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Hypoxia, Angiogenesis and Mechanisms for Invasion of Malignant Gliomas

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1. Introduction

Malignant gliomas are not only the most frequent primary brain tumor in the adult population, but also the most aggressive. Despite recent therapeutic advances, they remain associated with high morbidity and mortality. Determinant of key biological features of these tumors, like their response, resistance, and patterns of recurrence when challenged by standard and new therapeutic options. Glioblastoma multiforme (GBM), the most aggressive of the astrocytic neoplasms, is characterized by both necrosis as well as high rates of endothelial proliferation and neo-vascularization. that generates a high consumption of oxygen and nutrients, thus leading to local hypoxia. The latter activates a cascade of signaling pathways leading to: 1) angiogenesis, 2) enhanced motility/invasion, 3) changes in metabolism, and 4) the ability to survive oxidative stress. This is a dynamic and multifactorial process whose outcome, either death or survival, is dependent on the timing and the rates of each of these processes, as well as on the molecular characteristics of the tumor. Therefore, hypoxia-induced enhancement of motility/invasion may determine the outcome and response to therapy of a particular GBM. We seek a better understanding of the molecular and phenotypic effects of local hypoxia in GBM. Here, we review the definition of hypoxia and its molecular and phenotypic effects on cancer cells in general and then turn our attention to its impact on malignant glioma cells. We also examine the interplay between anti-angiogenesis, survival, and enhanced motility, and explore potential therapeutic implications. We close with unresolved questions and potential future directions.

2. Malignant gliomas

2.1. Overview of gliomas

Central nervous system (CNS) neoplasms are a diverse group that varies widely in terms of clinical presentation, aggressiveness, and response to therapy, with distinctions in histology and cellular composition being largely responsible for these differences (Brat and Mapstone 2003). Of the many different types of brain tumors, gliomas are the most frequent primary brain tumors in adults (Ricard, Idbaih et al. 2012). Lower grade tumors, such as grades I and II, are commonly well-differentiated with limited amounts of increased cell density and other abnormalities. Grade III astrocytomas are anaplastic and have increased vessel and cell density, cellular atypia, and increased mitotic activity. GBM, grade IV, is characterized by the presence of necrosis and endothelial proliferation (Brat and Mapstone 2003; Westphal and Lamszus 2011).

2.2. Significance of malignant gliomas

The annual incidence of malignant gliomas is approximately 4 to 5 per 100 thousand and this group accounts for approximately 70% of the total number of new cases of malignant primary brain tumors diagnosed in the United States each year (Wen and Kesari 2008; Wen, Macdonald et al. 2010). The overall incidence of gliomas is higher among males as compared to females (7.2 per 100,000 persons-years in males versus 5.0 per 100,000-person years in females) and it is also highest among Caucasians, as compared to other ethnic groups (Peak and Levin 2010). Malignant gliomas can occur in any age group however the incidence increases in the fifth decade of life and peaks at about 65 years of age (Brat and Mapstone 2003). GBM is the most aggressive glioma. Stupp and colleagues reported that patients treated with concomitant and adjuvant Temozolomide and radiotherapy had overall survival (OS) rates of 27.2 and 9.8 percent at 2 and 5 years, respectively (Stupp, Mason et al. 2005; Stupp, Hegi et al. 2009).

3. Hypoxia

3.1. Definition of hypoxia

Oxygen plays a central role in cell biology and human cells require constant and adequate supply of oxygen. Oxygen functions as the terminal electron acceptor in the process of mitochondrial respiration, the process by which ATP is generated for use in most biochemical reactions (Semenza 2012; Semenza 2012). Hypoxia is a term used to describe reduced levels of oxygen and can be defined as a condition in which the oxygen pressure in the environment is less than 5 to 10 mmHg (Lu and Kang 2010). Hypoxia typically ranges from 0.1 percent to 3 percent oxygen, with exact definitions varying according to individual researchers (Wang, Jiang et al. 1995; Semenza 1998; Ke and Costa 2006; Palazon, Aragonés et al. 2012). Normoxia for tissue culture experiments is considered approximately 21 percent oxygen. to

meet consumption. For example, a local environment including a large number of metabolically active cancer cells may be hypoxic when the oxygen supply is not enhanced; to prevent hypoxia, supply must be increased. Factors that contribute to hypoxia include low partial pressure of oxygen in the blood, reduced ability of blood to carry oxygen, reduced tissue perfusion, increased diffusion distances, or inability of cells to use oxygen (Hockel and Vaupel 2001). Hypoxia is threatening to the normal cellular environment and a series of highly regulated and coordinated response-mechanisms are necessary in order for cells to survive in hypoxic environments (Wheaton and Chandel 2011).

