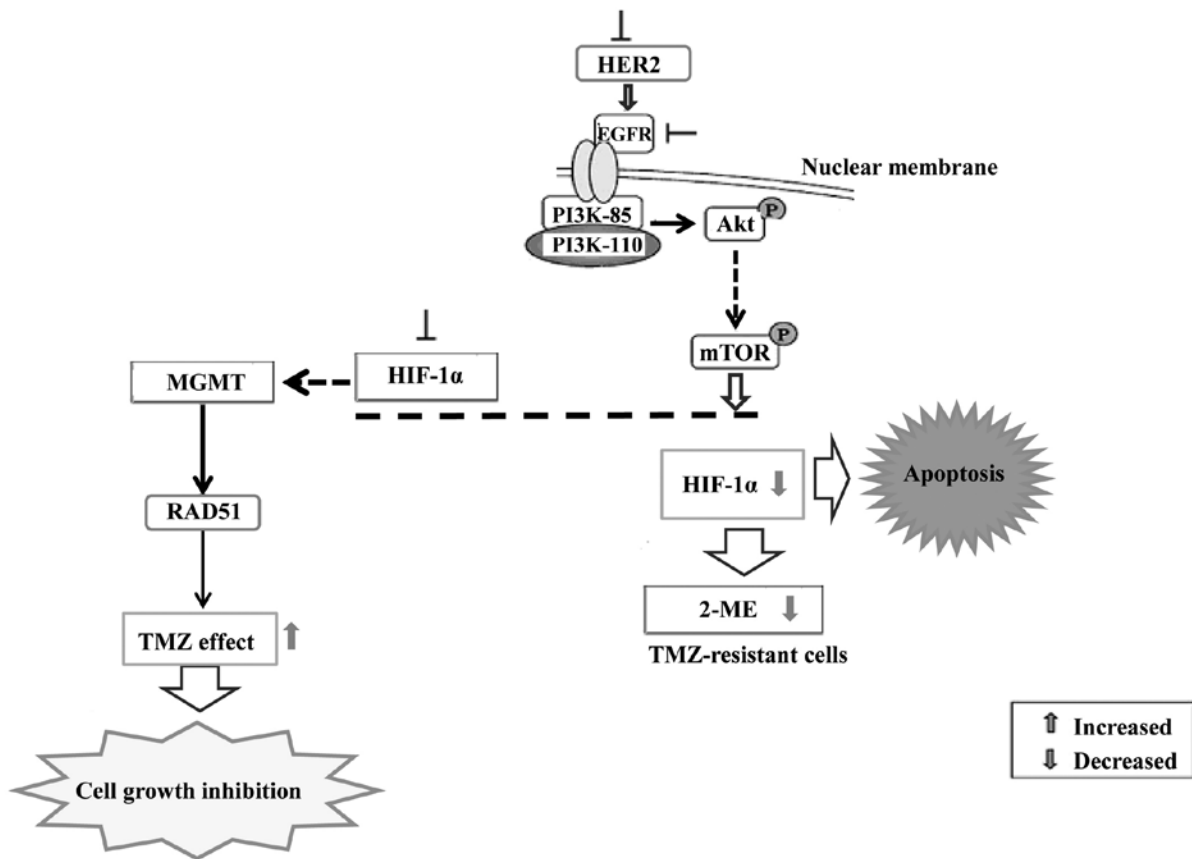


Figure 4. HIF-1 $\alpha$  is linked to drug resistance in GBM. HIF-1 $\alpha$  suppression results in the downmodulation of MGMT expression and decreases DNA damage repair ability by decreased RAD51 protein expression, and HIF-1 $\alpha$  knockdown or the HIF-1 $\alpha$  inhibitor 2ME can confer temozolomide (TMZ) sensitization to human glioma cells. HIF-1 $\alpha$  protein and 2-methoxyestradiol (2ME) were found to be higher in TMZ-resistant and HER2-transfected cells than in AI-sensitive cells. HIF-1 $\alpha$  expression in TMZ-resistant cells is likely regulated by the HER2 activated-PI3K/Akt/mTOR pathway, as its expression is inhibited by HER2 and kinase pathway inhibitors.

invasion/migration-suppressing, apoptosis-inducing and differentiation-promoting effects exerted by TMZ. HIF-1 $\alpha$  downregulation sensitized U251 glioma cells to temozolomide treatment via inhibiting MGMT expression and Notch1 pathway activation (65).

