Chapter 12
Implications of Oxygen Homeostasis for Tumor Biology and Treatment

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Abstract Tumors serve as a prototype system to study the role of the hypoxic microenvironment and gain insight in the regulation oxygen homeostasis. A series of biochemical and cell biological studies have significantly extended our knowledge of how tumor cells activate key regulatory mechanisms of oxygen homeostasis not only to adapt to the hostile tumor microenvironment but also to acquire a more aggressive tumor phenotype. Reduced oxygen levels and tumor-specific genetic alterations synergistically drive tumor progression by activating a key transcriptional system, the hypoxia inducible factors (HIFs). HIFs trigger a set of adaptive responses commonly associated with tumor malignancy including tumor angiogenesis, a shift in metabolism, proliferation, invasion, and metastasis. We and others could demonstrate that cancer stem cells are controlled by HIFs within a hypoxic niche, establishing an intriguing link between the well known function of hypoxia in tumor growth and stem cell biology. Additionally, HIF activation potentially conveys resistance to current tumor therapies including the evasive resistance phenotype observed after anti-angiogenic treatment. Together, these findings provide strong evidence that activation of the HIF system is a decisive step in cancer progression that critically shapes therapy response and clinical outcome. Recent insight into the precise mechanisms of oxygen sensing and signalling has offered new promising and potentially selective strategies to counteract this crucial pathway.

Keywords Anti-angiogenesis • Cancer stem cells (CSCs) • Epithelial mesenchymal transition (EMT) • Glioblastoma • Hypoxia • Hypoxia inducible factor (HIF) • Prolyl hydroxylase (PHD) • Tumor therapy

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12.1   Introduction

Oxygen is an indispensable substrate for aerobic metabolism and is therefore essential for the normal development and functioning of higher organisms. The efficient distribution of oxygen to tissues is a main function of the respiratory and cardiovascular systems. However, oxygen availability can vary greatly among individual cells and organs or during specific stages of development. While the arterial oxygen partial pressure is 90 mmHg (corresponding to ~12.5 %) under physiological conditions, differences in vascularization, tissue diffusion properties, and cell-specific oxygen consumption create a heterogeneous O_2 distribution, so that most tissues are exposed to lower oxygen concentrations. In particular, the brain experiences low O_2 levels down to 1 mmHg [24]. Reduced levels of oxygen (hypoxia) activate a set of adaptive responses which either enhance oxygen delivery or decrease oxygen consumption to promote survival under low oxygen conditions. Importantly, hypoxia arises not only in physiological situations, but is also a characteristic feature of various pathological conditions [1]. Particularly prominent among those is the process of neoplastic transformation and progression. Tumor growth and progression occurs as a result of the cumulative acquisition of genetic and epigenetic alterations in individual cells, followed by the selection of tumor cell clones with enhanced proliferation and survival potential. Once a tumor is formed, it creates a specialized microenvironment, which critically controls tumor progression. Highly proliferating tumors frequently outstrip their vascular supply leading to a tumor microenvironment characterized by low oxygen tension, low glucose levels, and an acidic pH. Tumor hypoxia is associated with an increased frequency of tumor invasion and metastasis and a poor therapy outcome. Notably, tumor cells not only adapt to survive under low oxygen, but also exploit hypoxia-induced mechanisms in order to promote their own growth and dissemination. Indeed, tumor hypoxia has become one of the main settings to study the mechanisms and functions of hypoxic signaling. Here, we will briefly summarize the mechanisms of cellular oxygen homeostasis and will focus on the function of hypoxic signaling in various aspects of cancer progression and resistance, as well as on possible strategies to target the hypoxic response as an anti-tumor therapy.