3.2. Hypoxia and tumor cells

One of the characteristics of cancer cells is deregulated, high cellular proliferation that ultimately results in structurally and functionally abnormal blood vessels that are unable to provide an adequate amount of oxygen to meet the increased metabolic demands of proliferation, which ultimately results in hypoxia (Vaupel, Kallinowski et al. 1989; Harris 2002; Semenza 2012). Although indirect evidence for hypoxia in human tumors was first reported in the 1950s, Peter Vaupel and colleagues were among the first researchers to demonstrate direct evidence of hypoxia in human cancers, as well as linking hypoxia with increased metastasis and poor prognosis in patients with squamous tumors of the head and neck, cervical cancers, and breast cancers (Thomlinson and Gray 1955; Hockel, Schlenger et al. 1999; Hockel and Vaupel 2001; Harris 2002).

Tumor cells generally respond to hypoxia in 1 of 2 ways: either cellular proliferation is restricted and apoptosis and necrosis may occur or the tumor cells adapt to the stress of the hypoxic environment and become more aggressive (Vaupel 2008). This adaptation towards a more aggressive phenotype is accomplished by regulating the expression of various genes that have critical roles in cellular events, which include cellular proliferation, differentiation, tumor glycolysis, angiogenesis, and metastasis and invasion (Vaupel 2004; Lu and Kang 2010; Semenza 2012). Additionally, tumor cells not only acquire more invasive and metastatic properties in response to hypoxia, but also become more resistant to standard treatments, such as chemotherapies and radiation therapies (Semenza 2012). Hypoxia-associated resistance to radiation therapy can occur when the partial pressure of oxygen in a tumor is less than 25-30 mmHg. The mechanism of this resistance is thought to be multi-factorial, however some proposed possibilities include decreased oxygen concentrations, higher levels of heat shock proteins, and the presence of cells with decreased apoptotic potential (Hockel and Vaupel 2001). Hypoxia-induced resistance to chemotherapy is likely affected by inhibition of cellular proliferation, decreased effectiveness of some agents in the setting of hypoxia, tissue acidosis, and/or the loss of apoptotic potential of cells (Hockel and Vaupel 2001).

4. Molecular signals of hypoxia

Hypoxia-inducible factor (HIF) is a transcription factor that plays a critical a central role in mediating the ability to adapt to low-oxygen concentrations (Wang, Jiang et al. 1995; Semen-

za 1998). One of the primary cellular events in response to the initial exposure to hypoxia is activation of hypoxia-inducible factor 1 (HIF-1), a hetero-dimeric basic helix-loop-helix protein, composed of 2 subunits: HIF-1 α , which is up-regulated in an oxygen-dependent manner, and HIF-1 β , which is constitutively expressed (Semenza 2000; Lee, Bae et al. 2004; Zhu, Zhou et al. 2011). Over-expression of HIF-1 α is seen in many cancer types associated with a poor prognosis, like malignancies of the brain, oropharynx, breast, cervix, ovary, and uterus (Semenza 2003; Lu and Kang 2010).

Hypoxia-inducible factor-2 alpha (HIF-2 α) is another mammalian protein that appears to play a role in the cellular response to hypoxia (Hu, Wang et al. 2003; Semenza 2012). HIF-2 α is structurally similar to HIF-1 α , sharing 48 percent of the overall amino acid identity (Hu, Wang et al. 2003; Ke and Costa 2006). Unlike HIF-1 α , which is ubiquitously expressed, HIF-2 α is mainly expressed in the lung, endothelium, and carotid body (Kaur, Khwaja et al. 2005; Ke and Costa 2006). recently been discovered; its splice variants appear to play a role as negative regulators of HIF-1 (Gu, Moran et al. 1998; Maki-no, Cao et al. 2001; Ke and Costa 2006). Other important transcription factors that are activated by hypoxia include cyclic-AMP-response element-binding protein (CREB), nuclear factor- κ B (NF- κ B), activating protein-1 (AP-1), and metal-responsive transcription factor-1 (MTF-1) (Harris 2002; Vaupel 2004).

