Chapter 12
Endothelial Cell Reactions to Oxygen: Implications for Cancer

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Abstract The blood vasculature establishes an important interface between the environment and the organism. Localizing in the inner surface of the tubular blood vessel network, endothelial cells allow the exchange of nutrients and oxygen, fostering aerobic cell metabolism of peripheral tissues. Oscillations in oxygen levels may pose significant tissue threats, and thus, endothelial cells have evolved intricate molecular mechanisms to sense and adequate perfusion with tissue metabolic demands. In healthy tissues, where nutrients and oxygen are delivered in abundance, endothelial cells are quiescent and form a smooth inner vessel surface with tight barrier functions. Vice versa, when nutrients and oxygen are scarce, endothelial cells activate migratory and proliferative mechanisms in order to sprout new vessel branches and nourish the hypovascularized tissue, in a highly regulated process called angiogenesis. Angiogenesis is revisited in several pathological conditions, such as cancer, where it plays a relevant role in malignance, namely, in the progression to the deadly metastatic disease. This chapter gives an overview on how oxygen availability shapes endothelial cell phenotypes and discusses its implications in cancer.

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J.-J. Feige et al. (eds.), *Molecular Mechanisms of Angiogenesis*,
DOI 10.1007/978-2-8178-0466-8_12,

12.1 Overview of Oxygen-Mediated Pathways

The high reduction potential of oxygen enables organisms to generate large amounts of energy during aerobic respiration. The evolution from unicellular to more complex multicellular organisms with an increased energy demand has thus been linked to the rise in atmospheric oxygen. This allowed the employment of oxygen as a terminal electron acceptor in the mitochondrial respiratory chain and the increased efficiency in energy production (Hedges et al. 2004). However, the reliability on oxygen for metabolic reactions has also led to a lethal dependency. In several disease conditions—such as stroke, myocardial infarction, neurological disorders, ischemia, reperfusion of transplanted organs, chronic lung disease, and cancer—perturbation of oxygen supply confirms that oxygen homeostasis is indispensible for the mammalian system (Bazan et al. 2002; Bergeron et al. 1999; Maxwell et al. 1997; Carmeliet et al. 1998; Semenza 2011). Moreover, the penalty associated to the use of oxygen for aerobic respiration is the production of reactive oxygen species (ROS) (Guzy and Schumacker 2006). If not properly scavenged by the cellular anti-oxidative defense systems, these metabolic by-products cause irreversible damage to DNA, proteins, and lipids, hence impairing crucial cellular performance and ultimately resulting in tissue demise (Cooke et al. 2003).

Given the dangers of unbalanced oxygen availability, oxygen-dependent mechanisms have evolved to sense oxygen tension and thus to assure a tight regulation of oxygen supply in case of its shortage. On the whole organism level, the complex blood vessel network enables the efficient distribution of oxygen over distances beyond its diffusion limit. Sensory structures like the carotid body ensure a fast response to disturbed oxygen tensions, resulting commonly in pulmonary vasoconstriction and dilation of systemic vessels (Lopez-Barneo et al. 2001). Within these sensory structures, chemoreceptor cells sense and acutely respond to modulations in oxygen tension via oxygen-sensitive ion channels (Peers 1997). At the cellular level, adaptation to changes in the oxygen supply is accomplished by oxygen-regulated transcription factors (Cummins and Taylor 2005). One of the key transcription factors responsible for adaptive responses to low oxygen pressure is the hypoxia-inducible factor (HIF) (Adams et al. 2009). Inseparably linked to oxygen homeostasis are the prolyl hydroxylase domain proteins (PHDs), which regulate the turnover of HIFs, and are true cellular oxygen sensors, since their catalytic activity is dependent on oxygen availability (Bruick and McKnight 2001; Epstein et al. 2001). Factor inhibiting HIF (FIH), like the PHDs, belongs to the iron- and 2-oxoglutarate-dependent enzymes and regulates the transcriptional activity of HIF, thus representing another component of the oxygen-sensing machinery (Mahon et al. 2001; Lando et al. 2002a). Other iron- and 2-oxoglutarate-dependent enzymes have also been implicated in serving important functions in oxygen sensing (Loenarz and Schofield 2011). Additionally, JmjC histone demethylases, responsible for epigenetic modifications, have been implicated in oxygen sensing and angiogenesis (Boeckel et al. 2011). With a less clear molecular mechanism, mitochondria are also linked with oxygen sensing through inhibition of PHD function (Kaelin 2005;

