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Review Article

Implications of Hypoxia in Glioblastoma (Gbm): Review of Current Concepts and Therapies

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Abstract

Mechanisms underlying the pathogenesis of brain tumors remain elusive due to their complex and genetically unstable nature, making it very challenging to develop effective therapies for patients diagnosed with high-grade malignant gliomas such as glioblastoma (GBM). Notably, the low survival outcomes observed in these patient populations are further exacerbated by physiological conditions such as hypoxia which activate molecular targets like hypoxia inducible factor-1 alpha (HIF-1α) to regulate processes that mediate resistance to radiation and chemotherapies. This concise review highlights the role of hypoxia GBM and emphasizes the importance of elucidating HIF-1α-mediated tumor resistance mechanisms as well as mechanisms that drive tumor survival in hypoxic conditions which can be targeted for refining standard-of-care therapy and improving clinical outcomes.

ABBREVIATIONS

ABTA: American Brain Tumor Association; HGG: High-Grade Gliomas; GBM: Glioblastoma Multiforme; TMZ: Temozolomide; WHO: World Health Organization; TCGA: The Cancer Genome Atlas; EGFR: Epidermal Growth Factor Receptor; TP53: Tumor Protein P53; IDH1: Isocitrate Dehydrogenase 1; PTEN: Phosphatase and Tensin Homolog; PI3KCA: Phosphatidylinositol 3-Kinase Catalytic Subunit Alpha; RB1: Retinoblastoma Protein 1; NF1: Neurofibromin 1; PDGFR: Platelet-Derived Growth Factor Receptor; CDKN2A: Cyclin Dependent Kinase Inhibitor 2A; NEFL: Neurofilament Light; GABRA1: *Gamma-Aminobutyric Acid Type A Receptor Alpha1 Subunit; SYT1: Synaptotagmin 1; SLC12A5: S*olute Carrier Family 12 Member 5; PI3K: Phosphatidylinositol 3-Kinase; Mtor: Mammalian Target of Rapamycin; MGMT: O-6-Methylguanine-DNA Methyltransferase; TERT: Telomerase Reverse Transcriptase; ATRX: Ataxia Thalassemia/Mental Retardation Syndrome X-Linked; BRAF: B-Raf; H3F3A: Histone 3 Family Member 3A; K27M: Lysine Converted to Methionine at Amino Acid 27; SF: Scatter-Factor; HGF: Hepatocyte Growth Factor; HR: Homologous Recombination; NHEJ: Non-Homologous End-Joining Recombination; HIF-1α: Hypoxia-Inducible Factor -1 Alpha; Bhlh-PAS: Basic Helix Loop Helix-Per-ARNT-Sim Family; HIF-1β: Hypoxia-Inducible Factor -1 Beta; N-TAD: N-Terminal Transactivation Domain; C-TAD: C-Terminal Transactivation Domain; ODDD: Oxygen Dependent Degradation Domain; Phds: Prolyl Hydroxylases; Pvhl: Von-Hippel Lindau Protein; Hres: Hypoxia Responsive Elements; CAIX: Carbonic Anhydrase 9; GLUT1: Glucose Transporter 1; VEGF-A: Vascular Endothelial Growth Factor-A; SDF-1: Stromal-Cell Derived Factor 1;MDM2: Mouse Double Minute 2; Hdacs: Histone Deacetylases; EZH2: Enhancer of Zeste Homolog 2

INTRODUCTION

According to the American Brain Tumor Association (ABTA) and the National Brain Tumor Society, approximately 700,000 individuals in United States are currently diagnosed with brain tumors [1,2]. Notably, about 78,000 new cases (including primary, invasive, malignant, and benign brain tumors) are projected by the end of 2016 [1,2]. These brain tumors encompass various forms and subtypes such as meningiomas, medulloblastomas, schwannomas, pituitary adenomas, and gliomas (glioblastomas, astrocytomas, oligodendrogliomas, and ependymomas) among many others [3,4]. The gliomas, the most aggressive form of brain tumors, affect approximately on average of 7.2 per 100,000 adults and 0.8 per 100,000 children every year [5]. High-grade gliomas (HGG) such as GBM are typically classified into two broad categories: primary and secondary GBMs [6]. Primary GBM accounts for approximately 90% of all GBMs and is defined as tumors that emerge due to *de novo* oncogenic transformation of normal glial cells [6]. The remaining 10% of GBMs are classified as secondary GBM due to their development and progression