12.2   The Hypoxic Response and HIF

Since their discovery in 1995 [105], the hypoxia inducible transcription factors (HIFs) have emerged as the key transcriptional system initiating adaptive responses to hypoxia. HIFs act as heterodimers composed of a shared, stable HIFβ subunit and specific oxygen-regulated HIFα subunits. The stability of the α subunits is mainly controlled by prolyl hydroxylase domain proteins (PHDs), which use O_2 as a substrate to hydroxylate HIFα and target it for proteasomal degradation [15, 23, 62], following ubiquitination by the E3 ubiquitin ligase pVHL [48, 70]. An additional level of oxygen-dependent control is conferred by another hydroxylase, factor

inhibiting HIF-1α (FIH1), which modifies asparaginyl residues and inhibits the interaction between HIFα and its transcriptional coactivators p300/CBP [51]. More recently a number of additional mechanisms that modulate the HIF pathway have been identified, including the heat shock proteins HSP90 and HSP70, the histone deacetylases Sirt1 and Sirt6, TCA cycle-related metabolites, nitric oxide, the PHD E3 ubiquitin ligases Siah1/2, the microRNAs miR-17-92, and miR-107, as well as a number of oncogenes or tumor suppressor genes, e.g. PI3K/Akt, mTOR, Ras, p53 (reviewed in [69]). This high level of complexity of HIF regulation, which often involves an elaborate set of negative and positive feedback mechanisms [41], allows for precise, fine-tuned control of hypoxia-mediated responses and highlights the central importance of HIF signaling in cellular homeostasis [3]. In addition, hypoxia initiates adaptive responses independent of HIF through other redox sensitive systems, including activation of the NF-κB pathway, or global protein synthesis inhibition through the AMPK/mTOR or PERK/eIF2α pathways [11, 100], although the mechanisms mediating the O_2 dependence of these processes are less well understood.

There are two principal HIFα subunits, HIF-1α and HIF-2α, which mediate the hypoxic response through transcriptional regulation of an ever-growing number of genes. Although the functions of HIF-1α and HIF-2α partially overlap, it is now clear that the two isoforms are differently regulated by oxygen, are expressed in distinct normal and neoplastic cell types, possess different target specificities and generally appear to have complementary rather than redundant functions, as described in more detail in the following sections.