4.1. Mechanisms of hypoxia-induced signaling

Under normal oxygen conditions, HIF-1 α is hydroxylated by prolyl hydroxylase (PHD). This hydroxylation leads to the recognition and binding of von Hippel-Lindau (VHL), which is part of an E3 ubiquitin ligase complex that targets HIF-1 α for degradation through a proteasomal pathway (Wheaton and Chandel 2011; Semenza 2012). hypoxic conditions, the hydroxylation of the HIF-1 α subunit by PHD is inhibited and, therefore, HIF-1 α is stabilized, resulting in its translocation to the cell nucleus where it dimerizes with the HIF-1 β subunit and forms the active HIF-1. Activation of HIF-1 subsequently results in the recruitment of transcriptional co-activators and this complex then binds to consensus hypoxia-responsive elements (HREs) within the promoter regions of target genes and initiates the transcription of a variety of hypoxia-responsive genes (Vaupel 2004). These genes can be grouped into 4 functional categories (see Figure), namely, survival/proliferation, metabolism, angiogenesis, and invasion/metastasis (Lu and Kang 2010).

4.2. Effects on cell proliferation and survival

Increased rates of cell proliferation and decreased rates of cell death are 2 of the defining differences between neoplastic cells and normal cells. HIF-1 plays an important role in cellular proliferation. Iyer *et al.* demonstrated that cells deficient in HIF-1 α have reduced rates of cellular proliferation as compared to wild-type cells under both normoxic and hypoxic conditions, with the degree of reduction worsened in hypoxia (Iyer, Kotch et al. 1998; Feldser, Agani et al. 1999). Additionally, several growth factors that contribute to increased cell proliferation are also HIF-1 target genes. insulin-like growth factor-2 (IGF2) and transforming-

growth factor- α (TGF- α) (Feldser, Agani et al. 1999; Krishnamachary, Berg-Dixon et al. 2003). Binding of these factors to their receptors, namely insulin-like growth factor receptor-1 (IGFR1) and epidermal growth-factor receptor (EGFR), respectively, leads to autocrine signaling, resulting in both increased cell proliferation and survival as well as increased expression of HIF-1 (Semenza 2003; Ke and Costa 2006). These autocrine signaling pathways are necessary for cancer progression (Semenza 2003).

4.3. Effects on metabolism

The uptake of glucose by metastatic cancer cells is markedly increased as compared to non-cancer cells and under hypoxic conditions cells switch from the oxygen-dependent metabolic pathway, the tricarboxylic acid (TCA) cycle, to the oxygen-independent pathway, glycolysis (Dang and Semenza 1999; Ke and Costa 2006). HIF-1 plays an active role in this metabolic response to hypoxia by up-regulating the expression of glycolytic enzymes, such as aldolase A, phosphoglycerate kinase 1, and lactate dehydrogenase, that convert glucose to lactate (Vaupel 2004). HIF-1 also induces the over-expression of glucose transporters, namely *GLUT1* and *GLUT3*, that facilitate the uptake of glucose by the cells (Chen, Pore et al. 2001; Ke and Costa 2006; Semenza 2012). While it is well known that HIF-1 stimulates glycolysis, it has also been demonstrated that it can actively repress mitochondrial function and oxygen consumption by inducing pyruvate dehydrogenase kinase 1 (PDK1). Papan-dreou *et al* nicely demonstrated that HIF-1-dependent block of oxygen utilization results in increased oxygen availability and in decreased cell death when total oxygen is limiting (Papan-dreou, Cairns et al. 2006). In a feedback loop, this leads to a shut-down of the formation of mitochondrial acetyl-CoA and oxidative phosphorylation (OXPHOS), which in turn reduces the generation of mitochondrial Reactive Oxygen Species (ROS) and contributes to the generation of lactate that can be used to acidify the microenvironment leading to increase invasion and migration. Additionally, a very elegant study by Guzy *et al.* (2005 Cell metabolism) found that functionality of complex III of the mitochondrial electron transport chain (ETC) is required for the hypoxic stabilization of HIF-1 α and HIF-2 α and that an increase in ROS links this complex to HIF- α stabilization (Guzy, Hoyos et al. 2005). This important study linked an oxygen-dependent pathway to the stabilization of HIF-1. Thus, while HIF-1 controls glycolysis and shuts-down OXPHOS, functional mitochondria play a role in the regulation of HIF-1.