from low-grade gliomas [6]. While the average 5-year survival rate is 85% or higher for patients with low-grade gliomas, patients diagnosed with primary GBM, have a 5-year survival rate of less than 5% [7]. Therefore, to enhance the quality of life and the standard-of-care therapy for these patients, it becomes imperative to understand the underlying mechanisms that play a role in the pathogenesis of GBM. This review highlights the role of how hypoxia contributes to the six hallmarks of cancers previously reviewed and outlines by Hanahan and Weinberg [8]: (1) induction of angiogenesis, (2) activation of invasion and metastasis, (3) resistance to cell death, (4) sustained proliferative signaling, (5) growth suppressor's evasion, and (6) replicative immortality. All these hallmarks play a role either directly or indirectly in hypoxia-driven tumor growth, and therapeutic approaches directed towards ameliorating hypoxia-driven mechanisms in GBM progression are currently under investigation [8].

Challenges facing GBM

Despite some advances in de bulking surgery, as well as, radiation, and chemotherapy regimens, improvements in progression-free survival have been minimal [7,9,10]. While numerous factors contribute to marginal improvement following brain tumor therapies, inherent and acquired resistance to treatment remains as a major obstacle [7,11]. Several reports suggest that a central mechanism of resistance in tumors such as GBM involves the pathophysiological condition of the tumor microenvironment commonly referred to as tumor hypoxia [7,9,11]. The concept of tumor hypoxia is illustrated by the fact that the median oxygen partial pressure of a tumor might be around 10 mmHg, whereas, in normal subcutaneous tissue, the median value for oxygen partial pressure ranges between 40 and 60 or 80 mmHg [12,13]. While normal physiological oxygen levels in the brain range between 0.5-7%, several studies have shown that there is intertumor and intratumor heterogeneity when it comes to oxygen levels in GBM which fall between 0.1%-2.5% oxygen [14,15].

GBM pathogenesis is attributed to multifactorial elements that play a role in promoting genetic instability. This makes investigating and uncovering mechanisms of GBM resistance even more challenging in regards to novel drug developments [16,17]. It is first critical to identify the molecular signatures and validate what these signatures predict in terms of dysregulated signaling networks. Importantly, how these networks are regulated under hypoxia will also be critical. Recent research initiatives by the World Health Organization (WHO) and research networks like the cancer genome atlas (TCGA) have identified specific molecular signatures that will aid in classifying and distinguishing specific GBM subtypes [4]. The TCGA uses large patient cohorts to characterize molecular signatures in GBM with the hope of using this information as a predictive correlative in conjunction with histological features [5,18]. Through genome profiling a number of key genetic drivers of GBM have been identified and include (Table 1). Epidermal Growth Factor Receptor (*EGFR*); Tumor Protein P53 (*TP53*); Isocitrate Dehydrogenase 1 (*IDH1*); Phosphatase and Tensin Homolog (*PTEN*); Phosphoinositide 3-Kinase Catalytic Subunit-alpha (*PI3KCA*); Retinoblastoma Protein 1 (*RB1*); Neurofibromin (*NF1*); Platelet-derived Growth Factor Receptor-alpha (*PDGFRA*); and Cyclin-Dependent Kinase Inhibitor 2A (*CDKN2A*) [19]. Moreover, this TCGA-based genome-wide profiling studies resulted in identification of molecular signatures that in conjunction with histological and clinical information help distinguish four different GBM subtypes: proneural, classical mesenchymal, and neural [5]. The most common genetic alterations for each subtype include proneural GBM - alterations of *PDGFRA, TP53, PI3KCA,* and *IDH1*; classical GBM - amplifications of *EGFR* and *CDKN2A* mutations; and, mesenchymal GBM - *NF1, PTEN*, and *RB1.* Neural GBM are characterized based on the presence of neuron markers such as neurofilament light (*NEFL), gamma-aminobutyric acid type A receptor alpha1 subunit* (*GABRA1), synaptotagmin 1 (SYT1),* and solute carrier family 12 member 5 (*SLC12A5)* [19]. Mutations are also present in growth factor receptor - dependent activation of receptor tyrosine kinase pathways such as MAPK and/or phosphatidylinositol 3-kinase (*PI3K*) pathways [20]. Approximately, 70% of GBMs have alterations in components involving the PI3K pathways and other components such as *PTEN* and mammalian target of rapamycin (*mTOR*) [20]. *Furthermore, epigenetic changes are typically found in proneural GBM subtype. This represents yet another layer of dysregulation where changes in methylation patterns of CpG islands rewire gene expression patterns* [5].