12.3   The Hypoxic Response and the Hallmarks of Cancer

Hypoxic signaling activates a large number of downstream biological responses, which together promote most of the defining properties of tumors (Fig. 12.1.) [4, 11]. One of the primary effects of hypoxia is the induction of a shift in cellular metabolism from oxidative phosphorylation to anaerobic glycolysis via HIF-mediated upregulation of glucose transporters and glycolytic enzymes [60]. This is accompanied by the upregulation of carbonic anhydrase IX (CA IX), transporters for lactate, H^+, HCO_3^- and other ions, as an adaptive mechanism of pH homeostasis, leading to a net acidification of the extracellular tumor environment. A decreased pH is a characteristic feature of many tumor types and has been shown to promote tumor growth and metastatic spread, at least in part through activation of extracellular matrix-degrading enzymes [18]. Another key response to tumor hypoxia is the stimulation of angiogenesis in order to improve the blood and oxygen supply of tumor cells. It is well established that tumors induce the formation of new vasculature as a key event in multistage carcinogenesis, a phenomenon termed "angiogenic switch" [8]. This is mainly accomplished through the upregulation of multiple angiogenic genes as direct HIF targets, including components of the VEGF, angiopoietin, and SDF-1 pathways [85].
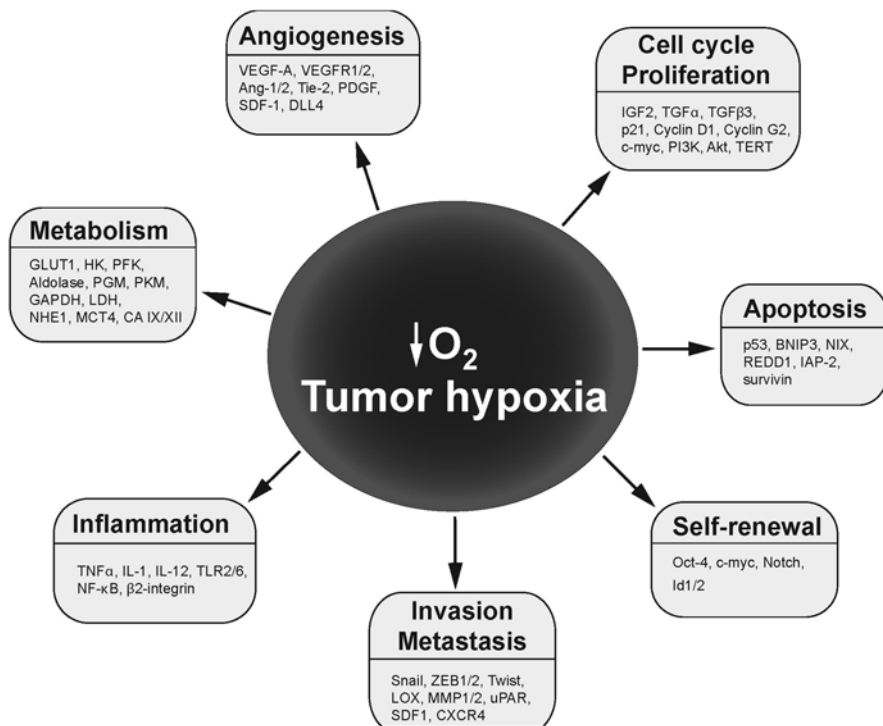
Fig. 12.1 Major aspects of tumor biology regulated by hypoxia. HIFs regulate multiple cellular processes relevant to tumor progression in response to hypoxia through trancscriptional upregulation of HIF-1α and/or HIF-2α target genes. In some cases, however, alternative mechanisms are involved, such as HIF-mediated protein stabilization (p53) or modulation of transcriptional activity (c-myc, Notch). *Ang-1/2* angiopoietin 1/2, *BNIP3* BCL2/adenovirus E1B 19 kDa interacting protein 3, *CA IX/XII* carbonic anhydrase IX/XII, *CXCR4* C-X-C chemokine receptor type 4, *DLL4* delta-like protein 4, *GAPDH* glyceraldehyde-3-phosphate-dehydrogenase, *GLUT1* glucose transporter 1, *HK* hexokinase, *IAP-2* inhibitor of apoptosis protein 2, *ID-1/2* inhibitor of DNA binding 1/2, *IGF2* insulin-like growth factor 2, *IL-1/12* interleukin 1/12, *LDH* lactate dehydrogenase, *LOX* lysyl oxidase, *MCT4* monocarboxylate transporter 4, *MMP1/2* matrix metalloproteinase 1/2, *NHE1* Na+/H+ exchanger 1, *NIX* NIP3-like protein X, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PDGF* platelet-derived growth factor, *PFK* phosphofructokinase, *PGM* phosphoglycerate mutase, *PKM* pyruvate kinase M, *PI3K* phosphatidylinositol 3-kinase, *REDD1* protein regulated in development and DNA damage response 1, *TERT* telomerase reverse transcriptase, *TGF* transforming growth factor, *Tie-2* tunica interna endothelial cell kinase 2, *TLR2/6* Toll-like receptor 2/6, *TNF* tumor necrosis factor, *SDF-1* stromal cell-derived factor 1, *uPAR* urokinase plasminogen activator receptor, *VEGF-A* vascular endothelial growth factor A, *VEGFR1/2* vascular endothelial growth factor receptor 1/2, *ZEB1/2* zinc finger E-box-binding homeobox 1/2

Hypoxia also plays an important role in the regulation of tumor cell proliferation and cell cycle progression, via the control of growth factors (e.g. TGFs, IGFs), oncogenes (c-myc), the PI3K/Akt pathway, p21, cyclins, and telomerase [88, 92]. The regulation of tumor cell death is another major aspect of cancer cell biology modulated by the hypoxic response. HIFs can activate proapoptotic genes such as

BNIP3, NIX, and REDD1 [92] and can stabilize p53 [6]. However, tumor cells develop various mechanisms to evade hypoxia-induced cell death, e.g. by HIF-dependent upregulation of anti-apoptotic molecules such as IAP-2 and survivin or by exerting a selective pressure to acquire p53 mutations under hypoxia [34, 36, 78]. Moreover, we have shown that HIF-1/2α induce the expression of PHD2 and PHD3, which act in a negative feedback loop even under low O_2 concentrations to protect tumor cells against hypoxia-induced cell death by dampening the HIF response [42].

