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REVIEW PAPER

A Dialogue between the Hypoxia-Inducible Factor and the Tumor Microenvironment

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Abstract The hypoxia-inducible factor is the key protein responsible for the cellular adaptation to low oxygen tension. This transcription factor becomes activated as a result of a drop in the partial pressure of oxygen, to hypoxic levels below 5% oxygen, and targets a panel of genes involved in maintenance of oxygen homeostasis. Hypoxia is a common characteristic of the microenvironment of solid tumors and, through activation of the hypoxia-inducible factor, is at the center of the growth dynamics of tumor cells. Not only does the microenvironment impact on the hypoxia-inducible factor but this factor impacts on microenvironmental features, such as pH, nutrient availability, metabolism and the extracellular matrix. In this review we discuss the influence the tumor environment has on the hypoxia-inducible factor and outline the role of this factor as a modulator of the microenvironment and as a powerful actor in tumor remodeling. From a fundamental research point of view the hypoxia-inducible factor is at the center of a signaling pathway that must be deciphered to fully understand the dynamics of the tumor microenvironment. From a translational and pharmacological research point of view the hypoxia-inducible factor and its induced downstream gene products may provide information on patient prognosis and offer promising targets that open perspectives for novel "anti-microenvironment" directed therapies.

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Abbreviations

2-OG	2-oxoglutarate
AMPK	adenosine monophosphate kinase
Ang-2	angiopoietin-2
bHLH	basic helix-loop-helix
BNIP3	Bcl2/adenovirus E1B19kD protein interacting protein 3
BNIP3L	Bcl2/adenovirus E1B19kD protein interacting protein 3 like
CA IX	carbonic anhydrase IX
COX	cytochrome c oxidase
CXCR4	cytokine receptor
HER2/Neu	epithelial growth factor 2
ECM	extracellular matrix
pHe	extracellular pH
ERK	extracellular-regulated kinase
FIH	factor inhibiting HIF-1
Glut	glucose transporter
HBO	hyperbaric oxygen therapy
HIF	hypoxia-inducible factor
HRE	hypoxia-response elements
pHi	intracellular pH
LDH-A	lactate dehydrogenase-A
LOX	lysyl oxidase
mTOR	mammalian target of rapamycin
MMP	matrix metalloproteases
LON	mitochondrial protease
MCT	monocarboxylate transporter
NHE	Na^+/H^+ exchanger

OXPHOS	oxidative phosphorylation
ODD	oxygen-dependent degradation
CBP	p300/CREB binding protein
pO_2	partial pressure of oxygen
PI3K	phosphatidylinositol-3-kinase
PHD	prolyl hydroxylase domain
PDK-1	pyruvate dehydrogenase kinase-1
TPZ	tirapazamine
TAD	transcriptional activation domain
TCA	tricarboxylic acid cycle
TSC1/ TSC2	tuberous sclerosis complex
VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau

Introduction

A tumor is not just a mass of individual cancer cells multiplied infinitely but is constituted of cancer stem cells, mature cancer cells, metastatic cancers cells, stromal cells, endothelial cells, macrophages…all embedded in an extracellular matrix (ECM). These cells must be considered within the context of the tumor matrix, in which they communicate with each other and interact strongly with their local microenvironment. To obtain a better understanding of the mechanisms of tumorigenicity, tumor development and metastasis an overview of the whole system integrating the characteristics that are fundamental for cell growth dynamics is essential. The tumor ecosystem can be seen as an assembly of constraints put on cancer cells to which they respond and adapt. The hypoxia-inducible factor (HIF) acts as a key feature in microenvironmental adaptation of tumor cells. We will describe the regulation of the expression and activity of HIF in focusing on specific environmental conditions including oxygen and nutrient availability as well as the pH and the extracellular matrix. We will examine how these constraints influence directly or indirectly the HIF signaling pathway, and in turn how the HIF downstream effectors can remodel the microenvironment.

Microenvironmental Oxygen Determines HIF-mediated Gene Profiling

Oxygen Distribution in Tumors

Through evolution, Mother Nature provided oxygen as a major resource for life. A cancer cell in close vicinity to a blood vessel is able to use the plentiful supply of oxygen carried by the blood to obtain energy through oxidative phosphorylation for rapid growth. Nonetheless, cancer cell oxygenation is limited by three convergent mechanisms: (1) As a tumor mass expands, cancer cells develop distant from blood vessels. The characteristic diffusion distance of oxygen results from the conjunction of the passive physical diffusion and active cellular oxygen consumption. Since this diffusion distance within tumor tissue is approximately 70 μm cancer cells proliferating at the periphery of blood vessels dispose of only a low oxygen availability [1, 2]. (2) Moreover, blood vessels in solid tumors are known to be highly disorganized and chaotic. This loss of coherent structural organization diminishes the oxygen profile of the tumor. To characterize the vascular network in tumors an original and very informative mathematical tool: the fractal character of the vascular system has been described [3, 4]. Histological studies have shown that the fractal dimension of blood vessels correlates with the nature of the tumor tissue. This dimension is higher in tumors than in non-malignant tissues, which reveals that the vessels present more irregularity and less homogeneity. Consequently perfusion and irrigation in tumors is often not optimal. (3) Finally the hematological status of cancer patients is frequently altered, either by the disease itself or by chemotherapy related toxicity. Hence these parameters converge to give inadequate irrigation of the tumor and thus low nutrient and oxygen availability. For example the median partial pressure of oxygen (pO_2) in the normal cervix is 42 mmHg compared to only 10 mmHg in squamous carcinomas [1, 2].

A tumor can be considered as a mosaic of blood vessels from which oxygen will diffuse into the tumor matrix, creating a myriad of gradients of oxygen concentrations within a hypoxic range. Clinical studies associate the hypoxic status of a tumor with bad prognosis and resistance to chemo- and radio-treatment [5]. Chemo-resistance can be explained partly by an inefficient diffusion of drugs to poorly irrigated areas due to an increase in internal fluid pressure. Furthermore, hypoxic radio-resistance is explained by the fact that radiotherapy uses oxygen to generate cytotoxic free radicals but also by active anti-apoptotic cellular mechanisms induced by hypoxia [6].

Impact of Environmental pO_2 on HIF Activity: Oxygen-Sensing

Hypoxia is a condition encountered in both physiological (embryonic development) and pathophysiological (ischemia diseases, diabetes, atherosclerosis, Alzheimer's disease, chronic obstructive pulmonary disease, inflammatory disorders, pre-eclampsia, psoriasis and cancer) situations. Cells exposed to a hypoxic stress must rapidly adapt, otherwise an imbalance in their energy supply/consumption ratio ensues. Hypoxia activates a global signaling network centered on the key element, the hypoxia-inducible factor (HIF) [5, 7, 8]. Thus low oxygen tension is the prototypic

regulator of HIF. This factor transcriptionally activates a panel of microenvironmental adaptation genes that contribute to rapid cell survival [5, 9, 10]. In addition to survival, the HIF signaling pathway confers on cancer cells an arsenal that allows them to become more aggressive. So, how is the transcriptional machinery activated in response to variations in the pO_2? HIF is a heterodimer composed of an O_2 regulated alpha subunit and a constitutively expressed beta subunit (Fig. 1). Several HIF alpha and beta isoforms have been described in mammals, where HIF-1α, HIF-2α and HIF-1β are the best characterized to date.