In comparison to adult GBM, an in depth characterization of pediatric GBM molecular signatures lags behind due to the rarity of the disease [21]. As more specimens are analyzed, however, progress is being made towards elucidating mechanisms involved in progression of pediatric GBM. Additionally, as reported by Sturm et al, the TCGA has identified molecular differences between adult and pediatric GBM [5]. In contrast to pediatric GBM, adult GBMs have a higher frequency of a methylated O-6-Methylguanine-DNA Methyltransferase (MGMT) promoter, increased EGFR amplification or overexpression, higher incidence of telomerase reverse transcriptase (TERT) mutations, alterations in the PTEN/AKT pathway, and higher mutations of IDH genes (Table 2) [21]. In addition, gain of chromosome 7 and loss of chromosome 10 is found in ~85% of adult GBMs while chromosome 7 gain and chromosome 10 losses are found in about 13-19% and 16-38%, respectively, in pediatric GBMs [5]. In contrast, pediatric GBMs typically have a higher incidence of *PDGFRA* amplifications/activation; mutations of *NF1,* Alpha Thalassemia/mental retardation syndrome X-linked *(ATRX*), TP53, B-Raf (BRAF); and mutations, of histone H3. 3A family member 3A *(H3F3A)* where lysine is mutated to methionine at amino acid residue 27 (K27M) [5,21]. Notably, the K27M H3F3A mutation is associated with poor prognosis in the pediatric GBM patient population [21].

Such genomic profiling and characterization of GBM are critical to development of effective therapeutic regimens specific to the particular GBM subtype. More investigations are now required to decipher in detail how combinations of genetic alterations in GBM promote resistance to therapy. This information will be critical for development of new strategic combinations of targeted agents that are efficacious but also have acceptable toxicity profiles.

Numerous factors contribute to the marginal improvement

Table 1: Key Genetic Drivers of GBM.

Gene	Function
EGFR	Encodes for epidermal growth factor receptor; Promotes growth, proliferation, and survival; Activates PI3K and/or MAPK pathways
TP53	Encodes for p53 protein; Tumor suppressor protein; Recognizes DNA damage; Induces DNA repair or apoptosis
IDH1	Encodes for IDH protein; Converts isocitrate to 2-ketoglutarate and produces NADPH which helps protect against ROS
PTEN	Encodes for PTEN protein; Tumor suppressor protein; Acts as a phosphatase Negatively regulates PI3K and AKT involved in survival
PI3KCA	Encodes for PI3K catalytic subunit alpha; Downstream of growth factor receptors like EGFR; promotes growth, proliferation, and survival
RB1	Encodes for RB1 protein; Tumor suppressor; Inhibits growth, proliferation, and survival
NF1	Encodes for NF1 protein; Tumor suppressor; Control growth and proliferation; Negatively regulates ras pathway
PDGFRA	Encodes for PDGFRA protein; Promotes growth, proliferation, and survival; Activates PI3K and/or MAPK pathways
CDKN2A	Encodes for p16 and p14arf proteins; Tumor suppressors; p16 inhibits CDK4 and CDk6 to inhibit cell cycle; p14arf activates p53; Controls growth

Table 2: Comparison of Somatic Genetic Alterations between Adult and Pediatric GBMs.

Gene	Function	Adult GBM	Pediatric GBM
MGMT methylation	MGMT: Rapairs alkylated guanine adducts	⇧	⇩
EGFR Amplification	EGFR: Epidermal growth factor receptor; promotes growth/survival/proliferation	⇧	⇩
TERT Mutation	TERT: Protects telemore shortening and protects cells from feath (apoptosis); Allows replicative mmortality	⇧	⇩
PTEN Mutation	PTEN: Tumor suppressor protein; acts as a phosphatase negatively regulates PI3K and AKT involved in survival	⇧	⇩
AKT Mutation	AKT: Serine/Threonine kinase downstream of growth factor receptor signaling pathways	⇧	⇩
PDGFRA Amplification	PDGFRA: Platelet-derived growth factor receptor; promotes growth/survival/proliferation	⇩	⇧
NF1 Mutation	NF1: Tumor suppressor; Controls growth and proliferation; negatively regulates ras pathway	⇩	⇧
ATRX Mutation	ATRX: Involved in chromatin remodeling	⇩	⇧
TP53 Mutation	TP53: encodes for tumor suppressor protein (p53) Recognizes DNA damage; Induces DNA repair or apoptosis	⇩	⇧
BRAF Mutation	BRAF: Proto-oncogene; promotes growth, survival, proliferation	⇩	⇧
H3.3A (H3F3A) Mutation	H3.3A: Variant histone 3	⇩	⇧