Solid tumors trigger an intrinsic smoldering inflammatory response modulated so as to create a protumorigenic environment, which plays a critical role at different stages of tumor progression [37]. Hypoxia/HIFs are central mediators of this process by modulating key aspects of immune cell function and inflammation to promote an immunosuppressive environment. This is mediated for example through the control of immune cell adhesion (via induction of β2 integrin), expression of toll-like receptors, production of NO, proinflammatory cytokines (e.g. TNF, IL-1, IL-12), and activation of NF-κB signaling [47, 76]. An additional central aspect of cancer progression that is under the control of hypoxia is tumor cell invasion and metastasis. Hypoxia can induce the process of epithelial-mesenchymal transition (EMT), which is thought to be an essential early determinant of metastatic dissemination [109]. Hypoxic signaling suppresses the expression of the epithelial cell adhesion molecule E-cadherin, an essential step of EMT [26], through HIF-1α or HIF-2α-dependent upregulation of EMT transcriptional repressors of the SNAI, ZEB, or TWIST families [27, 46, 59, 61, 110]. Furthermore, hypoxia can promote metastasis via upregulation of pro-invasive and metastatic HIF target genes including extracellular matrix-remodelling proteins such as lysyl oxidase (LOX) and urokinase plasminogen activator receptor (uPAR) [25, 58], matrix metalloproteinases [94] and the prometastatic chemokine receptor CXCR4 [79, 98, 111].

Additionally, hypoxia plays an essential role in the regulation of the self-renewal and differentiation of physiological stem cells in a variety of tissues, at least in part through the transcriptional activation of central stem cell regulators such as Oct4, Notch, and c-myc [53]. Importantly, work by us and others has demonstrated that hypoxia also has a critical function in the maintenance of cancer stem cells (CSCs), a population of tumor cells with properties of stem cells, that drives tumor initiation and progression [30]. Hypoxia promotes the self-renewal of CSCs, particularly in glioblastoma [40, 72, 91], while HIF knockdown blocks this effect and reduces CSC-mediated tumor growth [65, 73, 91, 95]. Current evidence indicates that HIF-2α is the main isoform to promote CSC maintenance as HIF-2α, but not HIF-1α, is highly expressed and strongly upregulated by hypoxia in glioma CSCs [65], enhances the CSC phenotype [91] and promotes tumor growth [40]. Moreover, HIF-2α regulates crucial signaling pathways that are involved in stem cell maintenance. HIF-2α interacts with and stabilizes the Notch ICD (intracellular domain), enhancing Notch signalling to control cellular differentiation [38]. Additionally, HIF-2α transcriptionally regulates Oct4 [21], a transcription factor that is important for the maintenance of the self renewal of embryonic stem cells and one of four factors necessary to induce pluripotency [99].