The basic helix-loop-helix (bHLH) domain in the N-terminal part of HIFα confers specific DNA recognition. DNA regions called hypoxia-response elements (HRE) are sequences characteristic of HIF downstream target genes. In hypoxia the heterodimer transactivates target HRE containing genes (1 to 2% of the genome is probably modulated by hypoxia). HIFα functional recruitment of RNA polymerase is driven by its transcriptional activation domains (TAD) where HIF-1α and HIF-2α share the distinctive feature of bearing two TADs: N-terminal (N-TAD) and C-terminal (C-TAD) (Fig. 1). The half-life of the HIFα protein in the presence of 21% oxygen is less than 5 min, and increases to 60 min as the O_2 concentration decreases to 1%. On reoxygenation, the HIFα protein is degraded after only a few minutes. As the O_2 concentration does not impact on the HIFα mRNA level, HIF is regulated exclusively posttranslationally by the pO_2.

The mechanisms by which cells sense and respond, through HIF, to environmental oxygen was revealed in the early 2000s. Dioxygenases catalyze protein hydroxylation, a reaction requiring ambient O_2, that leads to the formation of an −OH group on HIFα proteins. Oxygen-sensor dioxygenases target the HIFα subunit and are thus termed HIF-hydroxylases. Two types of HIF-hydroxylases have been characterized to date: (1) HIF-prolyl hydroxylases; though preferably referred to as prolyl hydroxylase domain (PHD) proteins since substrates other than HIF have been and are probably still to be identified, and (2) HIF-asparaginyl hydroxylase also called factor inhibiting HIF-1 (FIH) [11].

Three major PHD isoforms have been described and catalyze the hydroxylation of two prolyl residues of the HIFα subunit, an action that strictly depends of the pO_2 [12–14]. These two residues are located in the oxygen-dependent degradation (ODD) domain of HIFα and when hydroxylated this domain shows a strong affinity for the von Hippel-Lindau (VHL) protein, a component of a E3 ubiquitin ligase complex. The consequence is poly-ubiquitination and targeting of HIFα for degradation by the proteasome (Fig. 2). Thus an increase in HIFα protein levels in hypoxia is due to the inhibition of this oxygen-dependent degradation process.

The second oxygen sensor FIH catalyzes asparaginyl hydroxylation in the C-terminal TAD of HIFα when in the presence of oxygen [11] (Fig. 2). This results in a change in the local hydrophilic/hydrophobic balance of the protein and impairs the interaction between the hydroxylated C-TAD and one of its essential coactivators: p300/CREB binding protein (CBP) [15, 16]. This abolishes the C-TAD activity, however, inhibition is progressively alleviated as the pO_2 decreases towards anoxia.

Thus, HIF hydroxylation is a double locking system. In normoxia HIF is degraded by highly active PHDs but if a

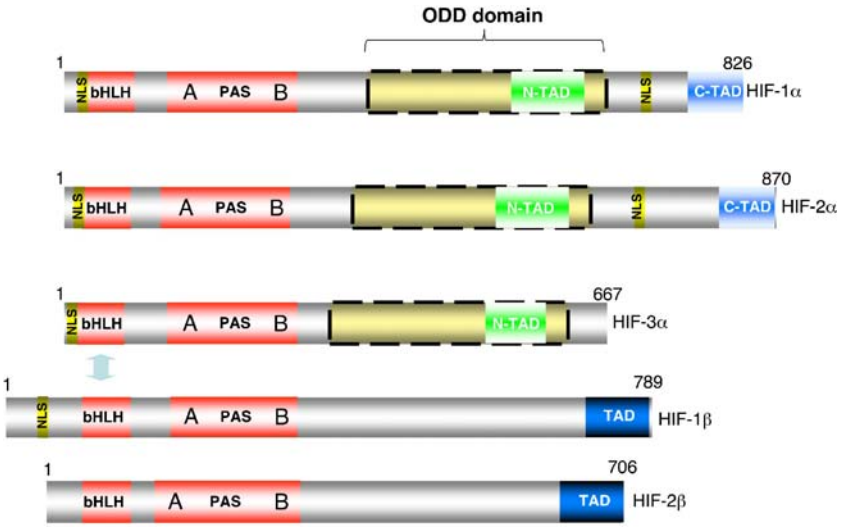

Fig. 1 Schematic of the structure of the three HIFα and the two HIFβ isoforms. NLS, nuclear localization signal; bHLH, basic helix-loop helix-domain; PAS, per arnt sim domain subdivided into PAS A and PAS B; ODD, oxygen-dependent degradation domain; TAD, transactivation domain. HIF-1α and HIF-2α have two distinct TAD, in the C- (*C-TAD*) and N- (*N-TAD*) terminal domains. The PAS and bHLH domains are dedicated to dimerization and recognition of target DNA sequences

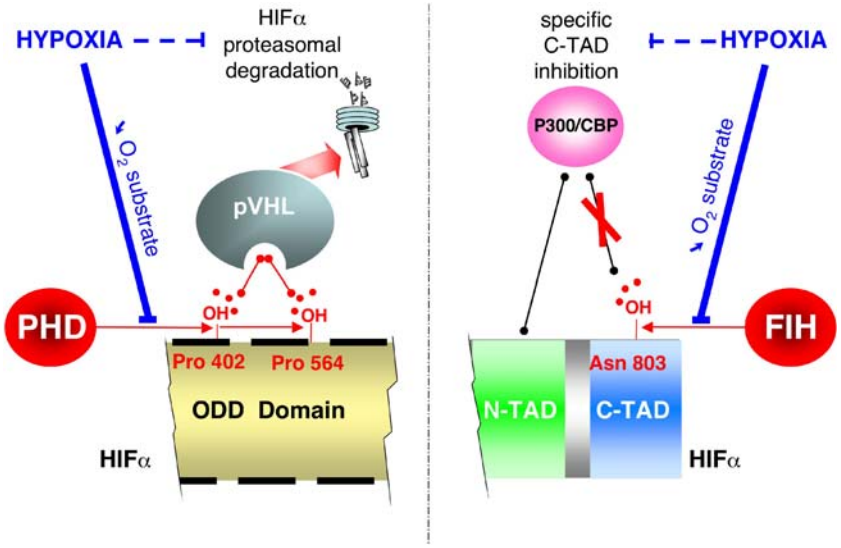

Fig. 2 Proline hydroxylation drives HIFα stability and asparagine hydroxylation drives HIF activity. *Left panel*: Under normoxic conditions, the interaction between two hydroxy-prolyls (Pro 402 and 564 for human HIF-1α on the schematic) and the VHL protein leads to the degradation of HIFα by the proteasome. Under hypoxic conditions, because of the lack of the oxygen substrate, the HIF-prolyl hydroxylase domain (PHD) proteins do not hydroxylate these two prolyl residues, leading to stabilization of HIF-1α. *Right panel*: In normoxia, the hydroxylation of an asparagine residue (Asn 803 for HIF-1α on the schematic) impairs interaction between the HIFα C-terminal transactivation domain (C-TAD) and its co-activator p300/CBP. Under hypoxic conditions, because of the lack of the substrate oxygen, HIF-asparagine hydroxylase (FIH) does not hydroxylate this asparagine residue, leading to increased activity of the HIFα C-TAD. Interestingly the N-TAD is not affected by asparaginyl hydroxylation. Symbols: ⟶, stimulation; ⟞, inhibition; ●—●, interaction

pool of HIF escapes this system it will nonetheless be inactivated by FIH. When the oxygen tension decreases, HIF is stabilized and becomes fully active *via* its two TADs (Fig. 2).