in outcomes following treatment in GBM patients, but resistance to current standard-of-care agents tops the list [7,11]. Several reports suggest that a major mechanism by which tumors such as GBMs mediate resistance to therapies involves the pathophysiological condition commonly referred to as tumor hypoxia [7,9,11]. Notably, tumor hypoxia has also been reported to be the driving force for inducing genetic instabilities such as increasing CD133+ brain tumor stem cell population, and by down-regulating DNA-repair proteins in a number of cancers including gliomas and GBM [13].

Hypoxia

Hypoxia is a state of insufficient or reduced oxygen concentrations due to improper regulation/balance between oxygen supply and consumption [1,22]. Reduced oxygen concentrations contribute to the onset of several pathological diseases among which include ischemia, anemia, and stroke [11].

GBM is characterized by its highly necrotic tissues, due to the reduced oxygenation from a vascular primary tumors or abnormal microvasculature within the tumor [11,23]. Under these conditions, tumors exploit hypoxia-induced factors that function in numerous biological processes to promote tumor growth, survival, and invasiveness via mechanisms involving tumorigenesis, angiogenesis, and vasculogenesis [11,23]. While genetic amplifications and oncogenic driver expression have been considerably studied in GBM, efforts in terms of elucidating the functional outcome of hypoxia in GBM is only recently beginning to be investigated [11]. It has been reported that the hypoxic tumor microenvironment exacerbates tumorigenesis due to increased CD133+ brain tumor stem cell populations residing in the hypoxic niche [11]. In terms of, increased genetic instability, loss of the *PTEN* gene in conjunction with hypoxia, can result in increased intratumoral necrosis [11]. This is of particular importance since several studies suggest there is a correlation between patient survival outcome and degree of GBM necrosis [11]. Hypoxia has also been reported to contribute to the migration of GBM tumor cells by induction of c-Met, a receptor for scatter-factor (SF)/hepatocyte growth factor (HGF) [11].

Additionally, hypoxia in GBM and other tumors can be characterized as acute or chronic [22]. Acute tumor hypoxia (perfusion-limited) refers to insufficient oxygenation due to variations in tumor blood flow due to transient decrease in perfusion [22]. On the contrary, chronic tumor hypoxia (diffusion-limited) originates from increased oxygen diffusion distance contributed by unorganized tumor microvasculature [22]. Chronic hypoxia is induced by the limited ability of oxygen to diffuse through tumor and tissue at distance of about 150-200μm from the source of the blood/oxygen supply, which results in necrotic GBM tumors [22]. Both acute and chronic hypoxia can occur simultaneously in different areas of a GBM, creating tumor microenvironments with spatial and temporal variability of oxygen concentrations [22].

Hypoxia is a key element within the tumor microenvironment and clearly contributes to the poor survival outcomes for patients diagnosed with GBM [9]. Extensive research initiatives are focusing on understanding hypoxia-driven resistance to certain chemotherapeutic agents and radiation therapy [9,11]. One mechanism by which hypoxia can mediate resistance involves failure to induce DNA strand breaks due to decreased production of oxygen radicals [23]. Tumors such as GBMs have adapted mechanisms to survive in the hypoxic environment through mechanisms that down-regulate proteins involved in DNA repair such as BRCA1 (homologous recombination), KU70 (non-homologous end-joining), and MSH2 (mismatch repair [13]. This down regulation of DNA repair leads to accumulation of damaged, unrepaired double-stranded DNA breaks and replication errors all of which if not repaired result in either death or in some cases genetic instability that leads to mutations that promote emergence of therapy-resistant clones [13]. Studies are still ongoing to identify the precise mechanisms by which hypoxia mediates resistance to therapies. However, it has been postulated that hypoxia-induced chemo resistance and radio resistance also involve mechanisms that upregulate hypoxia-induced molecular targets such as hypoxia-inducible factor-1 alpha (HIF-1α) which may directly or indirectly mediate the ability of the GBM tumors to maintain the six hallmarks of cancer cell survival.