4.4. Effects on angiogenesis

Lee *et. al* demonstrated that inhibition of HIF-1 activity by either anthracycline chemotherapy or acriflavine prevents tumor vascularization in mouse models, thus lending support to the idea that HIF-1 activity is critical in tumor vascularization (Lee, Qian et al. 2009; Lee, Zhang et al. 2009; Semenza 2012). Angiogenesis is a complex process by which the vascular system is formed through growth of new capillaries from pre-existing vessels (Wang, Fei et al. 2004). In addition, although there is great diversity in the factors and signals that contribute to angiogenesis, the chemical signal that appears to play the most critical role in the process is Vascular Endothelial Growth Factor, or VEGF. VEGF is a pro-angiogenic growth

factor that is secreted by many cells, including mesenchymal, stromal, and especially tumor cells. VEGF induces the migration of the endothelial precursor cells to sites of angiogenesis and is also responsible for the proliferation and differentiation of these cells (Ahluwalia and Gladson 2010). (Kaur, Khwaja et al. 2005). Interestingly, HIF-1 activates the transcription of VEGF by binding to the HRE in its promoter region (Forsythe, Jiang et al. 1996; Kaur, Khwaja et al. 2005). Hypoxia also increases VEGF production by stabilizing its mRNA; this effect is mediated by the 3' un-translated region of the mRNA (Onesto, Berra et al. 2004; Kaur, Khwaja et al. 2005). Moreover, HIF-1 up-regulates the expression of additional key molecules that induce angiogenesis, including stromal-derived factor 1 (*SDF1*), placental growth factor (*PGF*), platelet-derived growth factor B (*PDGFB*), and angiopoietin (*ANGPT*) 1 and 2 (Rey and Semenza 2010; Semenza 2012). These pro-angiogenic genes increase vascular density, thus supplying oxygen and nutrients to metabolically demanding tumor cells (Ke and Costa 2006).

4.5. Effects of hypoxia on tumor invasion and motility

Metastasis is a complex process that consists of a series of highly regulated, rate-limiting steps in which tumor cells gain more invasive properties. Metastasis begins with a change in tumor plasticity in a process called epithelial-mesenchymal transition (EMT). During this process there is a loss of epithelial cell characteristics and a gain of mesenchymal gene expression. In short, this process is characterized by the down-regulation of epithelial (E)-cadherin, involved in adherence junctions and endothelial stabilization, and the up-regulation of mesenchymal (N)-cadherin, which allows cells to become more motile (Lu and Kang 2010; van Zijl, Krupitza et al. 2011). the repression of (E)-cadherin (Imai, Horiuchi et al. 2003; Krishnamachary, Zagzag et al. 2006; Yang, Wu et al. 2008; Lu and Kang 2010). Next, tumor cells begin to disrupt the integrity of the basement membrane. This is accomplished via a proteolytic cascade that is put into motion by HIF-1 α -dependent up-regulation of enzymes such as cathepsin D (CTSD), urokinase-type plasminogen-activator receptor (uPAR), and matrix metalloproteinase-2 (MMP2) (Krishnamachary, Berg-Dixon et al. 2003; Lu and Kang 2010). Once the basement membrane is disrupted, tumor cells eventually penetrate the walls of blood vessels in order to become circulating tumor cells (CTCs) in a process known as intravasation (Lu and Kang 2010). (Gupta, Nguyen et al. 2007; Lu and Kang 2010). Hypoxia-induced expression of VEGF also increases the chance of intravasation through the effects of VEGF on increased microvascular permeability and interstitial fluid pressure (Sullivan and Graham 2007; Lu and Kang 2010).

Motility is also an important concept in the process of metastasis. Once the cell-cell junctions have been broken down by the down-regulation of (E)-cadherin, the cells are quickly able to reassemble the actin cytoskeleton into protrusive and invasive structures, such as lamellipodia and filopodia, that help them to migrate through the degraded extracellular matrix (ECM), into the blood vessels, and ultimately to their target location (Fathallah-Shaykh 2005). Actin-Related Protein 2/3 (Arp2/3), which is composed of 2 actin

related proteins and 5 structural subunits, is controlled by the Scar/WAVE complex, also known as the WANP complex, and is essential to the rapid assembly of actin networks that make up lamellipodia (Machesky 2008). Additionally, the up-regulation of the capping protein increases the protrusion activity of invasive tumor cells (Yamaguchi, Wyckoff et al. 2005). The formation of filopodia is controlled by proteins such as fascin, diaphanous, and Mena/VASP (Machesky 2008).

