Minireview

Hypoxia helps Glioma to fight Therapy

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Abstract:

Despite major improvements in the surgical management the prognosis for patients bearing malignant gliomas is still dismal. Malignant gliomas are notoriously resistant to treatment and the survival time of patients is between 3-8 years for low-grade and anaplastic gliomas and 6 - 12 month for glioblastoma. Increasing malignancy of gliomas correlates with an increase in cellularity and a poorly organized tumour vasculature leading to insufficient blood supply, hypoxic areas and ultimately to the formation of necrosis, a characteristic of glioblastoma. Hypoxic/necrotic tumours are more resistant to chemotherapy and radiation. Hypoxia induces either directly or indirectly (through the activation of transcription factors) changes in the biology of a tumour and its microenvironment leading to increased aggressiveness and tumour resistance to chemotherapy and radiation. This review is focused on hypoxia-induced molecular changes affecting glioma biology and therapy.

INTRODUCTION

Hypoxia in solid tumours

Evidence from experimental and clinical studies points to a significant role of hypoxia in solid tumours. Tumour hypoxia seems to be strongly associated with tumour propagation, malignant progression, and resistance to therapy. Hypoxia is probably the fundamental cause for the aggressive course of the disease in many patients and for the failure of conventional non-surgical treatment. Tumour growth requires the presence of a local vascular network supplying both oxygen and nutrients to the tumour cells. The diffusion limit for oxygen within tissue is around between 100 and 200μm [1, 2]. Characteristically, a highly proliferating tumour mass grows faster than the vasculature resulting in an avascular environment deficient of oxygen resulting in hypoxic conditions. Tumour hypoxia is defined as a result of insufficient oxygen (O_2) supply, which compromises biological functions [3]. Critical O_2 levels (hypoxic threshold) characterize the upper limit of a hypoxic range below which activities and functions progressively become restricted. These O_2 levels can span from 45-50mm in end-capillary blood to 0.02mmHg O_2 partial pressure in cytochromes (below this level cytochromes are no longer fully oxidized) [4]. Hypoxia and/or alterations in oncogene or tumour suppressor gene function can cause an 'Angiogenic Switch', a prominent feature in pathologic conditions like chronic inflammation and cancer, which results in the formation of new blood vessels. This newly formed microcirculation, however, is structurally and functionally disturbed and diffusion conditions are deteriorating, resulting in more severe hypoxia. Molecular signals triggered by this angiogenic switch include Hypoxia-Inducible factor 1 (HIF-1), Nuclear Factor-κB (NFκB) and AP-1. However, NFκB was found to be constitutively expressed in glioblastoma [5] and both NFκB andAP-1 are not activated by hypoxia in glioma [6] This paradox between hypoxia and increased microvascular density arises because the tumour vasculature is structurally and functionally abnormal.

Glioma

Gliomas are primary brain tumours, which are thought to derive from glial cells or their progenitors; in contrast to secondary brain tumours, which represent metastatic neoplasms derived from peripheral tumours e.g. in the breast or the lung. According to the grading system of the World Health Organization (WHO) gliomas are divided into grade I and II (low grade), grade III (anaplastic) and grade IV, which includes the most malignant neoplasm with an astrocytic phenotype, glioblastoma multiforme (GBM) [7]. GBM is the most common form of malignant glioma and is characterized by widespread invasiveness, tumour necrosis, and angiogenesis [8]. They account for 40% of all human primary central nervous system (CNS) tumours and mainly affect people aged between 45–70 years [9]. These neoplasms infiltrate normal brain parenchyma, destroy its function, and respond poorly to therapy. Even after receiving the most advanced treatment, including neurosurgery, radiotherapy, and chemotherapy, the mean survival is only 60 weeks [10]. Astrocytomas grade II and III, although being ultimately fatal, have a much lower growth rate and the survival time ranges from 3 to 8 years [11, 12]. Increasing malignancy of gliomas correlates with an increase in cellularity and tumour vasculature. Pathological examination of grade II astrocytomas characterizes them as highly infiltrative, non-angiogenic tumours that have the ability to co-opt blood vessels from the existing vasculature. Progression to grade III astrocytoma reveals a mild increase in vascular density, whereas, in glioblastoma (grade IV) a profound alteration in the biology of the tumour vasculature is noted [13]. GBMs are characterized by an accumulation of lactic and carbonic acid ('Acidosis', see below) and a usually poorly organized tumour vasculature resulting in an insufficient blood supply and hypoxic areas. Based on measurements of tissue hypoxia using the 2-nitroimidazol agent EF5, the percentage of tumour tissue pO_2 in glioma patients was calculated. Using a five-tier system ranging from physiological condition (pO_2 = 10%) to anoxia (pO_2 = 0%), low-grade astrocytomas (WHO grade 2) were characterized by modest cellular hypoxia ($pO_2 \geq 2.5$ %), grade 3 tumours by modest-to-moderate hypoxia (pO_2 = 0.5-2.5%) and most GBMs showed severe hypoxia ($pO_2 \leq 0.1$%) [14]. The presence