HIF-1α

HIF-1α is one of the best characterized hypoxia-inducible proteins and is a transcription factor that belongs to the basic helix-loop-helix-Per-ARNT-Sim (bHLH-PAS) protein family [11]. Through the bHLH and PAS motifs, HIF-1α can form a heterodimeric complex by interacting with its constitutively expressed co-subunit hypoxia inducible factor subunit 1 beta (HIF-1β) (Figure 1) [24]. HIF-1α is comprised of several regulatory motifs such as the N-terminal transactivation domain (N-TAD and the C-terminal transactivation domain (C-TAD) important for transcriptional activity (Figure 1). All of these domains play a specific role in regulating HIF-1α transcriptional activity, but its stabilization is primarily regulated by the oxygen-dependent degradation domain (ODDD) which encompasses prolines that get hydroxylated by oxygen-mediated enzymes known as prolyl hydroxylases (PHDs) (Figure 1) [25]. In normoxic states where sufficient oxygen is available, cells down-regulate the expression of HIF-1α by post-translational modifications. These modifications are achieved by exploiting PHDs and acetyl transferases for hydroxylation prolines 402 and 564 within the ODDD and by acetylating lysine 532 on HIF-1α, respectively (Figure 1) [24,25]. Ultimately these modifications enable von-Hippel Lindau protein (pVHL), an E3-ubiquitin ligase, to ubiquitinate HIF-1α and target it for proteosomal degradation (Figure 2) [24,26]. However, in pathophysiological conditions, where inadequate oxygen levels are maintained, cells have evolved mechanisms to help regulate their survival and proliferation by up-regulating HIF-1α-dependent genes that facilitate these processes under hypoxic conditions (Figure 2) [27,28]. This occurs by inhibition of PHD-mediated hydroxylation due to low levels of oxygen, which results in prevention of ubiquitination and ubiquitin-mediated proteasomal degradation of HIF-1α [27]. Subsequently, the stable HIF-1α is then able to translocate into the nucleus, bind to its transcriptional activating machinery (HIF-1β and co-activators) on the hypoxia-responsive elements (HREs) of target genes, and initiate transcription [28] (Figure 2).

Similarly, HIF-1α also contributes to tumorigenesis and angiogenesis by increasing expression of genes like carbonic anhydrase 9 (CAIX) and glucose transporter 1 (GLUT1)to promote favorable conditions for tumor cell growth/survival by regulating proper pH and glucose metabolism, respectively [22].

As mentioned previously, patients with hypoxic tumors are more likely to have poor prognosis and decreased survival outcomes compared to patients with non-hypoxic tumors [22]. Research efforts have focused on potential therapeutic approaches that target hypoxia and HIF-1α- induced mechanisms that promote tumor growth, invasiveness, and mediate resistance to therapy [22].

While this review primarily focuses on hypoxia-induced molecular changes which are predominantly mediated by HIF-1α, [11], it is important to note that hypoxia can also induce other factors that function independently of HIF-1α.

Role of Hypoxia in regulation of the six hallmarks of cancer

Hanahan and Weinberg previously outlined the six key hallmarks of cancer that need to be considered in development of new therapeutic approaches: (1) inducing angiogenesis, (2) activating invasion and metastasis, (3) resisting cell death, (4) sustaining proliferative signaling, (5) evading growth suppressors, and (6) enabling replicative immortality [8]. Notably, GBM and many other cancers, are characterized by hypoxia-driven mechanisms that can directly impact or potentiate the six proposed hallmarks. For instance, HIF-1α in hypoxic GBM tumor environment induces angiogenesis by up-regulating genes such as vascular endothelial growth factor -A (VEGF-A), a potent angiogenic factor that mediates formation of new blood vessels [9,11]. Up-regulation of VEGF-A increases vascularization in close proximity to the tumor cells providing sufficient blood supply and adequate oxygenation that enhances growth survival [9]. Similarly, HIF-1α and VEGF in hypoxic GBM environment have been reported to promote tumor invasion and metastasis by up-regulation of CXCR4, a chemo attractant receptor for the ligand stromal cell derived factor-1 (SDF-1) that is secreted by various tumors including GBM [29].