Other forms of posttranslational modification of HIFα including phosphorylation, SUMOylation, S-nitrosylation and acetylation (the mechanism of the latter remains controversial, reviewed in [17]), have been reported to influence stability and activity [18]. Such modifications may also contribute substantially to the hypoxic response of cells depending on the environmental conditions.

HIF-Induced Gene Profile Selectivity

Selective modulation of the spectrum of genes induced by HIF can occur in two ways. First, HIF-1α and HIF-2α can differentially transactivate a series of genes. For example *adrenomedullin* is a HIF-2 only gene in mouse stem cells, *carbonic anhydrase IX (ca9)* is a HIF-1 targeted gene in human HeLa cells, whereas *phd3* is regulated by both HIF-1 and HIF-2 in human cells [19, 20]. Relative HIF-1 and HIF-2 activities differ depending on the cell type. Second, through the bifunctional TAD activity of HIF-1α [20] (Fig. 3a). FIH specifically hydroxylates the C-TAD, and does not touch the N-TAD. So experimentally, FIH inhibits only a subset of HIF-1-target genes. Knowing this we put forward a model that divides the HIF-1 spectrum of genes into two categories: one that is C-TAD sensitive and the other N-TAD only sensitive. FIH would inhibit the C-TAD spectrum and as a result pilot the shift between these two categories of HIF-1 target genes. This double TAD regulation model can be easily transposed to HIF-2α, since this isoform is regulated in a highly homologous mode.

To fully understand the impact of this model, these data must be considered in the context of an oxygen gradient within a tissue. How do oxygen-sensors drive HIF activity in the physiological context? Studies into the enzyme activity showed that PHD and FIH have very different requirements in terms of the oxygen concentration. *In vitro*, the Km of PHD for O_2 is three times greater than that of FIH [21]. This corresponds respectively to oxygen concentrations of approximately 24% and 8% (compared to 2% for collagen prolyl hydroxylases which are consequently insensitive to hypoxia). This means that PHD should be inactivated in milder (theoretically 3 times more oxygen) hypoxia compared to FIH. However, experimentally, *in cellulo*, FIH activity has been reported at 0.2% oxygen whilst HIF-1α is maximally stabilized by complete inactivation of PHD [22]. Thereby there should be areas of mild hypoxia in the tumor where HIFα is stabilized by the inactivation of PHD but still partially inhibited by FIH-dependent hydroxylation of its C-TAD (Fig. 3b). In such areas the N-TAD repertoire of HIF-dependent genes should be induced. Further, under severe hypoxia, FIH should also be inactivated, thus releasing the C-TAD sensitive repertoire. Thus this model provides a link between the induction of a certain gene at a certain localization and the oxygen-dependent activity of the oxygen sensor FIH (Fig. 3b).

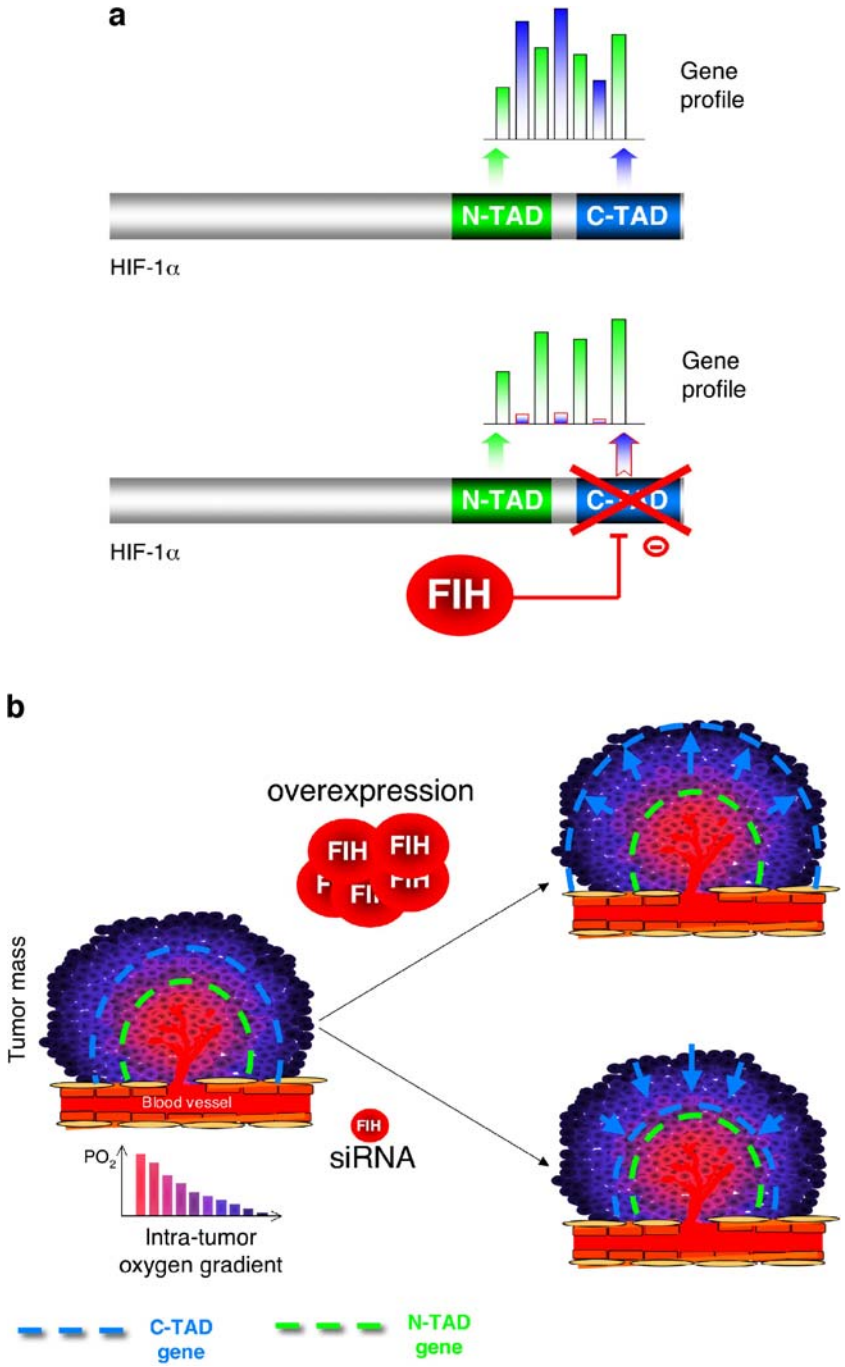

Fig. 3 Model that questions the molecular significance of the two HIF-1α TAD and the impact of the oxygen sensor FIH on the intra-tumor location of gene expression as a function of the oxygen gradient. **a** *Upper panel*: If the two HIF-1α TAD are functionally different they would target different genes. In *green* are represented the potential N-TAD-only target genes while in *blue* are represented the potential C-TAD sensitive genes. *Lower panel*: By targeting specifically the C-TAD, FIH would inhibit only a subset of HIF-dependent genes. Consequently, FIH would not be a pure inhibitor but rather a switch between two categories of HIF spectrum genes. **b** In accordance with our working model, overexpression of FIH should delocalize C-TAD sensitive genes (*blue dotted line*) to highly hypoxic areas. In contrast FIH inhibition by siRNA should delocalize C-TAD sensitive genes to moderately hypoxic areas in the vicinity of blood vessels (*lower panel*). In parallel, N-TAD only genes (*green dotted line*) should not be sensitive to modulation of FIH activity

Moreover it confers a new and more subtle role to FIH which is no longer considered as a pure inhibitor of HIF but rather as a discriminator between two categories of HIF-dependent genes: N- and C-TAD targeted genes.