Suppression of HIF-1 $\alpha$  expression by gene knockdown or by an inhibitor of 2-methoxyestradiol (2ME) increased the efficacy of TMZ on human pituitary adenoma cells (66). Furthermore, downmodulation of MGMT decreased DNA repair through a decrease in RAD51 protein expression. Thus, 2ME may be a useful adjuvant to enhance the efficacy of TMZ in the treatment of gliomas. HER2 or kinase inhibitors suppress the expression of HIF-1 $\alpha$  in cancer cells, suggesting that the HER2-driven PI3K/Akt/mTOR pathway is involved (Fig. 4).

Overall, hypoxia-induced resistance is implicated in treatment resistance not only to radiotherapy, but also to chemotherapy (67). Hypoxia induces resistance to several anticancer agents in neurons (68), but also in glioma cells (69). Moreover, stabilization of HIF-1 $\alpha$  in normoxia by cobalt chloride or suppression of HIF-1 $\alpha$  in hypoxia by various means (shRNAi, siRNA, dominant-negative HIF, small-molecule NSC-134754) did not induce drug resistance. Taken together, further evaluation of the regulation of HIF-1 $\alpha$  needs to be clarified in radiotherapy/temozolomide in clinical trials for patients with glioblastoma.

## 6. Strategies to overcome GBM by targeting HIF-1 $\alpha$

The importance of HIFs in cancer progression particularly in reduced oxygen conditions has led to the development of HIF-1 $\alpha$  and HIF-2 $\alpha$  inhibitors (70). Concomitant administration of inhibitors of HIF-1 $\alpha$  and its target genes and pathways may be novel strategy for treating GBM. However, a major difficulty in targeting HIF-1 $\alpha$  is the lack of specificity of the available inhibitors and most of them exhibit a number of off-target effects (71,72). Nevertheless, priority may be given to an inhibitor that could effectively reduce HIF-1 $\alpha$  levels and the expression of its target genes (VEGF, IGF2 and PDK1) rather than its cytotoxic effect. During the early stages in carcinogenesis, inhibition of the HIF-1 system may be beneficial particularly in reducing the development of resistance to cytotoxic and targeted drugs (Table I).

*Regulation of glycolysis and glucose metabolism.* Since aerobic glycolysis provides the major source of energy for cancer cell growth and proliferation, strategies targeting this process could be a promising therapeutic option. A synthetic Toll-like receptor (TLR) 7/8 ligand imiquimod (IMQ) was found to enhance aerobic glycolysis by upregulating HIF-1 $\alpha$  expression through ROS-mediated STAT3- and Akt-dependent pathways rather than through TLR7/8 signaling (73). Silencing of HIF-1 $\alpha$  repressed IMQ-induced aerobic glycolysis and

Table I. Strategies to overcome GBM by targeting HIF-1 $\alpha$ .