of more severe hypoxia in the primary lesion of gliomas correlated with an early, mostly local recurrence of the tumour, which have an even higher hypoxic fraction than the respective primary tumour [15].

Increased hypoxia ultimately leads to the formation of necrotic areas, which is one of the main characteristics of GBM. Necrosis can appear as a large central area (up to 80% of the total tumour mass in GBMs) or as pseudopalisades, which are multiple, small necrotic foci surrounded by densely packed small, fusiform glioma cells [16]. These densely packed tumour cells surrounding a necrotic area are not a result of hyper-proliferation or resistance to apoptosis, but represent hypoxic astrocytoma cells with enhanced MMP-2 and MMP-9 activity and a low proliferation rate, which have migrated away from a hypoxic/anoxic focus triggered by intravascular thrombosis [17, 18]. In a large histological analysis intravascular thrombosis was found in 92% of all primary resections of GBMs (88 cases), whereas it was uncommon in anaplastic astrocytomas (grade III) [19]. One of the strongest pro-thrombotic proteins is tissue factor, which is highly upregulated in malignant astrocytomas and its expression is correlated with tumour grade [20-22].

Although hypoxia is toxic for both tumour cells and normal cells, tumour cells undergo genetic and adaptive changes that allow them to survive and even proliferate in a hypoxic environment.

Hypoxia and Resistance

There are a number of adverse effects of hypoxia on the treatment of solid tumours, which can be direct (e.g. by decreasing the amount of oxygen -free radicals, which are necessary for an effective radiotherapy [23]) or indirect, through hypoxia-induced biological effects, like the activation of the transcription factor, Hypoxia Inducible Factor -1 (HIF-1), which regulates over 40 target genes. Hypoxia-induced effects include selection for more malignant and invasive neoplastic cells [17, 24-27], an increase in genomic instability, as well as a further enhancement of tumour progression and aggressiveness on the one hand and a reduction of sensitivity to radiotherapy and chemotherapy on the other [28-31]. A schematic representation of the relationship between hypoxia and resistance is

given in Figure 1. The direct and indirect effects of hypoxia in glioma are discussed in the following paragraphs.

DIRECT EFFECTS OF HYPOXIA

The oxygen enhancement effect:

Radiation therapy continues to be critical to the treatment of gliomas. The goal of ongoing research is to improve therapeutic efficacy while reducing toxicity. However, tumour hypoxia presents a severe problem for radiation therapy, because radiosensitivity is progressively limited when the partial oxygen pressure in a tumour is low. Under normoxic conditions radiation reacts with intracellular water in the presence of molecular oxygen resulting in the formation of free oxygen radicals, which cause severe DNA damage (oxygen enhancement effect), which cannot be repaired easily. In the absence of oxygen the amount of radiation needed to achieve the same results is three times higher than under normoxic conditions and DNA damage can be repaired much faster [23, 32].

Reactive oxygen and nitrogen

Under hypoxic conditions and after radiation high amounts of nitric oxide (NO) and superoxide ($O_2^{\cdot -}$) radicals are found in the tissue [33]; they are implicated in a wide variety of pathophysiological processes, e.g. vasodilatation and increased blood flow, increased vascular permeability, free radical injury to tumour cells, endothelial cells, and surrounding normal tissue. Matsumoto et al. demonstrated that radiation-induced NO radicals contributed to the induction of radioresistance *in vitro*, which could be prevented in the presence of inhibitors of NOS II (iNOS) or a scavenger of NO radicals [34].