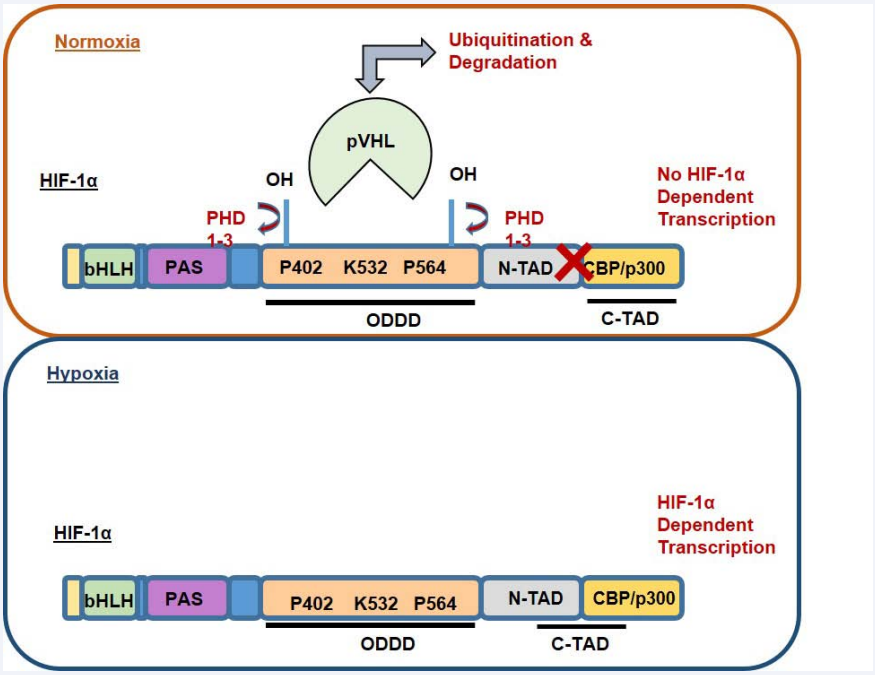

Figure 1 Structure of HIF-1α. In normoxic conditions, HIF- 1α becomes hydroxylated at proline residues 402 and 564 by PHDs. Collectively, all these hydroxylations result in degradation of HIF-1α and HIF-1α is not able to bind to coactivators (CBP/p300) to mediate target-gene transcription. In hypoxia, these enzymes responsible for hydroxylation are not activated. Therefore, HIF-1α expression is preserved and can mediate transcription via coactivators. Adapted from Yeom et al.; Cancers, 2011 [54].

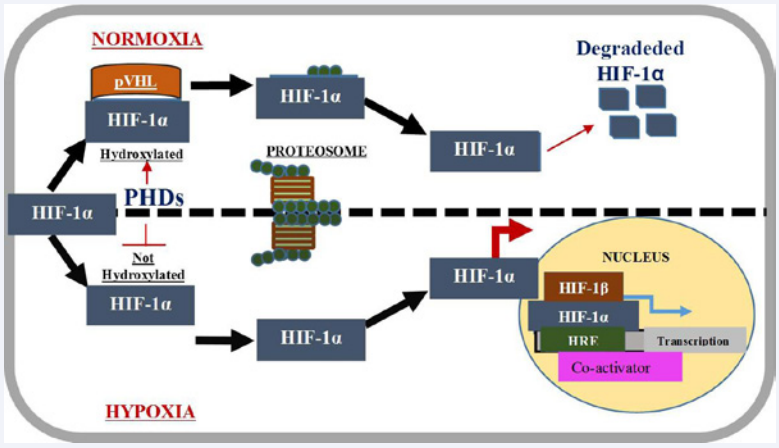

Figure 2 Role of HIF-1α in Hypoxia. In normoxia,HIF-1α will get hydroxylated by PHDs enabling E3 ubiquitin ligases such as pVHL to target HIF-1α for ubiquitination, ultimately, resulting in ubiquitin-mediated proteasomal-degradation [24]. Contrastingly, in hypoxia PHDs are unable to hydroxylate HIF-1α [24]. Absence of this hydroxylation, the pVHL-mediated ubiquitination and degradation does not occur [24,26]. Therefore, HIF-1α is stabilized and translocates into the nucleus to bind to HREs, HIF-1β, and coactivators on promoter region of its target gene to mediate their transcription [28]. Adapted from Shimoda and Semenza; American Journal of Respiratory Critical Care Medicine, 2011 [55].