Klimova and Chandel 2008). It has been proposed that under moderate hypoxia, ROS generated from complex III of the electron transport chain inhibits PHD activity and consequentially stabilizes HIF (Klimova and Chandel 2008). On the other hand, the mitochondrial oxygen consumption could contribute indirectly to the decrease of PHD activity. However, cells with a mutant cytochrome b, which produce ROS but do not consume oxygen, provide evidence for mitochondrial-mediated ROS production in HIF stabilization (Klimova and Chandel 2008).

When considering the oxygen-related responses on the cellular level, it is interesting to note that the vasculature itself not only serves as a well-defined oxygen distribution network but is also highly responsive to differences in oxygen tension. Indeed, endothelial cells in the active growing vasculature exhibit distinct phenotypes and molecular signatures that are dependent on oxygen gradients (Gerhardt et al. 2003; Claxton and Fruttiger 2004; Lu et al. 2004; Tammela et al. 2008). At the forefront of a sprouting vessel, tip cells are exposed to the lowest oxygen levels in comparison to the follower stalk cells, which experience higher oxygen tensions (described in more detail below). Oxygen signaling thus contributes to the endothelial cell fate and impacts on the overall angiogenic processes. Mechanistically, the initiation of the oxygen-sensing machinery encompasses a very complex and broad response—the HIF system itself already regulates the expression of more than a hundred target genes (Wenger et al. 2005). Multiple pro- and antiangiogenic molecules are induced providing compelling evidence that hypoxia is one of the key environmental cues that trigger blood vessel growth under physiological and pathophysiological conditions (Pugh and Ratcliffe 2003). Physiological angiogenesis is a tightly regulated process, where pro- and antiangiogenic players are accurately balanced to assure that the assembly of the new blood vessels occurs in an orderly fashion. Once this equilibrium is disturbed, the excessive production of specific angiogenic players will lead to abnormal vessel growth with long-lasting consequences as evinced by the chaotic structure and deficient function of tumor vessels (Papetti and Herman 2002). Gaining further mechanistic insights from physiological angiogenic processes, such as embryonic development, reproduction, or wound healing, might help to understand how these mechanisms are manipulated to serve pathophysiological situations.

12.2 Hypoxia-Inducible Factors Mediate Cellular Oxygen Signaling

Initially, oxygen sensing was believed to be confined to the specialized glomus cells of the carotid body, which in response to hypoxemia signal to the dorsal inspiratory center in the medulla oblongata to increase the volume and rate of breathing (Weir et al. 2005). We now know that virtually all nucleated cells in the body sense and respond to hypoxia. In the last two decades, a large amount of studies have been centered on the HIF system and its repercussion in the role of oxygen in health and disease.

This marked interest was captivated by the identification of the hypoxia-inducible transcription factor in 1995 (Wang et al. 1995) and the subsequent characterization of the prolyl hydroxylase domain proteins as regulators of HIF stability (Bruick and McKnight 2001; Epstein et al. 2001). Initially, mechanistic studies under hypoxia led to the discovery of a hypoxic responsive element (HRE; 5′-RCGTC-3′) in the 3′ enhancer of the erythropoietin (EPO) gene (Semenza and Wang 1992). This consensus sequence is very well conserved between different species, highlighting the evolutionary importance of the oxygen-sensing strategy.