In a series of experiments Cobbs et al. demonstrated the effects of nitric oxide and superoxide on the tumour suppressor protein p53 and the likely consequences for glioma progression [35-37]. Malignant CNS tumours express high levels of nitric oxide synthases (NOS), which regulate the

production of nitric oxide (NO). There are three isoforms, the constitutively expressed NOS I, and the inducible forms NOS II (also called iNOS) and NOS III; the latter represents a macrophage isoform, which is expressed at low level in tumour endothelial cells. NOS I and II expression and subsequently NO production are correlated with the grade of malignancy in gliomas, being highest in glioblastoma; whereas NOS reactivity and NO production in normal brain tissue are very low. Under hypoxic conditions NO reacts with superoxide forming the highly reactive compound peroxynitrite ($ONOO^-$) [38], which reacts with tyrosine residues of target proteins, e.g. c-Src or p53. The tumour suppressor protein p53 is a critical regulator of tumour progression. Although only 30% of glioblastomas show p53 mutations, most of the malignant gliomas show evidence of functional inactivation despite a positive immunoreaction to p53 antibodies. Cobbs et al. showed that physiological concentrations of peroxynitrite resulted in tyrosine nitration of p53 protein and loss of p53-specific DNA binding leading to functional inactivity of the p53 protein and survival of the tumour cell [35-37]. This mechanism demonstrates a hypoxia-induced proteome change leading to an increase of cells with diminished apoptotic potential, which has a substantial effect on radioresistance.

Treatment option: Replacement of the mutated *p53*-gene with the wild-type gene or over-expression of the wild-type gene was considered an effective treatment option and showed some anti-tumour effects on malignant glioma cells [39, 40]. However, one difficulty with this approach is that tumour cells with wild-type *p53* are highly resistant to *p53* gene transfer [41, 42] and it became apparent in a clinical study that it is difficult to eradicate malignant glioma by means of *p53* over-expression alone [41, 42]. Nashimoto et al. suggested a combined treatment with adenoviral p53 overexpression and mild hyperthermia based on the anti-proliferative effect they found in malignant glioma cells *in vitro* [43].

Another possibility was recently presented by Janjetovic et al. They reported a synergistic effect of NO (released from sodium nitroprusside, SNP) together with hyperthermia (43°C) on glioma cell death *in vitro*. SNP and hyperthermia caused oxidative stress and mitochondrial depolarization

leading to necrosis on one hand and to caspase-mediated apoptosis on the other [44]. Recent clinical trials using hyperthermia alone in recurrent glioma showed that this treatment was well tolerated and produced promising results [45, 46]

INDIRECT EFFECTS OF HYPOXIA

Hypoxia can influence the behaviour of tumour cells through the activation of transcription factors, e.g. hypoxia induced factor 1 (HIF-1) and early growth response gene 1 (egr1) resulting in expression changes of a large number of target genes, some of them affecting particularly glioma growth and are discussed below.

Hypoxia Induced Factor-1 (HIF-1)

The transcription factor HIF-1 is a major regulator of tumour cell adaptation to hypoxic stress leading to radio- and/or chemoresistance. The expression of HIF-1 is induced mainly by hypoxia and/or overactivity of signalling pathways, e.g. PI3K/AKT/mTOR or Ras/MEK/ERK. HIF-1 controls the expression of over 40 target genes by binding to hypoxia-responsive elements. HIF-1 plays a crucial role in many processes, including oxygen homeostasis, glucose metabolism, cell survival, invasion, homing and angiogenesis [47-49], which have been shown to influence radio-responsiveness (reviewed by [50]).

HIF-1 is a heterodimer composed of an α subunit and a constitutively expressed β subunit, which are basic helix-loop-helix-PAS (bHLH-PAS) domain proteins. While the HIF-1β subunit can dimerize with several different bHLH-PAS transcription factors, HIF-1α is unique to HIF-1. Under aerobic conditions HIF-1α is constantly ubiquinated and degraded by proteasomes, hence its low expression level in most cells [48]. Details about the mechanisms regulating the stabilisation and the activation of HIF-1α have been extensively reviewed [49, 51-54].

Although the most powerful inducer of HIF-1 stabilization is hypoxia, there are oncogenes and suppressor genes, which can control HIF-1α expression even under normoxic conditions; e.g. *in*

vitro experiments demonstrated that loss of function of the tumour suppressor gene *PTEN* can block the degradation of HIF-1α allowing the stabilisation of the HIF-1 heterodimer. Restoration of wt-PTEN to glioblastoma cells lacking functional PTEN markedly increased HIF-1α expression and the induction of HIF-1α-regulated genes [55]. Loss of PTEN is a characteristic in the malignant progression from low to high grade gliomas and can, therefore, contribute to tumour expansion through HIF-1 induced gene expression.