12.4 The Hypoxic Response and Tumor Progression

The brief overview provided above highlights the central role of hypoxic signaling in promoting the "hallmarks of cancer" [39, 88]. It is therefore not surprising that hypoxia and HIFs have been associated with tumor initiation and progression and a worse clinical outcome. It has been shown for a number of different cancer types that hypoxia correlates with a more aggressive tumor phenotype, including enhanced angiogenesis, metastasis, recurrence, therapy resistance, and decreased patient survival [49]. In comparison to adjacent tissue widespread HIF activation can be seen in various tumors types, correlating with tumor growth and progression. HIF overexpression in tumors is a result of hypoxia-dependent and hypoxia-independent mechanism such as oncogenic mutations or enhanced growth factor signalling. Various oncogenic mutations can directly lead to HIFα stabilization. For example, genetic alterations of pVHL are a characteristic feature of clear cell renal cell carcinomas and are linked to accumulation predominantly of HIF-2α [50]. In addition, activating mutations of PI3K, Akt, and Ras, as well as inactivating mutations of PTEN and TSC2 result in enhanced HIF-1α transcription, translation, or stabilization [14, 31]. An elevated expression of HIFs has been associated with multiple cancers, based on immunohistochemical analysis. HIF-1α has been found to be upregulated compared to the nonmalignant tissue in a broad variety of tumor types, including oligodendroglioma, breast, cervical, colon, ovarian, endometrial, lung, prostate, bladder, pancreatic, and oropharyngeal cancer [49, 92]. In most of these cancers higher HIF-1α levels have been associated with poor patient survival [11]. Elevated HIF-2α, on the other hand has been linked to worse prognosis in a distinct set of tumor entities, including clear cell renal carcinoma, non-small-cell lung carcinoma, head and neck squamous cell carcinoma, neuroblastoma, and glioma [65, 82].

In support of a distinct, nonredundant function of the two main HIFα isoforms, HIF-1α overexpression correlated with decreased patient mortality in head and neck cancer and non-small-cell lung cancer, in both of which HIF-2α showed the opposite association [92]. Furthermore, silencing of HIF-2α, but not HIF-1α, in a number of cancer cell lines reduced cell proliferation and tumor growth [29]. Such functional differences between HIF-1α and HIF-2α could be due to differential expression in the different tumor entities, as well as to the distinct sets of target genes controlled by the two isoforms [41]. In addition, the two HIFα subunits can modulate the activity of key oncogenes or tumor suppressors in opposing fashion. For example, HIF-1α antagonizes myc transcriptional activity, while HIF-2α promotes it [32]. Renal cell carcinomas that express HIF-2α but not HIF-1α upregulate myc target genes, have increased proliferation and enhanced resistance to replication stress [33]. HIF-1α and HIF-2α also have contrasting effects of the function of p53: while HIF-1α binds to p53 and stabilizes it [6, 75], HIF-2α indirectly suppresses p53, promoting radio- and chemoresistance [10, 86]. Compared to HIF-1α, HIF-2α accumulates at higher oxygen concentrations [43, 108], which more closely resemble the in vivo conditions under which tumors arise and grow. At the same time,

while HIF-1α gets only transiently upregulated under chronic hypoxia, HIF-2α levels remain elevated in these conditions [44]. In addition, HIF-2α appears to be the primary isoform regulating the self-renewal capacity of the CSC pool [65, 91], which may have different contributions to the progression of distinct tumor entities [54, 83].

Although HIFs are typically perceived as protumorigenic molecules, in some settings they can also act as tumor suppressors. For example, HIF-1α-deficient teratomas grow faster due to the refractoriness of the mutant tumor cells to stress-induced apoptosis [16]. Similarly, HIF-1α and HIF-2α were stabilized in Vhl−/− ES cells, but the resulting teratomas were smaller than in controls [68]. Furthermore, HIF-2α overexpression in glioma cells enhanced apoptosis and decreased tumor growth, whereas HIF-2α inhibition or genetic deletion had the reverse effect [2]. The complexity of HIF function in tumorigenesis was further highlighted by studies using a Kras mutant lung tumor model. Expression of nondegradable HIF-2α in this system increased tumor burden, angiogenesis, EMT, and decreased survival [55]. Paradoxically, deletion of HIF-2α in the same model also promoted tumorigenesis, whereas HIF-1α deletion had no apparent effect [71]. Such contrasting results indicate that the effect of HIFs on tumor progression is likely to depend on the cellular context as well as the precise extent of functional inhibition or activation of specific isoforms and the balance of competing signaling pathways that can be activated by their stimulation or suppression [69].