In summary, HIF gene induction is like a 'four-handed concerto' directed by two related but distinct transcription factors (HIF-1 and -2), each able to independently target genes with their two transactivation domains (N- and C-

TAD). Yet this model still needs further investigation to fully appreciate the variation in the repertoire of genes when induced in a more physiological situation.

The Microenvironment Impacts on HIF and HIF Impacts on the Microenvironment

So HIF is highly regulated by oxygen via prolyl- and asparaginyl-hydroxylation, but in turn, a series of HIF target genes impact on oxygen homeostasis. In the following section, we will describe how this reciprocity can be generalized to tumor features including oxygen, nutrients, pH and the extracellular matrix through a dialogue between HIF and the microenvironment.

HIF-Directed Modification of Oxygen Homeostasis

A number of HIF-induced genes directly influence environmental oxygenation (Fig. 4a). One of the best known is *vascular endothelial growth factor* (*vegf-a)* which favors the growth of new blood vessels into hypoxic zones [23]. Endothelial tip cells are capable of guiding the growth of blood capillaries towards hypoxic areas rich in VEGF-A. These endothelial tip cells emit long filopodia that are very rich in VEGF-R2 [24]. Hence the chemotactic gradient of VEGF-A is a guide for neo-vascularization to favor the sprouting of newly generated vessels toward poorly oxygenated areas. This phenomenon of angiogenesis, which is highly HIF-dependent [25], then substantially remodels the oxygen profile of the ischemic tissue or hypoxic tumor (Fig. 4b).

Another HIF-dependent molecule that is of great importance to blood vessel network remodeling is angiopoietin-2 (Ang-2) [26]. This protein antagonizes Ang-1 binding to its endothelial cell receptor Tie-2 and thereby prevents blood capillary maturation. The stabilization of the vascular network is dependent on activation of the Notch pathway, which results in quiescence of endothelial cells [27]. In this state, blood vessels are not sensitive to VEGF-A. So Ang-2, secreted from hypoxic regions of the tumor, by antagonizing its homologue Ang-1 destabilizes the organization of capillaries. This destabilization prior to blood sprouting is an essential step in making endothelial cells sensitive to VEGF-A and in initiating angiogenesis.

In addition to modulating extracellular oxygen homeostasis, HIF can impact on cellular respiration. It has been known for a long time that under low oxygen conditions cells shift from metabolism of glucose through oxygen-consuming oxidative phosphorylation (OXPHOS) in mitochondria to non-oxygen dependent glucose metabolism to lactic acid in the cytoplasm. This switch has now been revealed to implicate, at least in part, [28] HIF-induced

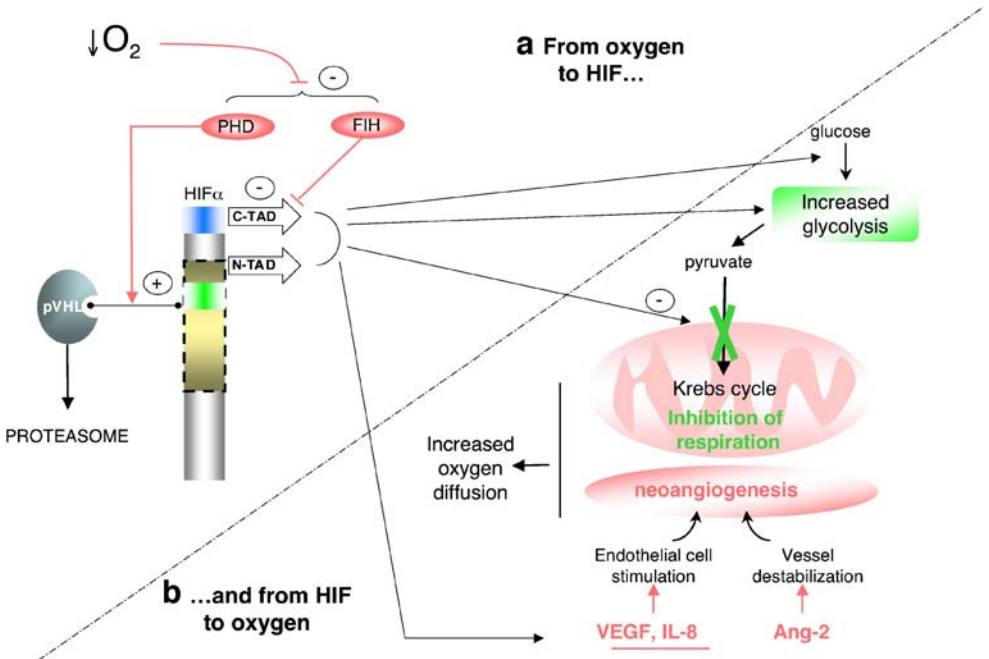

Fig. 4 From oxygen to HIF and from HIF to oxygen. **a** A drop in the partial pressure of oxygen results in the inactivation of PHDs and FIH. Consequently the HIF pathway is activated, due respectively to inhibition of proteasomal degradation and a release of the C-TAD activity. **b** HIF in turn impacts on oxygen homeostasis by a double mechanism touching metabolism (*green*) and oxygen distribution/angiogenesis (*red*). HIF increases the glycolytic flux and represses the entrance of pyruvate into the Krebs cycle. The consequence is a decrease in oxygen consumption by mitochondrial respiration. In parallel HIF promotes angiogenesis *via* stimulation of vascular endothelial growth factor (*VEGF*), interleukin-A (*IL-8*) and angiopoitin-2 (*Ang-2*) leading to endothelial cell stimulation and blood vessel destabilization. In combination, HIF simultaneously increases tissue perfusion and decreases local oxygen consumption, thus promoting oxygen diffusion through hypoxic areas

glucose capture, glycolytic flux and inhibition of OXPHOS (Fig. 4b). Thus, HIF has a dual action on oxygen homeostasis. First, it restores, in the long-term, the oxygen supply through neo-vascularization and it promotes rapid reduction of O_2 consumption by reducing cell respiration.

Impact of Environmental Nutrients on HIF

Even if oxygen is the main and prototypic driving force of the HIF pathway, other potential HIF regulators have been identified. The glucose concentration in tumors follows a gradient that is very similar to that of the pO_2 gradient described above. The topologies of these gradients are intimately linked to each other. Glucose, like oxygen, is delivered by the blood circulation and diffuses into tissues for cell capture.

The idea that microenvironmental nutrients could influence HIFα originated from the fact that the enzymatic reaction catalyzed by oxygen-sensors is dependent on the metabolite 2-oxoglutarate (2-OG). *In vitro* studies show that 2-OG is a co-substrate for HIF prolyl and asparaginyl hydroxylation by respectively, PHD [12] and FIH [11]. Thus, the PHD and FIH activities could be impaired by reduced levels of 2-OG. Since 2-OG is a product of the tricarboxylic acid (TCA) cycle (also Krebs cycle) and thus of glucose metabolism glucose availability could influence activity. The normal level of 2-OG ranges from 50 et 230 μM [29] while the *in vitro* Km for the PHDs has been estimated to be about 60 μM [30]. This level of affinity would classify the PHDs as nutritional sensors. Thus, through 2-OG the PHDs are sensitive to TCA cycle activity and the balance between aerobic and anaerobic metabolism.