Within gliomas the highest protein expression of HIF-1α was found in glioblastomas (GBM); in particular in hypoxic cells forming pseudopalisades around regions of frank necrosis and in invading cells [17, 18]. Most grade 3 astrocytomas showed strong staining for HIF-1α, as well; whereas in low grade gliomas (grade 2) HIF-1α immunoreactivity was scant [56].

HIF-1 and Radiotherapy

Radiotherapy is usually followed by a reoxygenation process in the tissue. Contrary to the assumption that reoxygenation would decrease HIF-1 levels, Moeller et al. found an increase in HIF-1 protein level following radiation [33, 57]. They identified two mechanisms for this phenomenon: In the presence of hypoxia (before radiation) the number of stress granules, which are protein-mRNA complexes, increase, leading to a blockage of the translation of HIF-1 mediated mRNAs into target proteins. During radiation these complexes disaggregate leading to a burst of HIF-1 regulated proteins. The second mechanism is based on the excessive formation of free radical species shortly after radiation, which results in an up-regulation of HIF-1 activity.

Despite a full course of radiotherapy, up to 90% of all glioblastomas relapse in close proximity to the resection cavity, an area, which is called the invasive edge characterized by high expression of HIF-1 in tumour cells [17, 18]. Wild-Bode et al. found that sublethal irradiation caused enhanced $\alpha_v\beta_3$ integrin expression and enhanced matrix metalloproteinase 2 and 9 activity. These up-regulations led to an increase in migration and invasion of tumour cells and to the recurrence of the tumour [58].

Hypoxia and irradiation also cause MMP-9 activation and increased secretion of VEGF and KitL from mast cells leading to tissue revascularization and mobilization of stem cells from their niche [59].

Treatment option: There is a growing number of HIF-1 inhibitors available, which had been evaluated for the usefulness as anticancer agents by G. Smenza [60], however, the complexity of the HIF-1 transcriptome makes it difficult to predict the effects of a certain HIF-1 inhibitor in a particular organism. E.g. Although HIF-1 induces expression of many genes that promote tumour cell survival, it also can induce the expression of genes that promote growth arrest [61] or tumour cell death [62].

Consequences of HIF-1 activation following radiation therapy are complex involving both tumour and endothelial cells. Some of these consequences are discussed in the following paragraphs.

HIF-1 Induction of VEGF

Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis and a highly specific mitogen and survival factor for endothelial cells [63-66]. Hypoxic pseudopalisading tumour cells express high levels of VEGF, which is mediated either through the HIF-1 or through an activated PI3 Kinase pathway as a result of receptor tyrosine kinase activation (e.g. EGFR) or through mutation or loss of *PTEN* [67, 68]. Therefore, the upregulation of VEGF expression in highly vascularized glioblastomas is probably the result of the accumulation of genetic alterations and hypoxia [12].

High expression levels of both HIF-1 and VEGF are found in glioma cell lines and throughout glial tumours suggesting that the expression is not only a physiological response to hypoxia but may represent a pathological up-regulation of these proteins in higher-grade brain tumours [69]. Ueda et al. showed that conditioned medium from hypoxic U251 glioma cell cultures blocked microvascular endothelial cell death induced by serum starvation. They found that VEGF, expressed by hypoxic

glioma cells, acted as survival factor activating nuclear factor kappa B (NFκB) expression in the endothelial cells, resulting in the expression of the antiapoptotic genes Bcl-2, Bcl-X_L, survivin and XIAP (X-chromosome linked inhibitor of apoptosis protein) [70]. A similar survival effect has been shown with conditioned medium from U87 [71] and C6 glioma cell cultures [72].

Although these findings have not yet been proven *in vivo*, it allows the hypothesis that tumour cells, under hypoxic conditions, enhance the survival of microvascular endothelial cells, which form new blood vessels (angiogenesis) to deliver oxygen and nutrients to the tumour cells.

Treatment option: Anti-angiogenic treatment approaches have long been developed for a variety of solid tumours, but only recently showed some success in the treatment of malignant glioma. The latest developments in the anti-angiogenic therapy for malignant gliomas has been comprehensively reviewed by Reardon et al. [73]. A phase II clinical trial using bevacizumab, a VEGF-A antibody, together with the cytotoxic drug irinotecan (CPT-11) showed promising results with moderate toxicities in recurrent glioma [74, 75].