5. Hypoxia and malignant gliomas

5.1. HIF in gliomas

In addition to decreased oxygen tension in brain and GBM tissues, the expression of HIF in gliomas can be affected by the activation of oncogenes, namely epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), and/or the loss of tumor suppressor function through p53 and phosphatase and tensin homolog gene (PTEN) (Brat and Mapstone 2003; Kaur, Khwaja et al. 2005). EGFR affects the expression of HIF by activating a series of pathways, the first of which is the Phosphatidylinositol 3-kinases (PI(3)K) pathway, which ultimately leads to increased HIF-1 α protein levels (Clarke, Smith et al. 2001; Kaur, Khwaja et al. 2005). EGFR amplification found in glioblastoma, as well as among other tumors, is also associated with a poor prognosis (Friedrick, Wang et al. 2000; Kaur, Khwaja et al. 2005). Platelet-derived growth factor (PDGF) has 3 isoforms and 2 receptors and of these, the PDGFA isoform and the PDGFR- α receptor are over-expressed by glial tumor cells (Brat and Mapstone 2003). Furthermore, PDGF is thought to enhance the transcription and secretion of VEGF (Brat and Mapstone 2003). The *PTEN* gene, located on chromosome 10, is a tumor suppressor gene that is mutated in 20 to 40 percent of glioblastoma (Li, Yen et al. 1997; Cantley and Neel 1999; Brat and Mapstone 2003; Kaur, Khwaja et al. 2005). Loss of function of the *PTEN* gene in gliomas is associated with increased HIF-1 α expression as well as tumor vascularization and Zundel *et al.* showed that over-expression of the gene in gliomas is associated with reduction in HIF-1 α expression (Zundel, Schindler et al. 2000; Kaur, Khwaja et al. 2005). The p53 gene, mutated in approximately 50% of gliomas and believed to be the "guardian of the genome," exerts a key role in hypoxia by enhancing the degradation of HIF-1 α , by promoting the interaction of the latter with MDM2, which enhances ubiquitination (Hollstein, Sidransky et al. 1991). Thus, expression of wild-type p53 down-regulates VEGF and therefore leads to inhibition of angiogenesis (Brat and Mapstone 2003; Hunter, Brat et al. 2003; Kaur, Khwaja et al. 2005). Interestingly, HIF-1 α stabilizes p53 by direct physical interaction. p53 activity is lost in gliomas by multiple mechanisms; mutations of p53 are common in gliomas (Brat and Mapstone 2003; Kaur, Khwaja et al. 2005). In addition, the MDM2 protein, which enhances p53 degradation by ubiquitination, is over-expressed in gliomas (Reifenberger, Liu et al. 1993; Brat and Mapstone 2003). Furthermore,

loss of p14^{ARF} function in gliomas leads to MDM2-mediated enhanced degradation of p53 (Nakamura, Watanabe et al. 2001; Brat and Mapstone 2003).

5.2. Hypoxia, angiogenesis, and GBM

GBM are highly cellular astrocytic neoplasms; they are histologically characterized by an area of central necrosis surrounded by a highly cellular rim of viable tumor (Brat and Mapstone 2003; Giese, Bjerkvig et al. 2003). GBM also display areas of microvascular hyperplasia as well as areas of necrosis with the appearance of tumor cells pseudo-palisading around the necrotic centers. The areas of microvascular hyperplasia are examples of accelerated angiogenesis and are thought to influence the biological behavior of malignant gliomas by favoring aggressive neoplastic growth. angiogenesis in the surrounding hypoxic areas. In fact, the VEGF concentrations in glioblastoma are 200 to 300 times greater than serum concentrations (Takano, Yoshii et al. 1996; Brat and Mapstone 2003). The pseudo-palisading cells have also been shown to over-express HIF-1 and also to secrete other pro-angiogenic factors in addition to VEGF, such as interleukin 8 (IL-8) (Rong, Durden et al. 2006; Onishi, Ichikawa et al. 2011). It is also important to note that newly formed blood vessels in malignant gliomas generally have increased diameters, are highly permeable, have thickened basement membranes, and have highly proliferative endothelial cells (Lopes 2003; Onishi, Ichikawa et al. 2011).