As previously mentioned, both EGFR and PDGFRA can be mutated in GBM leading to sustained activation of downstream growth-promoting pathways [5,30]. These mutations result in pro-survival signaling cascade by constitutive activation of their downstream tyrosine kinases involved in MAPK and/or PI3K signaling pathways [31]. Although, hypoxia or HIF-1α don't directly regulate hyper-activation of EGFR and/or PDGFRA, pro-survival signaling through these receptors enables GBM tumors to survive in the hypoxic tumor microenvironment. Additionally, growth factor receptor (EGFR, PDGFRA) -mediated PI3K signaling results in the downstream activation of AKT which promotes anti-apoptotic signals such as activation of the ubiquitin-ligase murine-double minute 2 homologue (MDM2) which is negative regulator of the tumor suppressor protein p53 [31,32]. Under normal circumstances, p53 acts as a tumor suppressor protein by inducing apoptosis in cells where DNA damage occurs [31]. The AKT-mediated activation of MDM2 enables MDM2 to inhibit p53-dependent transcriptional activity of pro-apoptotic genes (BAX, etc) and target p53 for degradation [32]. This is one mechanism by which GBM cells resist cell death, sustain proliferative signaling,

and evade growth suppressors such as p53 to promote survival in hypoxic conditions. However, the incidence of p53 mutations in GBM is about 87% which typically leads to inactivation of p53-dependent functions that control cell cycle arrest, DNA repair, and survival [31].

Due to TERT promoter mutations which result in TERT up-regulation in GBM, these tumors acquire the capability to enable replicative immortality by preventing shortening of the telomeres [33]. This telomere shortening occurs during DNA replication to help protect the ends of the chromosomes, however, through multiple cycles of DNA replication the telomere gets shorter and eventually is gone resulting in the cell death [33]. By upregulation of TERT, the telomeres are protected from shortening and replicate infinitely. Interestingly, TERT up-regulation also results in increased transcriptional activity even in harsh conditions such as hypoxic tumor environments [34].

Therapies for GBM

Radiotherapy in combination with TMZ treatment is the standard of care therapy especially in those individuals where the MGMT expression is low [35]. Since tumor hypoxia plays a major role in cancer progression and resistance, it is imperative to develop therapies that target hypoxia and/or HIF-1α mediated mechanisms that contribute to GBM pathogenesis, as well as, targeting mechanisms of GBM that allow these tumors to survive in hypoxic environments [36].

Owing to the novel technologies that enable detection of hypoxia in various tumors such as GBM and the concept that hypoxia may mediate resistance to therapies, there is great interest in targeting hypoxia and hypoxia-induced molecular targets such as HIF-1α [11]. Exploiting the use of small molecule inhibitors, protein destabilizers, and HIF-1α siRNA is one approach for targeted-therapy against HIF-1α [37]. While promising preclinical results have been obtained using these approaches *in vitro* and *in vivo*, they will be challenging clinically due to the reduced efficacy of the delivery systems [37]. HIF-1α mediated targets such as VEGF-A have also been of great interest for therapeutic options due to development of Bevacizumab, a recombinant humanized monoclonal antibody targeting VEGF-A [38,39]. However, it is possible that hypoxia-induced resistance to therapies still persists even after treatment with Bevacizumab due to the fact that hypoxia may have other HIF-1α independent mechanisms to induce VEGF-A expression by oncogenes such as K-ras as observed in hypoxic colon cancers [40,41]. In 2014, the New England Journal of Medicine reported from two clinical trials focused on assessing effects of Bevacizumab in newly diagnosed GBM patients that whether used as first line therapy or in combination with standard of care (TMZ and radiation), bevacizumab did not improve overall survival. Moreover, and in both trials, they concluded the drug prolonged progression-free survival in [42,43]. Similar to the VEGF-A growth factor signaling pathway, EGFR receptor signaling is also an important signaling cascade to target in GBM. Erlotinib is an FDA approved drug that targets EGFR for metastatic non-small cell lung cancer. It is currently being tested in combination with TMZ in a clinical trial for GBM patients (NCT00039494) [31]. Other neoplastic drugs such as AG1433 that targets growth factor receptor PDGFRA and inhibitors of the downstream intracellular signaling proteins such as BEZ235 which targets PI3K/AKT/mTOR are also under investigation in clinical trials for GBM [44]. Additionally, other PI3K inhibitors such as BKM120 are also in clinical phase trials for GBM [45]. NVP-BEZ235, a dual inhibitor of PI3K-mTOR inhibitors, has shown promising results in preclinical phases and has now progressed into phase 1/2 clinical trials for solid tumors including GBM [45].