	Various drugs and their targets	Regulated mechanisms	Refs.
Regulation of glycolysis and glucose metabolism	Imiquimod (IMQ)	IMQ enhances aerobic glycolysis by upregulating HIF-1 $\alpha$ expression at the transcriptional level leading to an alteration in the multidrug resistance (MDR) phenotype of GBM cells	(73)
	Dichloroacetate (DCA)	DCA diminishes acid production under hypoxic conditions through the alteration of glucose metabolism and with hypoxia-induced resistance	(76)
	Resveratrol	Resveratrol blocks the hypoxia-induced upregulation of phosphorylated signal transducer and activator of transcription 3 (p-STAT3), and upregulates miR-34a under hypoxia	(77)
HIF-1 inhibitors	Echinomycin and 'programmable' polyamides	Echinomycin and 'programmable' polyamides inhibit HIF-1 $\alpha$ and block hypoxia-induced expression of dynamin-related protein 1 (Drp1)	(80)
	KCN1 (3,4-dimethoxy-N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]-N-phenylbenzene-sulfonamide)	KCN1 is a novel class of arylsulfonamide inhibitors of the HIF-1 pathway. It inhibits HIF-1 $\alpha$ transcriptional activity through the disruption of the interaction between the HIF-1 $\alpha$ -p300/CBP in patterns that markedly attenuated HIF activity; KCN1 impairs the recruitment of HIF-1 $\alpha$ -p300/CBP to pre-assembled HRE-HIF complexes on the chromatin, and prevents hypoxia-induced transcription on the CH1 domains of p300 and CBP, which are predicted to block the interaction with HIF-1 $\alpha$	(70,71,81,82)
	OKN-007	OKN-007 decreases microvessel density (CD-31) and HIF-1 $\alpha$ levels in glioma models; decreased levels of VEGFR-2 could decrease SULF2 and PDGFR- $\alpha$ immunoexpression, and increase decorin expression	(83,85,87)
	CpG oligodeoxynucleotides (CpG ODN)	CpG ODN107 is tightly related to its anti-angiogenic activity via suppression of HIF-1 $\alpha$ /VEGF pathway	(91)
Histone deacetylase inhibitors (HDACIs)	Vorinostat, romidepsin	HDACIs regulate HIF-1 $\alpha$ with direct acetylation as a possible regulatory mechanism for stability of HIF-1 $\alpha$ and other proteins such as the chaperone Hsp90 involved in regulating the expression of VEGF angiogenic factors that promote tumor proliferation and metastasis	(96-99,103,104, 107,108)
	pan HDACI: suberoylanilide hydroxamic acid (SAHA), PCI-24781	HDAC activity is inhibited and HIF-1 $\alpha$ translation is repressed by treatment of SAHA; SAHA may induce the expression of other key protein(s) that negatively modulate HIF-1 $\alpha$ translation; PCI-24781 accelerates cell apoptosis by downregulating the expression of AKT, mTOR, p70 ribosomal protein S6 kinase (p70s6k), glycogen synthase kinase 3A and B (GSK3a/b) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), and enhances the accumulation of HIF-1 $\alpha$	(109,110)

Table I. Continued.

	Various drugs and their targets	Regulated mechanisms	Refs.
	Valproic acid (VPA)	VPA is a broad-spectrum inhibitor of class I and II histone deacetylases, which significantly inhibit HIF-1 $\alpha$ expression and cell migration and knock down histone deacetylase-2 (HDAC2)	(112)
Natural compounds or small molecule agents	Isoliquiritigenin (ISL)	ISL significantly inhibits VEGF expression in breast cancer cells via promoting HIF-1 $\alpha$ proteasome degradation and directly interacts with VEGFR-2 to block its kinase activity; ISL has a reversible inhibitory effect on DNA topoisomerase (TOP I) activity, reduces the rate of single-stranded DNA unwinding in tumor cells, and upregulates p21/WAF1 and p27 in inducing the apoptosis of U87 glioma cells	(115-117)
	Celastrol	Celastrol has a potent inhibitory activity against HIF-1 activation induced by hypoxia in cancer cell lines; coumestrol lowered tumor microvessel density (MVD) significantly in an SHG-44 xenograft model (112-1), and decreased the level of VEGFR-1 and VEGFR-2 expression	(119,120)

GBM, glioblastoma multiforme; HIF-1 $\alpha$ , hypoxia inducible factor-1 $\alpha$ .

sensitized cells to apoptosis due to a faster depletion of ATP and Mcl-1. Co-treatment with a glucose analog (2-DG) and an Hsp90 inhibitor (17-AAG) enhanced the effect of IMQ in inducing cancer cell apoptosis *in vitro* and inhibiting tumor growth *in vivo*. These observations support the use of inhibitors of HIF-1 $\alpha$  and glycolysis to enhance the antitumor effects of IMQ. Additionally, IMQ modulates the activity of the glioma-associated oncogene (GLI) transcription factors by engaging signaling components of other pathways. GLI1 is a direct transcriptional target of GLI2 and GLI3, serves as a robust and sensitive functional read-out for the Hedgehog (HH) pathway activity (74,75).

The anticancer action of sodium dichloroacetate (DCA) caused ambiguous effects varying from tumor growth stimulation to significant anticancer activity. Under hypoxic conditions, the anticancer efficacy of DCA against glioma C6 cells was significantly enhanced (76). DCA was found to inhibit the glycolytic pathway through glycolytic inhibition which in turn diminished acid production. Moreover, DCA treatment also led to an alteration in the multidrug resistance (MDR) phenotype of GBM cells. Thus, altering glucose metabolism in GBM cells with hypoxia-induced resistance could be enhanced by DCA.