In glioma, hypoxia plays a prominent role in several aspects. First, the characteristic necrotic regions which represent one of the key criteria for the histological diagnosis of glioblastoma (GBM) are associated with hypoxia [49]. Hypoxia and the activation of HIF also contribute to the second characteristic feature of GBM, the high degree of vascularization [2]; the GBM microcirculation, however, is leaky and functionally inefficient, failing to restore normal oxygenation [103]. Glioma cells overexpress HIF-1α and HIF-2α both in culture and in situ, especially in the perinecrotic pseudopalisading areas [49, 91]. Interestingly, the cells found in those regions have been implicated in hypoxia-induced migration away from the necrotic areas [12, 84]. Furthermore, the expression of classical HIF target genes like CA IX and glucose transporter 1 (Glut-1) correlates with higher brain tumor grade and poor response to treatment [28, 45, 52, 67, 89, 96, 102, 112]. Finally, as discussed above, hypoxia and HIF-2α play a particularly prominent role in the maintenance of glioma CSCs within a hypoxic niche, which is thought to be responsible for determining key aspects of GBM malignancy [30].

12.5 Hypoxia and Therapy Resistance

The capacity of hypoxia to protect tumor cells from radiation damage was first noted in the 1950s and has been extensively corroborated since then (reviewed in [11]). In GBM, for example, elevated hypoxia before radiotherapy is strongly

associated with decreased time to progression and patient survival [97]. In addition, hypoxic cells have an increased resistance to a variety of standard chemotherapeutic agents [101]. Hypoxic signaling converges on multiple pathways that contribute to therapy resistance. For example, hypoxia selects for cancer cell clones with mutant p53, a key mediator of therapy-induced apoptosis [34]. Additionally, in GBM cells hypoxia induces the activation of the antiapoptotic protein Bad and subsequent inhibition of programmed cell death [74]. Moreover, the multidrug resistance gene MDR1 is a direct HIF-1α target, which can mediate the efflux of chemotherapeutic drugs [20, 106]. The ability of hypoxia to increase the CSC pool, as discussed above, may provide an additional explanation for the decreased sensitivity of hypoxic tumors to treatment. Indeed, a series of studies have demonstrated that CSC have enhanced resistance to chemo- and radiotherapy [30]. This is due to a combination of properties characteristic of CSCs, including the high expression of ABC drug pumps, relative quiescence, resistance to oxidative DNA damage and enhanced DNA repair capacity [7, 90]. The increased resistance of CSCs, combined with the ability of only a very small number of CSCs to reinitiate tumor growth is thought to be a major reason for cancer persistence and relapse after treatment.

Anti-angiogenic therapies have become an established tool in the treatment of several cancers, including colorectal, lung, breast cancer, and GBM [66]. However, following the initial wave of enthusiasm, it has become clear that the inhibition of angiogenesis has complex consequences and smaller than expected benefits for cancer patients. Typically tumor shrinkage is initially observed, but this is followed by adaptation and renewed growth of the tumor, often resulting only in extension of progression-free survival, but not overall survival. The major problem underlying the relative inefficacy of angiogenic therapies is that tumors quickly adapt and manage to circumvent them. The mechanisms of this "evasive resistance" are poorly understood, but preclinical studies have started to suggest several possible explanations. By definition, anti-angiogenic agents are designed to curtail the blood supply of the tumor, thus inducing tumor hypoxia. This may provide one key explanation of the limited therapeutic efficiency of current anti-angiogenic drugs, since hypoxia activates a number of mechanisms that contribute to the evasive resistance following anti-angiogenic therapy. As discussed above, several alternative proangiogenic signals are HIF target genes. In addition, hypoxia enhances the recruitment of bone marrow derived cells to the tumor, which can promote the formation of blood vessels either through secretion of cytokines and growth factors or through direct differentiation into blood vessel cells, such as endothelial cells or pericytes [8]. Interestingly, inhibition of angiogenesis also elicits enhanced local invasion and distant metastasis in different tumor types, including breast tumors, pancreatic neuroendocrine tumors, melanoma, and glioma [22, 77]. The elevated hypoxia observed under these conditions [77] suggests several possible mechanisms for anti-angiogenesis driven metastasis, as outlined in the previous sections, however, the precise pathways involved in the evasive resistance phenotype remain to be elucidated.