A decrease in the amount of glucose provided to hypoxic cells was shown to result in a decrease in the level of HIF-1α [31] or to have no effect [32]. The explanation for this difference may lie in either the difference in cell lines or the level of hypoxia (0.1 or 1.0% oxygen) used. Thus, the sensitivity to glucose *in vivo* remains to be further clarified.

Another type of nutritional stress, amino-acid depletion, is able to decrease HIF protein expression by activating the adenosine monophosphate kinase (AMPK) and mammalian target of rapamycin (mTOR) pathway (Fig. 5a). In a situation of an energy imbalance and decrease in intracellular ATP, the tumor suppressor serine/threonine kinase LKB1 phosphorylates its downstream effector AMPK that has the potential to target the tuberous sclerosis complex (TSC1/TSC2) [33]. Once activated, the TSC1/TSC2 complex inhibits mTOR resulting in a decrease in protein synthesis. Even if it is generally accepted that a decrease in mTOR activity has a negative effect on HIFα expression, the mechanisms are controversial. Indeed, HIF protein synthesis has been shown to be upregulated by mTOR in various cell types [34, 35]. Genetic evidence reinforces this link at the level of translation between mTOR and HIF, by the presence of a 5′-terminal oligopyrimidine tract (5′-TOP) sequence in the 5′-untranslated region (5′-UTR) of HIF-1α. 5′-TOP sequences can be driven by the S6 ribosomal protein that is itself a downstream target of mTOR *via* p70S6Kinase. Yet it has been demonstrated that mTOR does not change HIF protein synthesis but modifies stabilization of HIFα in prostate PC3 cells [36]. This result is consistent with the fact that the HIF 5′UTR has been demonstrated to bear an internal ribosome entry site theoretically allowing HIFα to be translated even in the absence of mTOR driven CAP dependent translation [37]. Moreover, evidence indicates that HIF protein synthesis can be controlled by stimulation of an Akt-dependent but mTOR-independent pathway in PC3 cells [38]. To complete the picture, a feedback loop allows HIF to down-regulate mTOR *via* the hypoxia inducible REDD-1 protein by activating the TSC1/TSC2 signaling integrator complex [39]. In conclusion, a close link exists between mTOR and HIF, bringing together two fundamental microenvironmental constraints: nutrient and oxygen, which are central to generation of cellular energy.

Impact of HIF on Environmental Nutrients: from Autophagy to Metabolism

Nutritional deprivation induce a cellular catabolic response termed autophagy that can rescue cancer cells from cell death. Autophagy is a phenomenon where proteins, organelles and cytoplasm of a cell are engulfed into vacuoles and degraded into constituents for recycling for maintenance of metabolism and thus cell viability. Thereby, in tumor cells where apoptosis is defective autophagy promotes cell survival. However, it is paradoxical that autophagy is associated with increased tumorigenesis by a mechanism that remains to be defined. It has been suggested that autophagy protects cells from tumor necrosis and inflammation, and diminishes DNA damage in the tumor cell response to metabolic stress [40].

While HIF is a major actor in the cell survival response to hypoxia, HIF has also been associated with cell death. Indeed several studies have pointed to the implication of the HIF-induced putative BH3 only pro-apoptotic genes *Bcl2/adenovirus E1B19kD protein interacting protein 3 (bnip3)* and *bnip3 like (bnip3L)* in hypoxia-mediated cell death [41, 42]. We and others failed to reproduce the pro-apoptotic or necrotic cell death features of ectopically expressed BNIP3 or BNIP3L in various cell types including MEFs, MCF7, PC3 or LS174 cells. Therefore, we seriously questioned the cell death function of BNIP3/BNIP3L in a hypoxic environment and rather postulated on a positive role in activating the autophagic cell survival process [43]. Using a large panel of normal cells and tumor cell lines, we

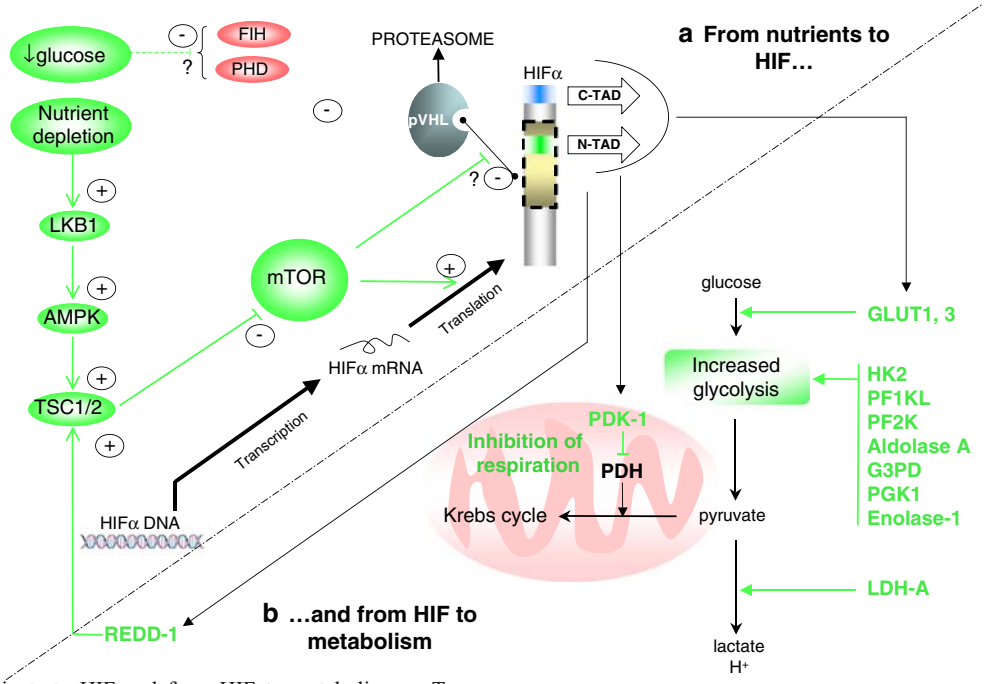

Fig. 5 From nutrients to HIF and from HIF to metabolism. **a** Two different pathways involved in the response to nutrients impact on HIF. First a decrease in the amount of glucose can inhibit the activity of FIH and PHD through decreased production of the co-substrate 2-oxoglutarate by the Krebs cycle. However, it is not sure that it is limiting *in vivo*. The question as to whether FIH and PHD might be nutritional sensors is still open to discussion. The second pathway responds to nutrient depletion reflecting a decrease in the ATP/AMP ratio. This nutritional stress activates the LKB1/AMPK/TSC1/TSC2 pathway resulting in an inhibition of mammalian target of rapamycin (*mTOR*). The action of mTOR on HIF is still a subject of debate. Essentially two options have been proposed: inhibition of HIF translation and/or inhibition of proteasomal degradation. Both converge in the activation of HIF under nutrient depletion conditions. **b** HIF can in turn impact on metabolism and cellular energy due to induction of a variety of target genes (here in *green*). It promotes glucose import *via* glucose transporters (*GLUT1, 3*) and it increases the rate of glucose consumption by inducing expression of glycolytic enzymes. HIF also promotes a shift to anaerobic metabolism by: favoring conversion of pyruvate to lactate through enhanced expression of the enzyme LDH-A and by increased expression of PDK-1 that counteracts the entrance of pyruvate into the Krebs cycle. Finally, through REDD-1, HIF stimulates the TSC1/2 complex, creating a negative feedback loop on mTOR. In summary, HIF allows cancer cells to shift to highly glycolytic anaerobic metabolism and to save energy by downregulating translation in a mTOR-dependent fashion