HIF is composed of the hypoxia-inducible alpha subunit (HIF-1α) and a constitutively expressed beta subunit (HIF-1β) (Wang et al. 1995). HIF-1β was previously identified as a binding partner of the aryl hydrocarbon receptor, thus being also referred to as aryl hydrocarbon nuclear translocator (ARNT) (Wood et al. 1996). Succeeding the cloning of HIF-1α, a closely related protein HIF-2α, sharing 48 % of amino acid identity, was discovered in 1997 (Ema et al. 1997; Flamme et al. 1997a; Hogenesch et al. 1997; Tian et al. 1997). To date three HIF-α proteins have been described. HIF-1α and HIF-2α are positively associated with HIF target gene expression, whereas the inhibitory PAS domain (IPAS), an HIF-3α isoform, is reported as a negative regulator of HIF signaling (Makino et al. 2001, 2002). HIF-α and HIF-β proteins contain a basic helix-loop-helix (bHLH) and Per-ARNT-Sim (PAS) domain in its N-terminal half (Wang et al. 1995). These domains are important for the α–β dimerization, whereas the downstream basic region enables specific DNA binding to the HRE sequence (Wang et al. 1995; Kinoshita et al. 2004). Subsequently a C- and N-terminal transactivation domain localizes within the C-terminal half of the HIF-α subunit, specifying the transcriptional activity of HIF (Jiang et al. 1997; Pugh et al. 1997; O'Rourke et al. 1999). Of note, an important negative regulation of HIF transcription activity is accomplished by FIH. This enzyme hydroxylates an asparagine residue in the HIF-α C-terminal transactivation domain, which therefore becomes unable to bind transcriptional co-activators such as the cAMP response element-binding protein CBP/p300 (Tian et al. 1997; Lando et al. 2002a, b).

There are a number of posttranslational regulations of the HIF protein such as hydroxylation, phosphorylation, acetylation, and sumoylation (Brahimi-Horn et al. 2005; Jeong et al. 2002; Richard et al. 1999; Sodhi et al. 2000; Minet et al. 2001; Lando et al. 2002b; Epstein et al. 2001; Bruick and McKnight 2001). Proline hydroxylation within the oxygen-dependent degradation domain (ODD) by PHDs is an important determinant of HIF protein stability. The von Hippel-Lindau protein (pVHL), part of an E3 ubiquitin ligase complex, recognizes the hydroxyproline in HIF and subsequently targets the transcription factor for proteasomal degradation (Maxwell et al. 1999; Ivan et al. 2001; Jaakkola et al. 2001).

In contrast to the constitutively expressed HIF-β subunit, HIF-α protein is very instable and its availability is highly dependent on the oxygen tension (Salceda and Caro 1997). In fact, HIF-α transcription and translational processes are not strictly regulated by oxygen, yet the rate of HIF turnover is an oxygen-dependent checkpoint. HIF-α is promptly degraded under normoxic conditions ($t_{1/2} = 5$ min) and accumulates

when oxygen levels drop, given the hampered degradation via the proteasome (Jewell et al. 2001). This allows HIF-α to translocate to the nucleus and to form a heterodimer with HIF-1β able to recognize and bind to the consensus sequence within the HRE of target genes (Wang et al. 1995; Wood et al. 1996). Subsequent recruitment of co-activators enables the initiation of the transcriptional complex, resulting in the expression of a number of HIF target genes (Kallio et al. 1998; Arany et al. 1996).

Interestingly HIF-1α and HIF-2α own overlapping and nonredundant functions (Hu et al. 2003; Sowter et al. 2003), and spatial-temporal differences in their expression do exist (Holmquist-Mengelbier et al. 2006). Whereas HIF-1α is nearly ubiquitously expressed, HIF-2α is more restricted to certain cell types, among them endothelial cells and glomus cells of the carotid body (Tian et al. 1997, 1998; Wiesener et al. 2003). HIF-2α was also shown to accumulate already at higher oxygen tension, supporting the concept that each isoforms might serve a specific function (Holmquist-Mengelbier et al. 2006).

12.3 The Function of Prolyl Hydroxylase Domain Proteins and Factor Inhibiting HIF as Oxygen Sensors

The oxygen-dependent regulation of HIF is a posttranslational modification event. One of the main factors involved in the cellular turnover of HIF proteins is the prolyl hydroxylase domain proteins (PHDs). PHDs belong to a family of nonheme iron- and 2-oxoglutarate-dependent enzymes (Bruick and McKnight 2001; Epstein et al. 2001; Ivan et al. 2001; Jaakkola et al. 2001). The hydroxylation reaction comprises molecular oxygen and 2-oxoglutarate as co-substrates and ferrous iron (Fe^{2+}) and ascorbate as cofactors. During the enzymatic reaction, one oxygen atom is used to form HIF-hydroxyproline, and the other is employed in the generation of succinate from the stoichiometric decarboxylation of 2-oxoglutarate. During this reaction, Fe^{2+} is bound to the active site of the PHD protein and oxidized; ascorbate is needed to reduce the iron during the reaction cycles, in a nonstoichiometric fashion (Hewitson et al. 2005). Hydroxylation occurs on prolines 402 and 564 within the LXXLAP sequence of the ODD in human HIF-1α and prolines 405 and 530 in human HIF-2α (Masson et al. 2001; Huang et al. 1998).