5.3. Hypoxia and invasiveness of GBM

As discussed earlier, cancer cell migration and invasion consists of a series of highly coordinated steps including dissociation of cellular adhesions, remodeling of the actin cytoskeleton, proteolytic degradation of the ECM, and formation of new adhesions. Brain invasion is a characteristic feature of malignant gliomas. In vivo studies of glioblastoma have shown that tumor hypoxia results in increased cell migration (Plasswilm, Tannapfel et al. 2000). Once again, the pseudopalisading cells found around the necrotic cores in GBM, which up-regulate HIF-1, appear to be hypoxic and migrating away from the necrotic core (Elstner, Holtkamp et al. 2007; Onishi, Ichikawa et al. 2011). discovery has revealed that the protein MINK, which inactivates ADF/cofilin by phosphorylation, is up-regulated in glioma cells as compared to normal brain cells (Fathallah-Shaykh 2005). Additionally, under hypoxic conditions, glioblastoma induce the expression of c-Met, which stimulates gliomas cells to secrete urokinase plasminogen activator (uPA), which converts circulating plasminogen into plasmin. The latter helps promote invasion by degrading ECM proteins as well as by activating matrix metalloproteins, including MMP2, up-regulated in glioma cells (Fathallah-Shaykh 2005; Martens, Schmidt et al. 2006; Eckerich, Zapf et al. 2007; Onishi, Ichikawa et al. 2011).

5.4. Hypoxia and cell survival in GBM

Microarray analysis of cultured glioma cells revealed over-expression of molecules that protect them from reactive oxygen species (ROS) and endoplasmic reticulum (ER)-induced apoptosis (Fathallah-Shaykh 2005). ROS, generated by the rapid multiplication of cancer

cells, cause a release of cytochrome c leading to apoptosis (Filomeni, Aquilano et al. 2003; Petrosillo, Ruggiero et al. 2003; Fathallah-Shaykh 2005). Glioma cells appear to acquire protective mechanisms against ROS-induced apoptosis. First, by up-regulating protective enzymes such as *AKR1A1* and *AKR1C1*, which belong to the aldo-keto reductase enzyme super-family (Sanli and Blaber 2001; Fathallah-Shaykh 2005). Second, by up-regulating several antioxidants, some of which include *PDG*, *TALDO1*, *AFG3L1*, *ANT2*, *GSTP1*, and *PRDX1* (Berggren, Husbeck et al. 2001; Nonn, Berggren et al. 2003; Fathallah-Shaykh 2005), nuclear factor κ B (NF- κ B) has been reported to be constitutively activated in cultured glioma cells and GBM surgical samples (Bharti and Aggarwal 2002; Robe, Bentires-Alj et al. 2004; Wang, Zhang et al. 2004; Fathallah-Shaykh 2005).

The ER, one of the cells largest organelles, is responsible for folding, glycosylating, and sorting proteins to their correct destinations, as well as for synthesizing lipids and cholesterol components of the cell membrane. When homeostasis is disrupted in the ER, mis-folded and un-folded proteins can accumulate with the ER lumen, resulting in *ER stress*, which shuts off protein synthesis leading to apoptosis. This response is beneficial to the cell as a safeguard against carcinogenesis because mis-folded proteins may acquire aberrant functions. On the other hand, bypassing this response is an important milestone in the development of cancer. Glioma cells appear to acquire pathways that help them recover from *ER stress* and avoid apoptosis. A normal ER stress-induced response ultimately leads to inactivation of the eukaryotic initiation factor 2 (eIF-2 α), which shuts off protein synthesis. By up-regulating activating transcription factor 4 (ATF4), malignant glioma cells appear to bypass the ER stress-induced protein synthesis inhibition. ATF4 induces the growth arrest and DNA damage-inducible protein (GADD34), which dephosphorylates eIF2 α , causing protein synthesis recovery (Connor, Weiser et al. 2001; Novoa, Zeng et al. 2001; Brush, Weiser et al. 2003; Fathallah-Shaykh 2005).

6. Linking hypoxia, invasion, and angiogenesis

6.1. Hypoxia, invasion, and angiogenesis in malignant gliomas

Hypoxia, invasion, and angiogenesis are interrelated through a variety of molecular pathways and feedback loops. divide and increase in number, they often out-grow their diffusion-limited oxygen supplies and hypoxia results. Once the tumor micro-environment becomes hypoxic, cellular and molecular signaling pathways are initiated in order to promote overall tumor survival. These adaptive responses include: 1) increasing cellular resistance to hypoxia, 2) favoring metabolic pathways that are optimal in hypoxia, 3) increasing motility and invasive properties in order to seek new oxygen supplies, and 4) initiating angiogenesis in order to create a new vascular supply, or a combination of these responses. (Shimizu, Eguchi et al. 1995). Therefore, there is evidence that local oxygen concentrations modulate a switch between proliferative phenotype and an invasive in GBM cells; this is

called the “Go or Grow” mechanism (Giese, Kluwe et al. 1996; Giese, Loo et al. 1996; Hatzi-kirou, Basanta et al. 2012).