demonstrated that hypoxia-induced BNIP3 and BNIP3L (1% O_2) were required to initiate autophagy. Whereas siRNA-mediated ablation of either BNIP3 or BNIP3L had little effect, combined silencing of the two HIF targets suppressed hypoxia-mediated autophagy. BNIP3 and BNIP3L are therefore major players of the HIF-mediated survival response in tumors and ischemic tissues. We propose a model in which the hypoxia-induced BNIP3/BNIP3L proteins are essential to disrupt the Bcl2/Beclin1 complex ([44], Mazure et al. in preparation). It is interesting to note that autophagy is triggered in conditions of full availability of nutrients (glucose, amino acids) and growth factors; the only limiting factor is oxygen. We propose that the pO_2 is, as for angiogenesis, the primary signal capable of preparing the tumor cell to endure prolonged nutrient starvation by inducing autophagy.

From a metabolic view point, the cellular energetic balance of hypoxic tumor cells is compromised by the shift away from OXPHOS, as mentioned above, since the alternative i.e. conversion of glucose to lactate produces less energy. To make up for lower ATP production tumor cells increase the capture of glucose and the flux of glycolysis through HIF-mediated induction of genes of glucose transporters such *as glut-1*and *glut-3,* and glycolytic enzymes like *pgk1* (Fig. 5b). In addition, HIF redirects the fate of pyruvate as a result of two concordant actions: (1) by upregulating the gene *lactate dehydrogenase-a* (*ldh-a*), the protein product of which favors the conversion of pyruvate to lactate [45] (2) by repressing OXPHOS through induction of the expression of pyruvate dehydrogenase kinase-1 (PDK-1), an inhibitor of pyruvate dehydrogenase (PDH), the enzyme that drives pyruvate into the TCA cycle for mitochondrial respiration [46, 47]. Finally HIF impacts on OXPHOS by modulating cytochrome c oxidase (COX) isoform expression [48]. In fact, oxygen is the final acceptor in the mitochondrial electron transport chain and COX, or more precisely COX-4 of complex IV, catalyzes the reaction with production of H_2O [49]. When this reaction is not sufficiently active oxygen is the final electron at the level of complex I or III that produces reactive oxygen species (ROS). The expression of COX4-1 and COX4-2, two isoforms of complex IV, is differentially regulated by HIF

through the up-regulation of the mitochondrial protease LON since LON in turn degrades COX4-1 [48]. Thus, in hypoxia COX4-2 substitutes for COX4-1, an isoform that is suggested to be more efficient in oxygen utilization. In this way cells exploit more efficiently low oxygen levels and avoid production of harmful ROS under hypoxic conditions.

Impact of Environmental pH on HIF

Another important feature of the tumor microenvironment is acidosis, which is a consequence of the boost in glycolysis leading to production of lactic acid and of defective vascular evacuation of metabolic lactic acid and CO_2 [50]. As for oxygen and nutrients, a gradient of acidosis is established from blood vessels to the periphery of a tumor. The decrease in oxygen and pH was examined along a length of 300 μm from blood vessels in a breast cancer xenograft mouse model and a drop in pO_2 from 12 to 0 mmHg and in extracellular pH (pHe) from 7.4 to 6.7 was reported [51]. The question as to whether pH can influence the HIF pathway was first examined through an indirect effect on the VHL protein, a component of the HIFα ubiquitin ligase complex [52] (Fig. 6a). Acidosis was shown to induce relocalization of the VHL protein into nucleoli. However, it is still not clear if a drop in pH on its own, is sufficient to allow HIF to bypass polyubiquitylation and thus escape proteasomal degradation. Results from our laboratory show that a low pH is not necessarily associated with HIFα stabilization (unpublished results), which was corroborated in a recent publication [53].

Low pH has also been shown to modulate two HIF-induced genes: *vegf* and *bnip3*. Both pH and hypoxia can independently induce *vegf* in human glioma cells [54] and the extracellular-regulated kinase (ERK) pathway is an independent intermediary candidate up-regulating the level of *vegf* in a pH-sensitive manner [55]. Although low pH does not increase BNIP3 protein expression it increases its activity in regulating cell death in cardiomyocytes [56]. It is possible to hypothesize that HIF-1 induces BNIP3 in moderate hypoxic regions whereas the pH gradient pilots BNIP3-directed cell death only in highly acidic/hypoxic areas [43]. Thus, low pH might be a major contributor in sensitizing cells to necrotic cell death. Nevertheless the link between HIF and cell death is a highly debated subject and the precise conditions of hypoxia-induced cell death in tumors still needs to be clarified [57].