In resistant tumors such as GBM, targeting other factors such as epigenetic components is a novel therapeutic approach. In a phase I/II trial focused on recurrent GBM patients, treatment with Vorinostat, an inhibitor of histone deacetylases (HDACs), resulted in a median overall survival of 5.7 months [35]. Additionally, histone methyltransferases such as enhancer of zeste homolog 2 (EZH2) have been reported to be important for maintenance of GBM cancer stem cells [46]. Therefore, targeting EZH2 through inhibitors like Tazemetostat, may provide another novel therapeutic approach for controlling GBM recurrence and progression.

Probing multiple therapeutic modalities

In terms of targeting hypoxic-dependent increases in HIF-1α, LaRusch and colleagues have reported that HIF-1α and p53 both bind to the same site in the hydrophobic pocket of MDM2. This has broad implications for attenuating VEGF production and angiogenesis, for the HIF-1α/MDM2 complex is involved in the increased transcriptional activity of VEGF [47]. Interactions of HIF-1α or p53 with MDM2 can be blocked by MDM2 protein-protein interaction inhibitors such as Nutlin-3a. In terms of therapeutic efficacy, Wang et al reported improved survival of mice with intracranial recurrent GBM tumors using combination therapy consisting of TMZ with the MDM2 inhibitor Nutlin-3a compared to TMZ alone [48]. To what extent second and third generation inhibitors can block binding of HIF-1α to MDM2 and block VEGF-mediated angiogenesis is not known. Utilization of preclinical models of GBM will provide a useful platform to evaluate sensitivity or resistance mechanisms that influence the efficacy of combination therapies [49].

Approximately 90% of GBM tumors have recurrence at the original primary site [50]. This can be attributed to the tumors' ability to evolve novel resistance mechanisms for circumventing the adverse effects of these therapies. It is important to note that even with current chemotherapeutic treatments, therapies that target hypoxia or HIF-1α inhibition, and improved surgical resection techniques, the survival for patients with GBM is only extended an additional 12-14 months following the time of diagnosis. Effects of TMZ-mediated DNA damage in GBM tumors can be diminished to a large extent by repair of the damage by the DNA repair protein MGMT as well as other DNA repair pathways [51,52]. Some GBM can express high levels of MGMT rendering them resistant to TMZ, while in other GBM methylation of the MGMT promoter blocks MGMT expression [50]. As previously stated, hypoxic tumor environment induces expansion of CD133+ glioma stem cells which play a role in progressive tumor proliferation/growth [51]. Notably, CD133+ glioma stem cells also express higher levels of MGMT than the bulk of the tumor which correlates with the increased therapeutic resistance to TMZ [53]. Drug discovery focused on tumor hypoxia is challenging and further exploration into target identification and underlying

mechanisms of action in a hypoxic tumor microenvironment will be critical for future progress.

CONCLUSION

Development of more effective therapies against a rapidly evolving, genetically unstable cancer such as GBM is critical. Tumor hypoxia, while being widely accepted as a key contributor for mediating resistance to radiation and chemotherapy, remains a pathophysiological condition that encompasses several unresolved questions for further explorations. Despite the recent advances made towards elucidating mechanisms for new therapies involved in tumor hypoxia within solid tumors such as GBM, the long-term survival outcome and prognosis still remains poor. This may be attributed to the fact that even after surgical resections of hypoxic tumors at the primary site, some of the hypoxic tumors cells remain and invasive cells are typically already residing in other areas of the brain parenchyma that are hypoxic [9]. By distinguishing the molecular genomics and phenotypic features between hypoxic and non-hypoxic tumors, potential predictive signatures, biomarkers, and neo-antigens could be discovered. These patient and tumor-specific differences could facilitate the classification of various tumor patients (GBM patients) into good versus poor responders of radiation and/or chemotherapy.

Standard-of- care treatment for GBM has been essentially unchanged for many decades. There is currently no cure for GBM and understanding how the hypoxic tumor niche regulates tumor growth and response to therapy will be critical to stabilizing disease and ultimately finding a cure. The next frontier of research in tumor hypoxia should focus on gaining a better understanding of therapeutic responses under hypoxia. To what extent personalized therapies will revolutionize cancer treatment holds promise but is still open for debate. To this end, more investigations focused on how molecular signatures of different GBM subtypes are regulated by a hypoxic tumor microenvironment are needed. New insights into the interplay of patient-specific tumor genetics and the hypoxic microenvironment may help advance new therapies that will improve the standard of care and quality of life for patients with devastating tumors such as GBM.

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