HIF-1 Induction of Tie2 Expression

In addition to VEGF blood vessel formation involves Tie2 receptor and its ligands angiopoietin 1 and 2 (Ang-1, -2). Ang-1, the primary ligand of the Tie2 receptor plays a role in the formation of tight junctions at the blood brain barrier [76] and in providing vessel stability complimenting the effects of VEGF [77, 78]. In gliomas, Ang-1 mRNA expression was found in tumour cells [79] and is not up-regulated by hypoxia [80]. The second ligand Ang-2 is a natural antagonist to Ang-1, which binds with similar affinity to Tie2, but blocks receptor activity. Ang-2 mRNA is expressed on tumour endothelial cells at sites of vascular remodelling, where it seems to block the stabilizing action of Ang-1 [78, 79, 81].

Tie2 expression was found in vascular endothelial cells and is upregulated in tumour endothelium in glioma [79]. Recently, Tie2 was also detected on Tie2 expressing monocytes (TEMs) [82, 83], which seem to play a crucial role in tumour angiogenensis. Lewis et al. [84] have comprehensively

reviewed the function and regulation of TEMs. TEMs have been identified in a variety of human tumours where they represented a small subset of tumour-infiltrating leukocytes, which are found largely in perivascular and avascular, viable areas of human tumours, but are very rare in non-neoplastic tissues adjacent to tumours [82, 83]. Human TEMs can be distinguished from tumour associated macrophages (TAMs) by the presence of specific surface antigens Tie2, CD45, CD33, CD13, CD11b, and CD11c. TEMs express inherent pro-angiogenic activity by supporting the sprouting of blood vessels in a paracrine manner during the angiogenic process [84].

In the orthotopic mouse glioma model the tumour initially grows by co-opting the pre-existent vasculature; however, the co-opted blood vessels eventually regress, leading to a secondarily avascular tumour and tumour cell loss [81]. The remaining tumour is rescued by *de novo* vessel formation primarily at the tumour periphery. In the same glioma model, De Palma and co-workers showed that TEMs are recruited to the angiogenic tumour site and promote the formation of new blood vessels by, e.g. production of high levels of the pro-angiogenic factor basic fibroblast growth factor (bFGF) [83]. Elimination of TEMs completely prevented the angiogenic phase of human gliomas grafted in the mouse brain and caused widespread tumour necrosis and regression.

In the hypoxic tumour microenvironment Tie2 expression on TEMs is up-regulated, possibly making the monocytes more responsive to Ang-2 in these angiogenic areas [85]. Ang-2, under hypoxic conditions has several effects on TEMs through the activation of the Tie2 receptor: It can stimulate TEM migration [82, 85] and modulate their cytokine secretion. For example, the down-regulation of tumour necrosis factor-α (TNF-α) secretion by TEMs resulted in enhanced tumour and endothelial cell survival, thereby promoting metastasis and angiogenesis. Furthermore, Ang-2 inhibits the expression of the antiangiogenic cytokine interleukin-12 by TEMs [85] resulting in the down-regulation of the anti-angiogenic activities of tumour-infiltrating macrophages.

HIF-1 Induced Autophagy

Differential action of the HIF-1 subunits can cause gene-profile selection and promote cell proliferation or death. HIF-1 mediated expression of BNIP3 (Bcl-2/adenovirus EIB 19kDa-interacting protein 3) and BNIP3L (BNIP3-like), members of the Bcl-2 family of cell death factors, is upregulated during hypoxia and causing autophagy. Whether this autophagic response has a pro-apoptotic or a pro-survival effect on cells is still under discussion. Azad et al. found an autophagic response to hypoxia (<1% O2) followed by cell death in two glioma, two breast cancer and one embryonic cell line. Hypoxia-induced cell death was reduced in the presence of the autophagy inhibitor 3-methyladenine, but not in the presence of the caspase inhibitor z-VAD-fmk [86]. On the other hand, Pouyssegur and his group propose a pro-survival effect through hypoxia-induced autophagy [49, 51, 87]. Autophagy allows cells to recycle intracellular organelles for nutritional purposes [88]. Nutrient-depleted cells, e.g. hypoxic cells, have a supply of lipids, amino acids and sugar through catabolism of organelle components e.g. from ribosomes and mitochondria, and therefore, a survival benefit. Komata et al., however, published that hyperthermia-induced autophagy caused G_2M cell cycle arrest, rather than induction of cell death [89], which explains the moderate anti-glioma effect of hyperthermia on cell lines.