When considering angiogenesis as the adaptive mechanism in glial cells, it is necessary to evaluate the relationship between the rate of angiogenesis and the rate of cellular division. If cellular division proceeds at a rate faster than angiogenesis, then oxygen supply cannot meet metabolic demands causing not only local necrosis, but also dissemination of tumor cells by enhanced brain invasion. On the other hand, if angiogenesis proceeds at a rate faster than cellular division then oxygen supplies are sufficient to meet metabolic demands and no necrosis occurs, which is characteristic of anaplastic astrocytoma.

6.2. Anti-angiogenic agents as treatment for malignant gliomas

Both prospective and retrospective clinical trials have shown that Bevacizumab, an anti-angiogenic agent, is effective as a single-agent against recurrent glioblastoma as compared to historical controls of salvage chemotherapy (Friedman, Prados et al. 2009; Kreisl, Kim et al. 2009; Raizer, Grimm et al. 2010). The Roche-sponsored AVAglio international multicenter phase III clinical trial, comparing standard of care to standard of care plus Bevacizumab for the treatment of newly-diagnosed GBM, is completed; the results will be presented at The Society for Neuro-Oncology 2012 meeting. It is noteworthy that Bevacizumab has a positive significant impact on progression-free survival times (Norden, Drappatz et al. 2009; van den Bent, Vogelbaum et al. 2009; Keunen, Johansson et al. 2011).

Interestingly, *in vivo* GBM models have shown that anti-angiogenic agents enhance tumor cell migration/invasion. Keunen and colleagues studied a human GBM xenograft model, derived from patient tumor spheroids, in rats. The results revealed that Bevacizumab induces vascular remodeling that leads to increased tumor cell invasion into normal brain parenchyma, associated with a hypoxic tumor microenvironment and a glycolytic metabolism (Keunen, Johansson et al. 2011). Plasswilm *et al.* implanted cultured glioma cells in a chicken embryo model and showed that local hypoxia promotes tumor spread and the development of secondary tumor cell bulks (Plasswilm, Tannapfel et al. 2000).

6.3. Implications for treatment of malignant gliomas

Treatment with Bevacizumab also has important implications for how tumor progression is defined. Traditionally, tumor progression has been measured according to the MacDonald criteria which define progression by increase in contrast enhancement. However, as a result of blood-brain barrier stabilization secondary to anti-angiogenic agents, contrast enhancement is no longer a reliable measure of progression. Therefore, the Response Assessment in Neuro-Oncology (RANO) Working Group developed the RANO criteria as a means to address potential progression by non-enhancing T2/FLAIR (fluid attenuated inversion recovery) lesions as well as by increases in contrast enhancement in malignant gliomas (Province, Han et al. 2011). The use of anti-angiogenic agents, namely Bevacizumab, has also raised many questions about patterns of recurrence in malignant gliomas. Additionally, although there is some conflicting data on this subject secondary to poor study design and low study

power, there is evidence that patients who are treated with Bevacizumab exhibit more diffuse or distant recurrence patterns as compared to those who do not receive Bevacizumab. Once again, the possibility of more diffuse and distant recurrence patterns may be explained by the “Go or Grow” mechanism, as explained above, with further supporting evidence derived from the glioblastoma xenograft model, also detailed above (Keunen, Johansson et al. 2011; Hatzikirou, Basanta et al. 2012).

Hypoxia-mediated enhancement of brain invasion has profound biological and clinical implications on GBM, in particular in the context of treatment with anti-angiogenic drugs. Recall that necrosis is a hallmark of GBM; thus, one can safely assume that, by the time of the initial clinical presentation, GBM cells have already invaded the surrounding brain parenchyma because of local hypoxia. This feature of GBM may limit the effectiveness of local therapeutic options, like radiation therapy and surgical resection. Furthermore, one could argue that, though anti-angiogenic drugs prolong progression free survival times, they may create a more aggressive and invasive tumor by hypoxia-mediated enhancement of motility, which delays clinical progression. These patients may experience a so-called “honeymoon period,” in which they may appear stable; however, once they recur, the rate of progression of Bevacizumab-treated tumors may be very acute secondary to the large volume of invaded brain. This rationale may explain the limited effects of Bevacizumab on overall survival times.

7. Future directions

The Figure depicts the effects of HIF-1 α on key cellular functions. Though some of the signaling molecules are known, we seem to be only looking at the tip of the iceberg. More research is needed to fit the pieces of the puzzle. What other molecules are activated by hypoxia to influence the 4 phenotypes shown in the Figure? How do they relate to HIF-1? Are there feedback loops between the phenotypes? That is, does motility affect metabolism and vice versa? We have presented a succinct view of the molecular basis of the four phenotypes; however, a detailed analysis is not within the scope of this chapter.