Resveratrol, a natural compound in food, has anticancer effects, antioxidant properties and an impact on glucose metabolism. It markedly suppressed glioma U87 and U251 cell migration and invasion under a hypoxia condition and higher doses led to stronger blockage (77). It has been proven that

hypoxia promotes aggressiveness, angiogenesis and resistance in glioma. Mechanically, hypoxia-induced upregulation of phosphorylated STAT3 was blocked by resveratrol. Notably, miR-34a was downregulated under hypoxia, but upregulated by resveratrol which consequently inhibited hypoxia-induced migration and invasion possibly via the p-STAT3/miR-34a axis and this effect was both time- and dose-dependent.

**HIF-1 inhibitors.** The close relationship between HIF-activated gene products and tumor progression/metabolism pinpoints HIF-1 $\alpha$  as an attractive therapeutic target. Several studies have already established that inhibition of the HIF-1 pathway can inhibit malignant characteristics such as glucose metabolism in gliomas (78,79). Indeed, a number of small-molecule inhibitors of HIF-1 $\alpha$  signaling are already undergoing clinical trials. The search for new HIF-1 $\alpha$  inhibitors continues. Echinomycin and 'programmable' polyamides inhibited HIF-1 $\alpha$ , and blocked hypoxia-induced expression of dynamin-related protein 1 (Drp1). Notably, Drp1 inhibitor Mdivi-1 efficiently attenuated hypoxia-induced mitochondrial fission and migration of U251 cells (80). One of the lead inhibitors is KCN1 [3,4-dimethoxy-N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]-N-phenylbenzene-sulfonamide], which is a new class of arylsulfonamide inhibitors of the HIF-1 pathway, inhibited HIF-1 $\alpha$  transcriptional activity through the disruption of the interaction between the HIF-1 $\alpha$  subunit and transcriptional co-activator p300/CBP (70,81). The latter acts as a bridge



between transcription factors to the transcriptional machinery. p300/CBP has a critical role in HIF function since blocking HIF-1 $\alpha$ -p300/CBP interaction markedly attenuates HIF activity (71). A recent study also found that KCN1 impaired the recruitment of these co-factors to preassembled HRE-HIF complexes on the chromatin, and prevented hypoxia-induced transcription in malignant glioma tumor xenografts. Mechanistically, KCN1 on the CH1 domains of p300 and CBP are predicted to block the interaction with HIF-1 $\alpha$  (82).

HIF-1 $\alpha$  inhibitor OKN-007 reduced HIF-1 $\alpha$  expression even under hypoxia, acted on GLUT-1 and MIB-1 to decrease cell proliferation, and increased apoptosis through cleavage of caspase-3 (83). OKN-007, previously known as NXY-059, is a very effective compound against *in vivo* adult glioma models (84-86), and it is currently undergoing clinical trial assessment as a new investigational drug for recurrent adult GBMs. OKN-007 is a small molecule that can traverse the blood-brain barrier and also has anti-inflammatory, antioxidant, pro-apoptotic (87,88) and anti-angiogenic properties (85,86). It was also previously demonstrated that OKN-007 is an effective anti-angiogenic compound *in vivo*, by directly decreasing microvessel density (MVD) (CD-31) and HIF-1 $\alpha$  levels in both F98 and U87 glioma models (85), and *ex vivo* by directly decreasing the levels of VEGFR-2 in C6 rat gliomas (86). OKN-007 was also able to decrease the levels of VEGFR-2 in a preclinical GL261 mouse glioma model (89). OKN-007 treatment substantially decreased VEGFR-2 levels in a GL261 glioma model, and is considered as an anti-angiogenic therapy in human gliomas. Furthermore, OKN-007 was able to significantly decrease SULF2 and platelet-derived growth factor receptor- $\alpha$  (PDGFR- $\alpha$ ) immunoexpression, and significantly increased decorin expression in responsive mice. This study indicates that OKN-007 may be an effective anticancer agent for some patients with GBMs by inhibiting cell proliferation and angiogenesis, possibly via the PDGFR $\alpha$  pathway, and could be considered as an additional therapy for pediatric brain tumor patients (90).

CpG oligodeoxynucleotides (CpG ODNs) are specific inhibitors to knock down gene expression. CpG ODN107 in combination with radiotherapy significantly decreased MVD, the VEGF level and HIF-1 $\alpha$  expression in orthotopic implantation glioma. In conclusion, CpG ODN107 significantly increased the radiosensitivity of U87 human glioma cells *in vitro* and *in vivo*. The radiosensitizing effect of CpG ODN107 was tightly related to its anti-angiogenic activity via suppression of the HIF-1 $\alpha$ /VEGF pathway (91). The mRNAs of some target genes were also downregulated. Thus, CpG ODN may be effective therapeutics in the future.