Treatment option: While most of the results mentioned above were generated in *in vitro* settings, hyperthermia has shown promising results in recent clinical trials on recurrent glioma [45, 46] and might be enhanced by concurrent application of a NO-releasing agent [44].

HIF-1 Regulated Metabolism

Beside being hypoxic the tumour micro-environment of tumours is usually highly acidic due to the Warburg effect (Acidosis). The energy production of normal cells includes the metabolism of pyruvate through oxidative phosphorylation in the mitochondria. Cancer cells, however, use

predominantly anaerobic glycolysis for energy production. This shift seems to be regulated by HIF-1 in inducing glycolytic enzymes, which metabolise pyruvate to lactate in the cytoplasm [90]. Although, this yields less adenosine triphosphate (ATP) per molecule of glucose, it is compensated by a HIF-1 mediated increase in expression of glucose transporters (Glu-1, Glu-2) and glycolytic enzymes leading to increased uptake of glucose and rate in glycolysis. The accumulation of lactate leads to a lowered intra-cellular pH, which has been associated with metastasis, proliferation and drug resistance [91]. The same authors also showed that acidification of the tumour micro-environment is attributed to the accumulation not only of lactic acid, but also of CO_2, suggesting a possible contribution of carbonic anhydrase 9 (CA9)**.** CA9 is induced by hypoxia and its expression is correlated with malignancy in gliomas [92-95]. There is evidence that CA9 is involved in the regulation of both extracellular and intracellular pH [96, 97] suggesting that CA9 activity is needed for the survival of hypoxic cells and that its inhibition may represent a promising therapeutic approach.

An increased rate of glycolysis also evokes an upregulation of Aquaporin-1 (AQP1) expression [98]. Aquaporins are a family of water-selective transmembrane transport channels that allow rapid movement of H_2O along osmotic gradients across the hydrophobic cell membrane. AQP1 and AQP4 protein, the most studied of aquaporins, are highly expressed in glioma patient biopsies [99] and in reactive astrocytes in peritumoral tissue [98, 100]. AQP1 seems to be involved in growth and migration, whereas AQP4 enhances cell adhesion. Despite their different biological roles both proteins are considered as possible contributors to brain edema, which is a characteristic feature of malignant gliomas.

Additionally, Fukuda et al. reported that HIF-1 optimizes low levels of respiration in mitochondria by regulating the ratio of isoforms of cytochrome c oxidase, which represent components of the electron transport chain [101]. Thus, HIF-1 induced adaptive responses ensure that the energy requirements of the tumour cells are met.

HIF-1 and Chemoresistance

Hypoxic cells seem to be more resistant to chemotherapy than oxygenated tumour cells [102]. Contributing factors for this might be:

a) a diffusion barrier due to the distance between drug supplying blood vessels and tumour cells;

b) the requirement of a majority of chemotherapeutic for rapidly proliferating cells to induce cytotoxicity. The growth rate of hypoxic cells, however, is slower than that of well oxygenized tumour cells; therefore, the anti-tumour effect of cytotoxic drugs is diminished in a hypoxic environment [103];

c) the hypoxia-induced expression of ABC (ATP binding cassette) transporters on endothelial and tumour cells. ABC transporter proteins actively (driven by ATP hydrolysis) expel cytotoxic drugs from the cell, maintaining the drug level below a cell-killing threshold. The ABC proteins are grouped into seven sub-classes, ranging from ABCA to ABCG. The three best characterized representatives are ABCB1 (Pgp, MDR1), ABCC1 (MRP1) and ABCG2 (BCRP, MXP, BMDP). They share the ability of recognizing and translocating a large number of structurally diverse, mainly hydrophobic, compounds. An important characteristic of the blood brain barrier is the expression of ABCB1, ABCC1 and ABCG2 on brain endothelial cells controlling the transfer of various compounds like the Taxanes, Adriamycin and Vincristine, into the CNS and therefore, inhibiting drug delivery to the tumour site [104, 105].

Comerford et al. found that hypoxia could activate the *mdr1*gene via binding HIF-1α resulting in the expression of ABCB1 and the development of a drug-resistant phenotype. This was shown *in vitro* on tumour cells, multicellular spheroids and on primary cultures of human microvascular endothelial cells [106, 107].

Treatment option: The dual effect of ABC transporters, protecting the brain from toxic effects on the one hand and developing resistance of tumour cells on the other, complicates the use of ABC transporter inhibitors.