There are three PHD proteins (PHD1-3), also called egg-laying defective nine homolog (EGLN)—from its essential egg-laying function in C. elegans. Hydroxylation activity on proline 564 is higher for all PHDs, notably PHD3 is principally inactive at proline 402 (Hirsila et al. 2003; Appelhoff et al. 2004). Interestingly, PHDs also differ in their selectivity for HIF-1α versus HIF-2α hydroxylation. While PHD2 exhibits a higher hydroxylation activity on HIF-1α than on HIF-2α, PHD1 and PHD3 preferentially hydroxylate HIF-2α (Hirsila et al. 2003; Appelhoff et al. 2004). Additionally, FIH was shown to more potently hydroxylate HIF-1α thus contributing to the distinct HIF-1 and HIF-2 specific functions (Bracken et al. 2006).

PHD2 is known to be the main HIF regulator under normoxic conditions (Berra et al. 2003). Nevertheless, there is growing evidence that the relevance of PHDs is cell context dependent, the relevance of the respective PHD protein might shift. Therefore, PHD functionality might be designated by cell-type specificity, HIF isoform availability, and environmental cues. For instance, PHD2 and more prominently PHD3 are induced in an HIF-dependent manner in hypoxia (Metzen et al. 2005; Pescador et al. 2005). Interestingly though HIF-1α and HIF-2α differentially induce PHD2 and PHD3. Whereas HIF-1α induces both PHD2 and PHD3, HIF-2α only enhances PHD3 expression (Aprelikova et al. 2004; Henze et al. 2010). Remarkably, PHDs themselves suppress HIF transcriptional activity, further adding to the complexity of the system (To and Huang 2005; Hopfer et al. 2006). When associating with ING4, a tumor suppressor gene, PHD2 was shown to inhibit HIF-dependent transcription of angiogenic cytokines (Ozer et al. 2005).

Besides the canonical oxygen-dependent control of HIF, other mechanisms account for the regulation of the HIF response, e.g., receptor-mediated pathways. Similarly, PHDs were also shown to be involved in HIF-independent regulations. Examples of this are the negative modulations of the transcription activity of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) through either hydroxylase-dependent or hydroxylase-independent inactivation of the inhibitor of kappa-B kinase (IKKβ) in different cell contexts (Chan et al. 2009; Cummins et al. 2006; Berchner-Pfannschmidt et al. 2010; Takeda et al. 2011; Xue et al. 2010). A number of other PHD protein interactors have recently been reported and might represent novel downstream effectors of oxygen sensing (Wenger et al. 2009).

12.4 Role of Oxygen Signaling in Physiological and Pathophysiological Angiogenesis

Over the years it became clear that tumors hold a numerous strategies to build their own vasculature (Leite de Oliveira et al. 2011). Two of the most relevant processes are as follows: vasculogenesis, which refers to the de novo formation of blood vessels from endothelial precursor cells during embryonic development, and angiogenesis, which defines the process of new blood vessels sprouting from preexisting ones (Risau and Flamme 1995; Flamme et al. 1997b). In the embryo, angiogenesis occurs after the primary capillary plexus has been formed and is responsible for the assembly of a hierarchical network of larger vessels that branch into smaller ones in order to irrigate all parts of the developing organs. In the adult, angiogenesis is a relatively uncommon event, almost exclusively seen in the ovarian cycle or remodeling processes upon injury such as wound healing. Angiogenesis is initiated when cells in avascular areas experience low oxygen tension and mount adaptive responses mainly mediated by the stabilized HIF heterodimer. A number of very-well-coordinated events guarantee the establishment of new blood vessels from an existing vascular network. This involves destabilization of the preexistent vessels by loosening the attachment of pericytes and digestion of the basement membrane and

extracellular matrix that surrounds the blood vessels. Endothelial cells are then activated to be able to proliferate and migrate towards the avascular/hypoxic areas. Finally, tip cells from neighboring sprouts anastomose to bridge tubular loops. Upon the deposition of a basement membrane and the coverage by mural cells, vessels stabilize allowing an efficient blood flow. The oxygen levels rise and, in consequence, angiogenesis is shut down.