**Histone deacetylase inhibitors (HDACIs).** Histone deacetylases are enzymes that can regulate protein functions by removing their acetyl groups. Histones are typically deacetylated resulting in stronger binding to DNA. These enzymes can also regulate transcription factors such as HIF-1. The latter has been reported to upregulate histone demethylases (92).

The importance of HIF-1 in GBM has sparked a search for their inhibitors. They include small molecule inhibitors (93-95) and HDACIs (96). The latter not only suppressed HIF-1 $\alpha$  activity, but also the expression of HIF-regulated genes (97,98). Among the HDACIs developed, vorinostat, romidepsin and

suberanilohydroxamic acid (SAHA) are most promising and are now approved for treating GBM (99). Vorinostat is an orally active, potent inhibitor of HDAC activity that crosses the blood-brain barrier. Among the pleiotropic effects of HDAC inhibitors is the ability to attenuate inflammation, an action seen at concentrations lower than those required to slow cancer cell growth (100,101). The combination of vorinostat and tranlycypromine reduced GSC viability and displayed efficacy in a U87 xenograft model (102).

Inhibition of histone deacetylases not only causes hyperacetylation of histones, but other proteins as well such as the chaperon Hsp90 (103,104). Hsp90 regulates the expression and stability of HIF-1 $\alpha$  that in turn regulates VEGF that promotes glioma proliferation and metastasis (105,106). The translation of HIF-1 $\alpha$  is mediated, at least in part, by the PI3K/Akt pathway since inhibition of this pathway is sufficient to reduce normoxic HIF-1 $\alpha$  protein levels (107). HDACIs suppress constitutive Akt activation resulting in decrease HIF-1 $\alpha$  protein levels (108,109). The inhibition of histone deacetylase may vary with time. The pan-HDACI PCI-24781 accelerates cell apoptosis by downregulating the expression of AKT, mTOR, p70 ribosomal protein S6 kinase (p70s6k), glycogen synthase kinase 3A and B (GSK3a/b) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), and enhances the accumulation of HIF-1 $\alpha$  (110).

Another broad spectrum HDACI valproic acid (VPA) exhibited significant anticancer activity in gliomas (111). It inhibited HIF-1 $\alpha$  expression and cell migration and knock-down of histone deacetylase 2 (HDAC2) could mimic these effects (112). In glioma tumors, HDACIs mainly induced cytostasis and apoptosis (113,114). A number of mechanisms have been proposed to explain the HDACI-induced apoptosis but how these inhibitors work is still not fully understood. Unraveling the molecular actions of HDACIs on HIF-1 $\alpha$  may not only increase our understanding of the HIF signaling pathways but also allow the development of novel and more specific treatment options for GBM.

**Natural compounds.** A number of compounds derived from natural sources have anticancer properties that are linked to HIF-1 $\alpha$ . For example, a dietary chalcone-type flavonoid called isoliquiritigenin (ISL) was found to suppress sprout formation in VEGF-treated aortic rings. It also inhibited VEGF expression in breast cancer cells by fostering HIF-1 $\alpha$  proteasome degradation, and blocking VEGFR-2 kinase activity by binding to it directly. ISL inhibited breast cancer growth *in vivo*, suppressed VEGF/VEGFR-2 signaling, elevated apoptosis ratio but with minimal toxic effects (115). It has been reported that ISL had a reversible inhibitory effect on DNA topoisomerase I (TOP I) activity, reduced the rate of single-strand DNA unwinding in tumor cells, and upregulated p21/WAF1 and p27 in inducing the apoptosis of U87 glioma cells (116,117).

Celastrol, a phytoestrogen from the root of *Tripterygium wilfordii*, is a potent inhibitor of HIF-1 $\alpha$  that can lead to strong dephosphorylation of mTOR and its effectors, as well as the ERK pathway (118). It lowered tumor microvessel density (MVD) significantly in an SHG-44 xenograft model (119), and decreased the level of VEGFR-1 and VEGFR-2 expression (120). In a xenograft tumor model using Hep3B cells, celastrol effectively inhibited HIF-1 $\alpha$  protein expression leading to decreased tumor growth.