12.6   Strategies for Therapeutic Targeting of Tumor Hypoxia

Based on the realization that hypoxia plays a key role at various steps of tumor progression and resistance, substantial effort has been invested in targeting or exploiting tumor hypoxia as an anti-cancer therapeutic strategy (Fig. 12.2). Early attempts were aimed at preventing hypoxia by increasing tumor oxygenation during irradiation, but the clinical efficacy of such interventions was unsatisfactory [11]. Given the relevance of the hypoxic tumor fraction in shaping the tumor phenotype, a different strategy is to take advantage of the hypoxic state of tumor cells in order to selectively eliminate them. Several chemical classes of agents have been proposed which can be specifically converted from a nontoxic to a toxic form by reduction under low oxygen conditions [13]. An example of such a hypoxia activated prodrug is tirapazamine. In hypoxic cells, it gives rise to free radical species which block the function of topoisomerase II and lead to double-stranded DNA breaks. Phase III clinical trials of tirapazamine in combination with chemotherapy have demonstrated benefits in lung cancer patients [104]. Another bioreductive prodrug, the potent DNA intercalator and topoisomerase poison AQ4N, has shown selective activation in hypoxic regions in phase I trials [5]. Other classes of drugs (e.g. CB 1954 or SN 23862) are designed to release more stable cytotoxins upon reduction, which can diffuse away from hypoxic cells and kill additional cells in the tumor, in a "bystander effect" [13].
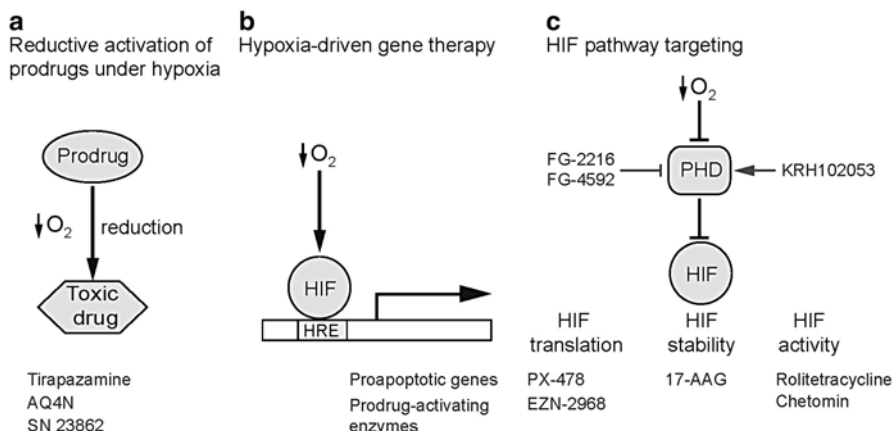

Fig. 12.2 Strategies for therapeutic targeting of tumor hypoxia (**a**) The hypoxic state of tumor cells can be exploited for the chemical conversion of prodrugs into a toxic form under the reducing conditions created by the shortage of O_2. (**b**) The stabilization of HIFs under hypoxia can also be used for driving the expression of cytotoxic gene products in hypoxic cells. (**c**) A third strategy is to interfere with the tumor promoting functions of hypoxic signalling, e.g. by specific targeting of components of the HIF pathway. Shown below the schemes are specific compounds or types of genes that are being explored in preclinical studies or clinical trials. FG-2216 and FG-4592 are PHD inhibitors currently in clinical trials for renal anemia [9, 17, 69]