During this process, endothelial cells are exposed to gradients of oxygen (Papetti and Herman 2002). A drop in oxygen tension reduces PHD and FIH activity and stabilizes HIF-1α and HIF-2α, which activate a transcriptional response (Wong et al. 2013). In contrast to other cell types, however, HIF-1α only gets stabilized at very low oxygen tensions, whereas HIF-2α can even activate transcription in cooperation with the transcription factor ETS under normal oxygen conditions (Dutta et al. 2008; Le Bras et al. 2007). In addition, endothelial-specific deletion of HIF2α in adult mice causes vascular disruption and permeability. Altogether, these observations suggest that HIF-2α is required for the maintenance of a stable, quiescent vasculature, likely also when tissue oxygenation is restored to the normal level (Mazzone et al. 2009; Skuli et al. 2009). Consistently, deletion of HIF-2α specifically in the endothelium increases angiogenesis in cancer and ischemia; however, the newly formed vasculature is immature and poorly functional, supporting the idea that HIF-2α has an inhibitory effect on vessel sprouting while promoting vessel maturation (Skuli et al. 2009, 2012). Vice versa, HIF-1α is more relevant in hypoxia when angiogenesis is needed to restore tissue oxygenation. In cancer, endothelial-specific deletion of HIF-1α results in loss of the angiogenic potential, with reduced endothelial cell proliferation and migration (Tang et al. 2004).

In light with the current model, when VEGF is released in response to a situation of hypoxia, the endothelial cell that senses the highest VEGF concentration in the neighborhood of the hypoxic region engages on the highest VEGFR2 signaling. Thus, this endothelial cell will extend filopodia and will start to migrate towards VEGF, acquiring a "tip cell" phenotype that leads the new sprout at the forefront (Gerhardt et al. 2003). As the tip cell moves towards the hypoxic area, PHDs and FIH become inactivated, favoring HIF-1 and/or HIF-2 accumulation. HIF-1α is required for VEGF and VEGFR2 induction and further maintains the tip cell phenotype (Skuli et al. 2012; Tang et al. 2004). VEGFR2 signaling induces the expression of the Notch ligand DLL4 on the surface of the tip cell membrane. Binding of DLL4 from the tip cell to NOTCH2 expressed by the adjacent endothelial cells prevents these cells to become tip cells (Hellstrom et al. 2007). Instead, endothelial cells trailing behind the tip cell become "stalk cells," by proliferating in response to VEGF and thus allowing the elongation of the new sprout. At this stage, since lumen formation has not occurred yet, blood flow is not present; thus, these cells are most probably under hypoxia, although less severely than at the tip cell position. Nevertheless, how this affects HIF signaling remains unclear. Literature evidence supporting the proliferative function of HIF-1α and the maturation function of HIF-2α would suggest that both factors are stabilized (Skuli et al. 2012). VEGF signaling triggered by HIF-1 is then weakened by HIF-2 and/or NOTCH2-mediated transcription of VEGFR1 and NOTCH2-mediated suppression of VEGFR2

(Chappell et al. 2009; Jakobsson et al. 2010; Mazzone et al. 2009). In a dynamic process, this final event will lead towards a mature quiescent vasculature, where endothelial cells acquire a "phalanx cell" phenotype, favored by HIF-2α accumulation, and the consequent increased expression of VEGFR1 and VE-cadherin, further decreasing VEGFR2 signaling and tightening the endothelial barrier (Mazzone et al. 2009). As suggested above, this step might start in hypoxia but perpetuate at higher oxygen tension since HIF-2α mediates VE-cadherin and VEGFR1 transcription in normoxic conditions as well (Dutta et al. 2008; Le Bras et al. 2007).