To circumvent the blood brain barrier, local delivery techniques have been developed, e.g. convection enhanced delivery (CED), which involves the infusion of therapeutic agents via surgically implanted catheters using a pressure gradient to achieve a greater volume of distribution. The use of CED for the treatment of malignant glioma is reviewed by Ferguson et al. [108]

Early Growth Response Gene 1 (Egr-1)

The early growth response gene-1 (Egr-1) is a tumour suppressor which plays an important role in cell growth, differentiation and apoptosis. Activated Egr-1 can induce the expression of several target genes (e.g. bFGF, platelet derived growth factor (PDGF), synapsin I and synapsin II) that play key roles in astrocyte cell proliferation and neuronal differentiation and plasticity [109]. Low levels of Egr-1 protein were found in primary cultures developed from astrocytoma grade III and IV, being lower in glioblastoma and tissue carrying *wt* p53 [110]; however, Egr-1 expression can be rapidly induced by microenvironmental stimulants, including hypoxia, growth factors, and hormones [6]. Franken and his co-workers found that radiation induced Egr-1 expression *in vitro* within the first 2 hours after treatment of glioma cells, and that induction of EGR-1 was correlated with reduced repair of lethal lesions and increased repair of sublethal lesions [111]. This finding together with enhanced $\alpha_v\beta_3$ integrin expression and enhanced matrix metalloproteinase 2 and 9 activity after sublethal radiation [58] (see HIF-1 and radiotherapy) could explain the inevitable recurrence of the tumour seen in glioma therapy.

The hypoxic induction of tissue factor expression in human malignant glioma also depends on the upregulation and enhanced transcriptional activity of Egr-1. Tissue Factor (TF) is the primary initiator of blood coagulation *in vivo.* High levels of TF are found in malignant gliomas, in particular in pseudopalisading cells around necrotic areas, and its expression is correlated with

histological grade and the extent of necrosis [20]. TF levels are up-regulated by hypoxia, PTEN loss and over-expression of epidermal growth factor (EGFR), the latter two represent characteristic events during the progression from anaplastic astrocytoma to GBM [112, 113].

Treatment option: An over-expression of Egr-1 through recombinant adenovirus infection almost completely abolishes glioma cell growth *in vitro*, regardless of the mutational status of the p53 gene [110]. Chung and his c-workers found that amitryptiline, a tricyclic antidepressant drug that has been used for the treatment of depression, induces Egr-1 expression in rat C6 glioma cells. The effectiveness of tricyclic antidepressant drugs in the treatment of gliomas is currently evaluated [114].

RESISTANCE AND CANCER STEM CELLS

Emerging evidence points to cancer stem cells being the perpetrator exhibiting treatment resistance of a particular cancer. The concept of a stem cell in cancer has been long hypothesized, however, only recently their identification was successful [115]. Ignatova et al. were the first to report about the existence of stem-like cells in human brain tumours [116], which was later confirmed by Singh et al [117, 118]. Following that various other groups reported about sub-populations in tumour specimen and glioma cell lines, which exhibited stem-like characteristics [119, 120]. There is evidence that cancer stem cells play a crucial role in radioresistance (see for review [121]), however, the relationship between hypoxia, cancer stem cells and resistance is not yet fully understood.

CONCLUSION

Hypoxia has a strong influence on the characteristics of a tumour. Directly or indirectly hypoxia helps the tumour cell to adapt to less favourable conditions and in some cases even supports survival and progression. Hypoxia-induced changes on the tumour cell genome and proteome can produce a phenotype, which is resistant to apoptosis and cytotoxic damage. This phenotype might

be even more protected through complex hypoxia-induced effects on the tumour microenvironment, e.g. on endothelial cells, rendering chemotherapy and radiation ineffective. The greatest obstacle for an efficient therapy is the heterogeneity of the disease within one single individual and between individuals with the same tumour type. Therefore, a detailed analysis including identification of known therapeutic targets of a particular tumour followed by an individualized treatment approach might result in a more successful outcome.

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Key Words:

Brain tumour, glioma, hypoxia, resistance, chemotherapy, radiotherapy.