An alternative set of approaches in preclinical development aims to exploit tumor hypoxia for the selective activation of gene expression. Such hypoxia-targeted gene therapy approaches involve constructs containing HIF-binding sequences (hypoxia response elements (HREs)), which are virally transduced into tumor cells and drive transcription of therapeutic genes in cells that experience hypoxia. Examples of such strategies include the hypoxia-dependent expression of proapoptotic genes [87] or of prodrug-activating enzymes [93]. Others have designed conditionally replicative oncolytic viruses that are specifically activated in hypoxic cells, causing their lysis [81]. A somewhat different approach consists in the coupling of diphtheria toxin to the oxygen-dependent degradation domain (ODD) of HIF-1α [57]. Under normoxic conditions such a fusion protein would be targeted for proteasomal degradation following ubiquitination of the ODD, however, under hypoxia it would be stabilized allowing the toxin to kill the hypoxic cell.

The largest group of hypoxia-targeting agents in current development are centered around HIF and the molecules that regulate its stability and function. Being a transcription factor, HIF represents a challenging target, but several different approaches have provided interesting hits. Interestingly, a surprisingly broad array of established drugs has been shown to suppress HIFα stability or activity. Examples include the HSP90 antagonists geldanamycin and 17-AAG, the histone deacetylase inhibitors trichostatin A and FK228, the DNA intercalating agents doxorubicin, daunorubicin, and acriflavine, the topoisomerase inhibitor topotecan, cardiac glycosides, as well as inhibitors of central signal transduction pathways like Ras/MAPK, PI3K/Akt, and mTOR [63, 64, 80]. While there is evidence that some of the anticancer effects of these drugs may be mediated by HIF inhibition [63, 64], the diversity of "nonselective" compounds that block HIF may rather be seen as an indication of the central role of this protein in the control of cellular homeostasis, than as an optimal strategy for the design of HIF-targeted therapies. More specific strategies to suppress HIF activity include the blockade of HIF-1α/HIF-1β dimerization or HIF binding to p300/CBP, which has been achieved with small molecule inhibitors such as rolitetracycline, chetomin, or YC-1 [80]. A specific inhibitor of HIF-1α translation, PX-478, exhibited antitumor activity against human xenografts and is currently in phase I clinical trials [56, 107]. The RNA antagonist of HIF-1α, EZN-2968, inhibits tumor cell growth and is also being tested in phase I clinical trials [35, 69]. A further possibility for suppressing HIF function is to promote activation of PHDs. For instance, a potent small molecule activator of PHD2, KRH102053, has been shown to decrease HIF-1α levels in tumor cells [19]. In principle, a variety of RNAi or gene therapy approaches, e.g. aimed at the silencing of HIFs, expression of dominant negative HIF mutants or overexpression of PHDs are potentially powerful alternative treatment strategies, provided that safe and efficient methods for clinical delivery become available.

It has to be noted that nearly all therapeutic agents so far have been targeted against the more ubiquitous family member HIF-1α. However, as discussed in the previous sections, HIF-2α plays a dominant role in some tumor types or subpopulations of tumor cells, such as CSCs. Small molecule inhibitors have been identified, which suppress HIF-2α translation in renal cell carcinoma cells through a mecha-

nism dependent on an iron response element in the 5′ UTR of the HIF-2α mRNA; however, the same compounds also decreased the levels of HIF-1α, albeit via unrelated mechanisms [113]. Therefore concentrating greater efforts on specifically targeting the HIF-2α isoform remains an important objective for future drug discovery screens. In addition, given that under some circumstances HIFs can also elicit tumor suppressive functions (see above), exploring HIF activating strategies, e.g. by using PHD inhibitors, may in some cases also prove valuable. Our own work, for example, has shown that PHD inhibition in GBM cells facilitates cell death induction by staurosporine or TRAIL [42].

Our growing understanding of the mechanisms mediating the hypoxic response and the signaling pathways involved in its regulation have allowed us to more fully comprehend central aspects of tumor cell biology and malignant progression. Our deepened knowledge of O_2 homeostasis in tumors has formed the basis for the design of novel therapeutic strategies targeted at hypoxic signaling, which carry the potential to become a powerful weapon in our battle against cancer.

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