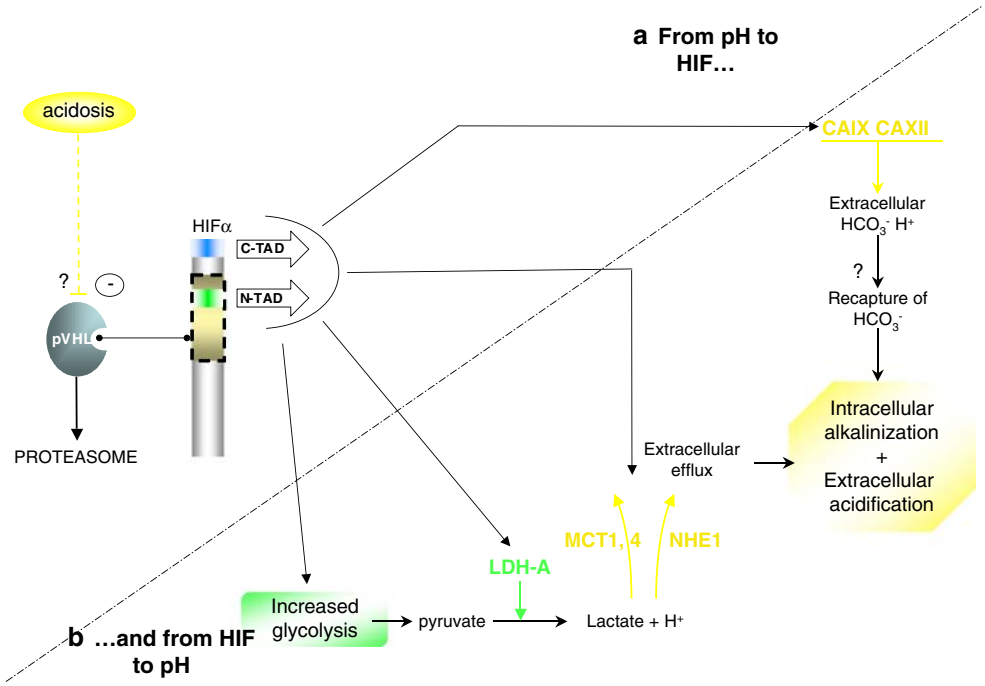

Fig. 6 From pH to HIF and from HIF to pH. **a** It has been proposed that acidosis could protect HIFα from proteasomal degradation by sequestering the component of the VHL E3 ubiquitin ligase complex in nucleoli. **b** HIF acts both directly and indirectly on the pH. On the one hand, by stimulating glycolysis (*green*) it favors the production of lactate and protons that could potentially acidify the intracellular medium. On the other hand HIF promotes intracellular alkalinization (*yellow*) by activating monocarboxylate transporter (*MCT1, 4*) and Na^+/H^+ exchanger (*NHE1, 6*) that are able to evacuate lactate and protons, respectively. Simultaneously, the membrane carbonic anhydrases (*CA*) CA IX and CA XII isoforms acidify the extracellular matrix by converting CO_2 and H_2O into HCO_3^- and H^+. Our group hypothesizes that while protons acidify the extracellular medium, bicarbonate could be recaptured in order to alkalinize the cell. In summary HIF compensates for the intracellular production of lactic acid associated with anaerobic metabolism by a process of intracellular alkalinization that is linked to extracellular acidification

HIF-Directed Modification of pH

Thus, hypoxia and acidosis cohabit in tumors but both undermine cell survival. In response, HIF triggers an adaptation strategy that leads to induction of specific genes dedicated to pH homeostasis (Fig. 6b). The gene products include the Na^+/H^+ exchanger (NHE) that extrudes protons from the cytoplasm at the expense of the Na^+ gradient and the monocarboxylate transporter (MCT) that evacuates lactic acid. The expression of the MCT4 [58], NHE1 [59] and NHE6 [47] isoforms is induced in hypoxia and the exchange activity of NHE1 is increased [58, 60]. MCT can transport lactate in both the intracellular and extracellular direction. Thus, lactate may be considered as both an acidic waste product and a source of energy, as has been shown for muscles and hypothesized for neurons [61]. In a tumor it may be envisaged that a cancer cell excretes lactate to be taken-up by neighboring fibroblasts for subsequent use by the TCA cycle. In fact, it has been shown that fibroblasts in contrast to cancer cells express a high level of PDH and a low level of PDK1 [62]. Thereby fibroblasts through an oxidative utilization of lactate may promote extracellular lactate clearance. The intracellular transport of lactate by endothelial cells that express active MCT1 [63] in tumors may also modify the microenvironment through promoting angiogenesis [64].

In addition, the membrane bound carbonic anhydrases (CA) CA IX, one of the most HIF-sensitive genes, and CA XII convert environmental CO_2 into bicarbonate which may alkalinize the intracellular pH (pHi), possibly through capture by Na^+-dependent and -independent Cl^-/HCO^{3-} exchangers [65].

Impact of the Extracellular Matrix on HIF

The extracellular matrix (ECM) indirectly impacts on all these environmental characteristics. Oxygen and nutrient diffusion are dependent on the stroma density, vasculature and cellular three-dimensional organization. Beside these physical characteristics, the chemical composition of the stroma can also be a determining feature (Fig. 7a). For instance, growth factors can modulate the HIFα protein level. Insulin like growth factor 1, epithelial growth factor and epithelial growth factor 2 (HER2/Neu) have been shown to induce HIF-1α expression in respectively, colon carcinoma cells (HCT116) [66], prostate cancer cells (DU145, PC-3, PPC-1, and TSU) [67] and breast cancer cells (MCF-7) [35]. These three factors increase the HIFα protein level *via* the phosphatidylinositol-3-kinase (PI3K)/Akt pathway targeting mTOR. However, the ERK pathway is also a candidate for HIF modulation by growth factors since HIF activity is stimulated through ERK-dependent signaling [68]. At least two mechanisms might explain HIFα up-regulation by ERK: the co-activator p300 that enhances HIF activity by binding to the C-TAD of HIF may be targeted by phosphorylation leading to a more favorable p300-HIF-TAD interaction [69], and/or HIF targeted phosphorylation may promote HIF-1α nuclear localization [70]. In addition, vasoactive hormones such as angiotensin II that are secreted by stromal cells can promote both HIF translation in a PI3K-dependent manner and HIF transcription through the action of the diacylglycerol-sensitive protein kinase C in vascular smooth muscle cells [71].

HIF-Directed Modification of the Extracellular Matrix

A series of hypoxia induced phenomena impact on the ECM. Indirectly, hypoxia-related acidosis can modulate the composition and architecture of the ECM, which in turn impacts on three-dimensional cellular organization and promotes metastasis (Fig. 7b). In a more direct fashion, hypoxia, through HIF is a modulator of lysyl oxidase (LOX) [9, 72], an enzyme that catalyzes collagen and elastin crosslinking [73]. Furthermore, stimulation of LOX by HIF is an essential intermediary of hypoxia-promoted metastasis [72, 74]. Surprisingly the canonical action of LOX on collagen and elastin might not be the key to this pathway but rather peroxide production, a side product of LOX activity, and focal adhesion kinase activity may be at the center of this pathway. However, the question as to whether LOX is associated positively or negatively with tumor development has hardly been discussed [72, 74–76], yet this ECM modifier is strongly induced by hypoxia. The significance of LOX in cancer should be reconsidered taking into account the hypoxic status of a given tumor.

Another family of proteins that modifies the ECM and that is directly involved in metastasis is the matrix metalloproteases (MMP). These proteins participate in the destruction of the ECM, an action known to be essential for cellular migration. MMP-2 and membrane type 1-MMP1 expression [77, 78] has been shown to be responsive to HIF-1 and HIF-2, respectively. In the same context, metabolic changes and HIF action have been shown to play a role in adhesion, in particular in interaction with vascular endothelial cells through a selectin- and integrin-mediated pathway [79]. Evidence for HIF negative regulation of E-cadherin might also provide another clue to elucidate how cancer cells lose contact and proceed to epithelial to mesenchymal transition leading to metastasis [80–82]. This process is particularly relevant in renal cell carcinoma where the loss of the ubiquitin ligase VHL protein function correlates with overexpression of HIF. Nonetheless the intermediary signal linking HIF and E-cadherin remains to be identified. Another group of HIF-dependent genes impacting on the remodeling of the ECM include, fibronectin, cathepsin D and urokinase plasminogen activator [5]. Yet another series of genes products also induced by HIF has