Thus, the balance between VEGFR1 and VEGFR2 is important to define the endothelial cell phenotype, and it is highly regulated by NOTCH signaling (Jakobsson et al. 2010). Less clear is the role of HIF-1α and HIF-2α in these processes, although suggestive findings highlight the relevance of these two transcription factors in endothelial cell phenotype determination (Skuli et al. 2009, 2012; Tang et al. 2004). Given the different affinities of PHDs and FIH for oxygen (Km = 100–250 μM for PHDs; Km = 90 μM for FIH), one could speculate that FIH inactivation is instrumental in anoxia, thus in the migratory, non-perfused tip cell, whereas PHD inactivation is more relevant during mild changes in oxygen tension as it likely occurs along vessel elongation and so at the level of the stalk/phalanx cells. Endothelial cell-specific deletion of PHD2, which results in preferential HIF-2-mediated transcription of VE-cadherin and VEGFR1, represents an important example illustrating tumor endothelial normalization with improved vessel perfusion and oxygenation (Mazzone et al. 2009). Besides PHDs and FIH, HIF in endothelial cells can also be regulated by other factors such as FGF and EGF, which are able to stabilize HIF-2 (Dutta et al. 2008). However, how cytokine-mediated HIF regulation affects endothelial cell behavior still remains largely unknown, leaving open opportunities in this research field.

Also at the level of the deadly metastatic spread of tumors cells, HIF-1 and HIF-2 seem to play distinctive roles. While endothelial-specific deletion of HIF-1α diminishes lung metastasis, HIF-2α loss enhances the metastatic spread. This effect was attributed to HIF isoform-specific regulation of nitric oxide (NO) homeostasis, with HIF-1α loss resulting in reduced NO release and HIF-2α deletion enhancing it. The contrasting HIF-1-/HIF-2-dependent regulation of NO release results in opposite effects on endothelial tumor cell transmigration. An HIF-1α-deficient endothelium enhances, and an endothelium with disrupted HIF-2α expression inhibits, the transmigration of tumor cells (Branco-Price et al. 2012). As outlined above, genetic deletion of PHD2 results in tumor vessel normalization, namely, improved endothelial lining, barrier, and stability, mediated by an HIF-2-driven response. These structural changes result in a better tumor vessel perfusion and thus oxygenation. As a consequence tumor cell intravasation and metastasis are reduced (Mazzone et al. 2009). There are no reports so far for a causal link between endothelial-specific PHD1 and/or PHD3 deletion and vessel (mal)function. Overall, PHD inhibition in tumor endothelial cells promises a beneficial outcome for cancer treatment.

Besides the classical HIF-mediated events, other mechanisms have been considered in angiogenesis as the recently discovered ataxia-telangiectasia-mutated kinase (ATM) activation in response to ROS accumulation in the sprouting vasculature.

Notably, this VEGF-independent mechanism seems to be present only in pathological angiogenesis (tumor and retinopathy), as ATM is not active in normal vessels. Another study reported that increased oxidative stress secondary to the loss of Ubiad1 was responsible for endothelial regression and consequently cardiovascular defects in a zebrafish model. Given the transversal presence of oxidative stress in several vascular-related pathologies, further insights on ROS signaling in this context would be important to better understand the basis of potential therapeutic strategies.

Tumor angiogenesis was envisioned to be essentially regulated by cancer cells expressing pro-angiogenic factors in response to hypoxia and lack of nutrients. There is now abundant evidence that stromal cells in the tumor microenvironment are instrumental in switching on angiogenesis, such as the tumor-associated macrophages (TAMs), which tend to accumulate in or adjacent to poorly vascularized, hypoxic sites (Lin and Pollard 2007; Murdoch et al. 2005). TAMs react to hypoxia by increasing the expression of HIF-mediated pro-angiogenic genes such as VEGF, promoting dysfunctional angiogenesis and further increasing tumor hypoxia. This connection is illustrated by the specific deletion of VEGF in macrophages, which attenuates tumor angiogenesis and leads to a normalized tumor vasculature (Stockmann et al. 2008). Similarly, production of placental growth factor (PlGF), a member of the VEGF family, promotes angiogenesis (Rolny et al. 2011). Because of the surge of cytokines they release, TAMs are involved in acquired resistance to anti-VEGF(R) agents (Fischer et al. 2007). Remarkably, besides hypoxia, also TAM-derived cytokines such as angiopoietins, IL4, and IL12, can promote an angiogenic/arteriogenic phenotype by mimicking a situation of pseudohypoxia consisting in the downregulation of PHD2 or upregulation of HIF (Hamm et al. 2013; Takeda et al. 2010, 2011).