Although preclinical studies have revealed a molecular link between hypoxia, angiogenesis, and invasion, the clinical implications of these relationships are still not well known and future studies are needed. Drawing from what has already been established regarding the effects of anti-angiogenic agents on increased migration and invasion in malignant gliomas, a plausible direction for future therapeutic development would include blocking not only angiogenesis but also invasion and migration. motility agents in malignant GBM will result in significant improvement in PFS as well as overall survival times by blocking two of the primary mechanisms for their survival.

8. Conclusion

From this chapter, it is our hope that the reader has gained an understanding of the extensive role of hypoxia in brain tumors, with specific regard to its role in angiogenesis, invasion, metabolism, and overall survival of the tumor. Furthermore, although the use of anti-angiogenic agents as therapeutic options for malignant gliomas has provided some promising results, it has also revealed the need for further molecular research into the mechanisms for invasion of malignant gliomas, especially as it relates to the hypoxic environments created by anti-angiogenic drugs. Hopefully, this combination of efforts to decrease both angiogenesis and invasion will result in the development of more promising therapeutic agents to treat this disease.

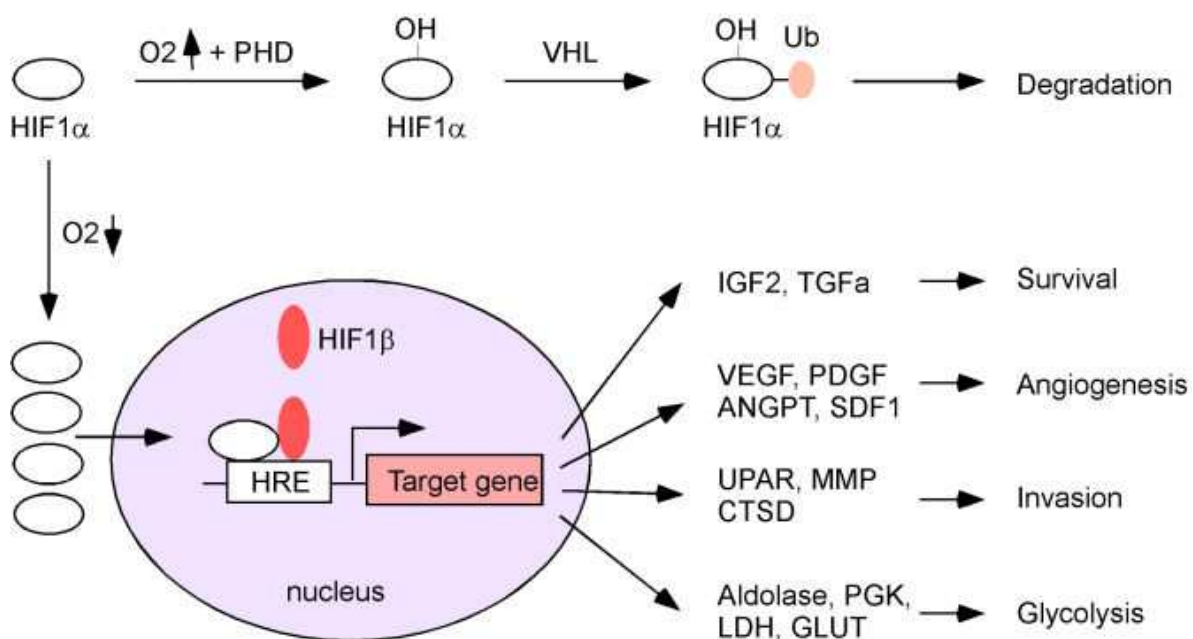


Figure 1. Cartoon illustrating signaling and degradation of HIF-1 α in hypoxia. Under normal oxygen supply, one of the two proline residues (Pro-402, Pto-564) in HIF-1 α is hydroxylated by prolyl hydroxylase. Once hydroxylated HIF-1 α is recognized by VHL, an E3 ubiquitin (Ub) ligase, which targets HIF-1 α for degradation through the proteasomal pathway. In hypoxic conditions, hydroxylation is inhibited causing the accumulation of HIF-1 α , which translocates to nucleus where it associates with HIF-1 β then binds to the HIF responsive Element (HRE) of target genes activating their transcription. The HIF targeting genes modulate cell survival, metabolism, angiogenesis, and tumor invasion. PGK: phosphoglycerate kinase, LDH: lactate dehydrogenase.

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