Figure 1

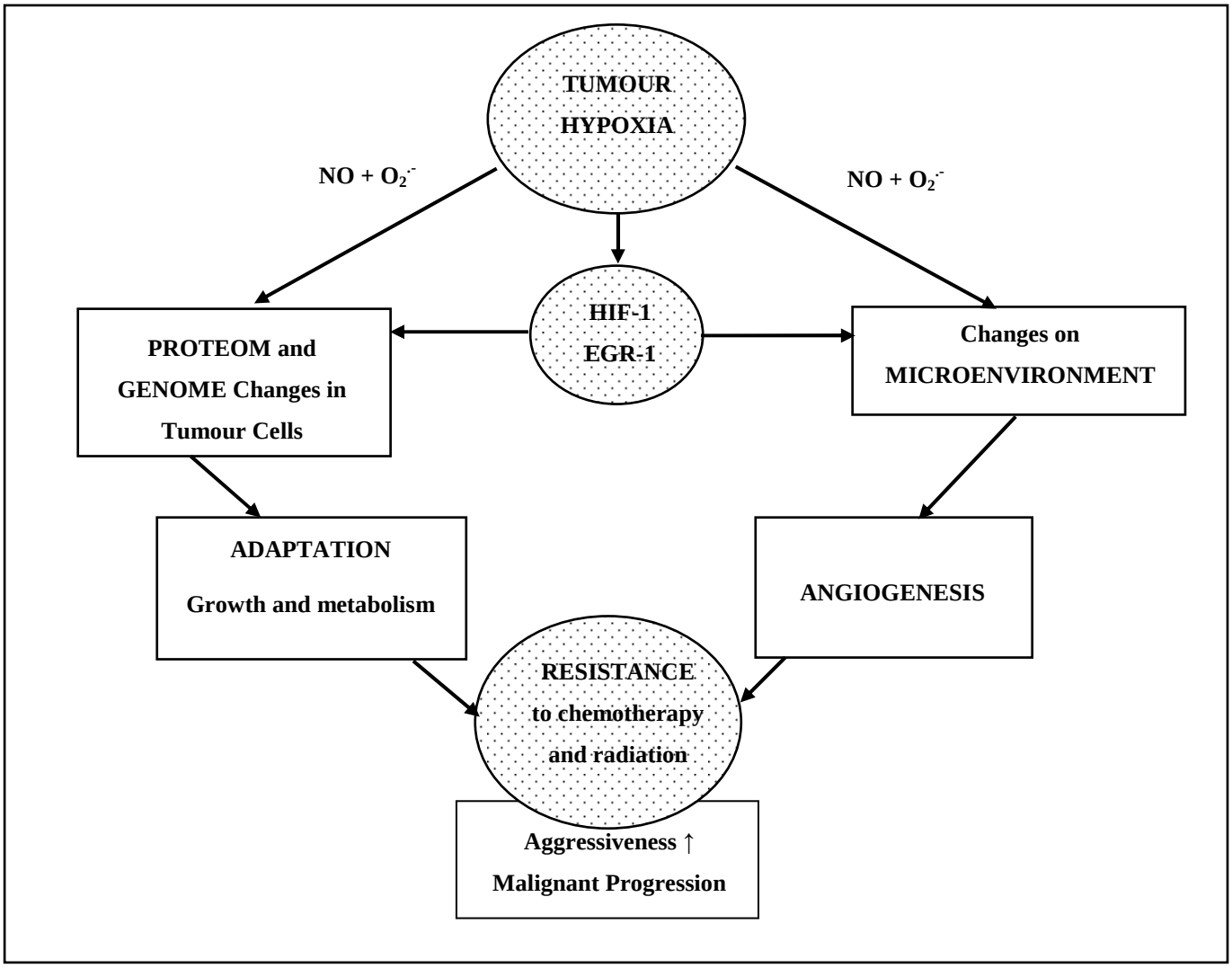
TUMOUR
HYPOXIA
NO + $O_2^{\cdot-}$
NO + $O_2^{\cdot-}$
HIF-1
EGR-1
PROTEOM and
GENOME Changes in
Tumour Cells
Changes on
MICROENVIRONMENT
ADAPTATION
Growth and metabolism
ANGIOGENESIS
RESISTANCE
to chemotherapy
and radiation
Aggressiveness ↑
Malignant Progression

Figure Legend:

Figure 1:
Schematic representation of hypoxia-induced changes in tumour cells and on the tumour microenvironment: Hypoxia causes either directly (NO and O2.-) or indirectly (HIF-1 or Egr-1) changes on the tumour microenvironment as well as in the proteome and genome of the tumour cells. These changes lead to increased aggressiveness and progression of the tumour, a new tumour vasculature (angiogenesis) and –ultimately to resistance to radiation and some chemotherapies.