12.5 Oxygen-Sensing Pathways as Future Therapeutic Targets

The connection between disturbed oxygen homeostasis and pathological conditions supports the notion that oxygen signaling pathways are co-opted to assist disease progression. Interfering in these signaling pathways holds the promise of novel and advantageous treatment options. The first evidence for potentially improved therapeutic prospects when targeting oxygen-sensing enzymes stems from drug-mediated inhibition of PHDs, which stimulates angiogenesis and generates a more mature vascular network. Genetic studies, however, allow a more profound understanding of how interfering in the function of one specific oxygen-sensing enzyme might alter disease progression.

Of utmost importance for cancer therapeutics is the finding that chemotherapeutic treatments in combination with genetic deletion of PHD2 lead to a beneficial outcome (Leite de Oliveira et al. 2012); on the one hand, because tumor growth and metastasis are reduced, and on the other hand, as chemotherapeutic side effects on

healthy organs are diminished. Endothelial-specific PHD2 deletion alone was previously shown to normalize the tumor vasculature and reduce metastatic tumor cell spread, however, without influencing the primary tumor growth (Mazzone et al. 2009). Remarkably, PHD2 inactivation synergizes with suboptimal doses of chemotherapeutics resulting in the reduction of tumor growth and metastasis, holding the promise for improved chemotherapeutic regimens. Normalization of the tortuous tumor vasculature through the inhibition of PHD2 function allows a better delivery of chemotherapeutic agents to the primary tumor thus leading to more effective eradication of malignant cells. Importantly, the advantageous combination of PHD2 inactivation in endothelial cells and chemotherapy is also present regardless of PHD2 levels in cancer cells, as inhibition of this oxygen sensor would likewise target different tumor cell compartments when achieved in a pharmacological setting (Leite de Oliveira et al. 2012). Nonetheless, pharmacological intervention on the FIH/PHD/HIF axis warrants caution. FIH and the respective PHD isoforms have been shown to differentially regulate HIF-1α and HIF-2α. Given the nonoverlapping and even opposing functions of HIF-1α and HIF-2α in tumor endothelial cells, namely, their isoform-specific regulation of metastasis (Branco-Price et al. 2012), one requirement for the successful use of drugs inhibiting these oxygen sensors is their selective activity for the desired enzyme. This might not only bypass undesired side effects due to the induction of unexpected HIF isoform-mediated functions; it might also circumvent effects evoked by interference in HIF-independent PHD or FIH-specific signaling pathways, as there is increasing evidence for HIF-independent interaction partners of oxygen-sensing enzymes that might critically alter the response (Wenger et al. 2009).

However, given the exceeding importance of oxygen signaling in angiogenic processes and their relevance in therapy-induced responses, pharmacological exploitation of oxygen-sensing pathways might serve as a gold mine for future drug discovery.

It is well described that chemo- and radiotherapeutic treatments are linked to ROS production. This indirect mode of action might eliminate cancer cells concomitant with the direct effect of chemo- or radiotherapy. However, adversely ROS also threats the survival of healthy tissue (Anscher et al. 2005; Berthiaume and Wallace 2007; Pabla and Dong 2008). By experiencing DNA damage, formerly intact cells might be endangered to undergo oncogenic transformation when exposed to ROS. In such a scenario, inhibition of PHD2 has been shown to be an advantage in an HIF-1-/HIF-2-driven detoxification program. ROS are assumed to inhibit PHD function via oxidation of the iron (II) bound in the active core of the protein, required for its enzymatic function. By inhibiting PHD function, cells are preadapted to these stress conditions, thus resulting in a more efficient response to oxidative damage and an overall beneficial outcome. Nevertheless, one has to take into consideration that in the studies performed so far, genetic deletion of PHD2 occurred before initiation or in the initial phases of tumor growth (Mazzone et al. 2009; Leite de Oliveira et al. 2012). Thus, it will be significant for future therapeutics whether such treatment regimen will prove effective on a well-established (metastatic) tumor.

In conclusion, exploring the activity of oxygen-sensing enzymes in the endothelium is a promising area for drug discovery, thus offering the possibility for novel treatments tailored to diseases implicating oxygen disturbances.

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