However, the hypoxic microenvironment in glioma is reductive in nature, and the effects of natural compounds on HIF-1 $\alpha$  protein are unclear in GBM. This is being exploited to selectively activate drugs such as ISL or celastrol targeting HIF-1 $\alpha$  in GBM is still a promising option since it regulates key cellular processes such as angiogenesis.

## 7. Conclusions and perspectives

It is important to ascertain how HIF-1 plays such a significant role in GBM since silencing HIF-2 $\alpha$  alone impairs tumor growth *in vivo* (121,122). Conflicting views are expressed in the scientific community regarding the roles of HIF-1 $\alpha$  and HIF-2 $\alpha$ . Some report that HIF-1 $\alpha$  is a tumor-suppressor gene while others report that HIF-2 $\alpha$  is an oncogene. Functional studies have shown that overexpression of HIF-1 $\alpha$  can suppress tumor growth while suppressing it enhances tumor growth (123). A recent study also indicated a novel signaling mechanism mediated by HIF-2 $\alpha$  in regulating invasiveness and stemness characteristics. It suggested that under hypoxic conditions, U87MG and A172 glioma cells acquire more migratory potential by increased Pan Mena and Mena INV expression as a consequence of this HIF-2 $\alpha$  mediated increase in Oct-4 and Sox-2 (124). These properties may help glioma cells to form a new nidus after local invasion or metastasis.

Metabolic reprogramming is now an established fact in GBM biology. In this process, HIF-1 plays a crucial role in switching energy metabolism from OXPHOS to glycolysis particularly under hypoxic conditions to provide GBM cells a survival advantage. In many types of cancers, poor prognosis is associated with abnormal levels and activity of HIF-1. Therefore, HIF-1 and its mediated metabolic pathways may be promising targets for treating GBM (125). Drugs such as DCA, and IMQ are effective in inhibiting HIF-1 expression and its activity and thus block tumor growth (73,76). Drugs targeting metabolic enzymes downstream of HIF-1 are also effective inhibitors of GBM progression. Therefore, combination therapy using both groups of drugs may provide an even more effective treatment regimen.

As HIFs are a group of transcription factors that regulate a large number of target genes, it is possible that they can act in an opposite fashion. How they work in a particular context depends on the shift in their balance between tumor-suppressive and oncogenic properties. At early stages of GBM development, HIF-1-mediated anti-apoptosis may be important. As time progresses, more mutations accumulate leading to more signaling pathways being re-programmed eventually leading to evasion of apoptosis. The anti-apoptotic function of HIF-1 becomes non-essential at later stages of GBM development and the balance may shift towards more tumor-suppression leading to a selective pressure on eliminating HIF-1. Nevertheless, targeting HIF-1 $\alpha$  is still a promising option since it regulates key cellular processes such as angiogenesis and epithelial to mesenchymal transition which are important for metastasis (126,127). HIF-1 $\alpha$  can further enhance the already activated signaling pathways in GSCs supporting their enrichment in GBM (35,128). Targeting HIF-1 $\alpha$  directly, indirectly or eliminating the hypoxic regions in gliomas may be workable for treating aggressive GBM (129,130).

A growing body of evidence supports the facilitating role of HIF-1 $\alpha$  in GBM progression/metabolism. Targeting HIF-1 $\alpha$  is a potent strategy for GBM, particularly as HIF-1 $\alpha$  is the key transcription factor responsible for the transactivation of a wide array of genes, many of which enhance the survival and metabolism of GBM cells. For example, Akt/mTOR is one of the major oncogenic pathways that shift the balance of HIF-1 $\alpha$  accumulation (131,132). A number of drugs are now in clinical trials but they only show low to moderate activity against gliomas (117,119).

Owing to the notable improvements in blocking the HIF-1 function, it may be expected to interfere with multiple attributes of tumor cells and eventually lead to tumor regression. Not surprisingly, significant efforts and resources have been invested into identifying small molecules that may potently and specifically inhibit HIF-1 $\alpha$ . Despite this, new inhibitors of the HIF-1 pathway, preferably with a defined mechanism of action, need to be identified, and we have yet to determine which agents may have the best antitumor efficacy and safety profile. Furthermore, drugs targeting these regulators of the HIF-1 system, which eventually degrade them in GBM cells, may be the future for developing novel treatment strategies.

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