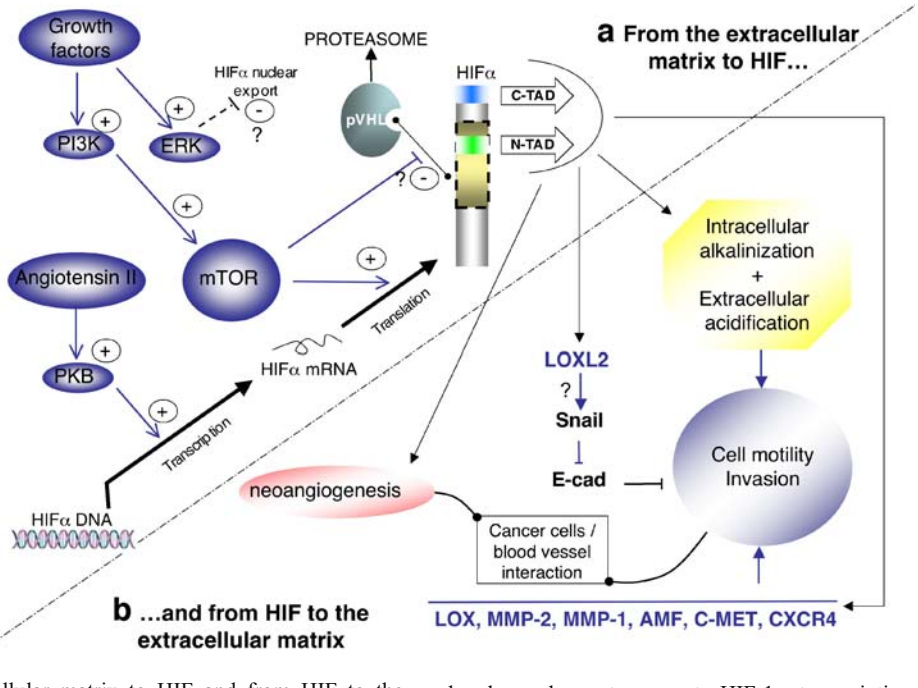

Fig. 7 From the extracellular matrix to HIF and from HIF to the extracellular matrix. **a** The extracellular matrix (*ECM*) is an essential component of the physicochemical environment of the cell, and includes fibrous proteins, glycoconjugates, growth factors and hormones. The chemical composition of the ECM can impact on HIF through growth factor stimulation. It has been shown that growth factors, by activating phosphatidylinositol-3-kinase (*PI3K*), can target mTOR and consequently HIF. As discussed above, the action of mTOR on HIF is a subject of debate. Essentially two options have been proposed: inhibition of HIF translation and/or inhibition of proteasomal degradation. Both converge to activate HIF. Growth factors could also activate HIF through the extracellular-regulated kinase (*ERK*) pathway. It has been proposed that phosphorylation of HIF by ERK could indirectly increase HIF activity by inhibiting the export of this transcription factor from the nucleus. As a consequence the activation of HIF target genes would be more efficient when HIF is phosphorylated. Moreover the vasoactive hormone angiotensin II has been shown to promote HIF-1α transcription through a protein kinase B (PKB) dependent mechanism. Finally, the physical density of the ECM could also indirectly influence the HIF pathway by modulating the oxygen diffusion length (not shown here). **b** A variety of HIF-induced genes have been shown to directly play a role on cell motility, invasion and extracellular matrix modulation (*blue*). A hypothetical mechanism for the loss of cell junctions under hypoxia could implicate the HIF-dependent activation of lysyl oxidase-like 2 (*LOXL2*) leading to stimulation of Snail. This transcriptional inhibitor could then downregulate E-cadherin (*E-cad*) and promote invasion. However, the subject of the ECM is also linked to other HIF-dependent actions, for instance, extracellular acidification could participate in mechanisms of invasion. Finally, the growth of neovessels, under the control of HIF, is also a crucial element in the penetration of cancer cells into the circulation. Autocrine motility factor (*AMF*); chemokine receptor CXCR4; receptor tyrosine kinase c-Met; lysyl oxidase (*LOX*); matrix metalloproteases (*MMP*)

been shown to promote cell invasion: the autocrine motility factor [83], vimentin, the receptor tyrosine kinase c-Met [84], the stromal derived factor-1, keratins 14, 18 and 19 and the cytokine receptor CXCR4 [85, 86].

Finally, the inflammatory status of the tumor stroma is a crucial element of the microenvironment and the HIF pathway can modulate the cellular inflammatory response. On the one hand, by promoting glycolysis, lactate production and thus extracellular acidification, HIF indirectly inhibits a subset of inflammatory actors: human cytotoxic T lymphocytes [87] (which are sensitive to lactate) and natural killers [88] (inactivated at low pH). On the other hand, HIF is a powerful driving force behind the action of tumor associated macrophages (TAM). As a result of over activation of the HIF pathway that increases glycolysis TAM are supplied with the energy necessary to migrate to tumor sites [89]. Consequently, TAM have a propensity to move toward areas distant from the circulation such as hypoxic and peri-necrotic zones [90]. In addition, they contribute to tumor aggressiveness through their ability to promote angiogenesis, extracellular matrix remodeling and cancer cell migration [91, 92].

This large body of data reveals the global role of HIF in tumor cell adaptation to microenvironmental stress. Commencing with a decrease in the pO_2 as the main regulator, this transcription factor initiates a cascade of events that modifies profoundly the microenvironment: tissue perfusion, metabolism, acidosis, matrix remodeling, proteolysis, and cellular migration. Taken together these data place HIF at the center of potential anti-environmental directed therapies.

Anti-cancer Therapy: A Few Hints on How to Disrupt Hypoxic Microenvironmental Adaptation

Clinical findings indicate that both hypoxia [5] and the characteristics of the tumor microenvironment [93] are related to the aggressiveness of a cancer.

In vitro, a hypoxic environment represents a harmful cellular stress situation giving rise to an unfavorable energetic balance, which should lead to diminished cell proliferation. Nonetheless, clinical studies have shown that the global hypoxic status of tumors is not correlated with a low rate of cancer cell proliferation. On the contrary, the degree of hypoxia in tumors positively correlates with bad prognosis [1]. These data indicate that severe hypoxia and a hostile microenvironment exert on tumor cells a drastic selection pressure leading to the emergence of tumor clones able to survive and migrate out of the nutrient-deprived environment. Therefore, from a therapeutic point of view, it is important to identify and antagonize the most pertinent adaptation mechanisms that allow tumor cells to escape this hostile milieu. Although this is a big challenge, basic knowledge of hypoxia signaling should allow development of rationalized novel anti-cancer strategies.

An Oxygen Centered Anti-microenvironmental Strategy

Based on the fact that O_2 is the main regulator of the HIF pathway, and that clinically hypoxic tumors are associated with poor patient survival, interfering with oxygen homeostasis constitutes an interesting anti-cancer approach. However, hypoxia is both a cytotoxic stress and a stimulus in initiating adaptation. The balance between the good and the bad side of hypoxia is very fine and might be tumor type dependent.

An illustration of the difficulty in foreseeing and controlling the precise effects of oxygen can be found in the controversy touching hyperbaric oxygen therapy (HBO). This type of treatment consists in submitting patients to 100% oxygen at a pressure higher than one atmosphere. The idea is to decrease the hypoxic score of the tumor. Contradictory results have emerged both from clinical investigation and animal experimentation [94]. HBO therapy can be pro- or anti-proliferative, or even inactive. On the one hand, oxygenation decreases the HIF microenvironmental adaptation response, but on the other hand it brings precious fuel to cancer cells.

The goal of anti-angiogenic approaches is to disturb vessels and consequently the oxygen profile in the tumor. By inhibiting VEGF [23, 95] or the endothelial VEGF-receptor [96–98] the formation of new vessels, a crucial process in the dynamics of tumor growth, is impaired. However, this approach paradoxically has limitations since anti-angiogenic agents will increase the hypoxic score of tumors, which is likely to increase cell virulence (Fig 8).

Another strategy consists in considering oxygen as a landmark of the HIF adaptive pathway where oxygen is a kind of environmental messenger dictating HIF function. An interesting strategy would be to specifically target hypoxic cells. In fact, their location, distant from blood vessels contributes physically to their resistance to classical chemotherapy (delivered by the circulation) and radiotherapy (that uses oxygen as an intermediary target to generate

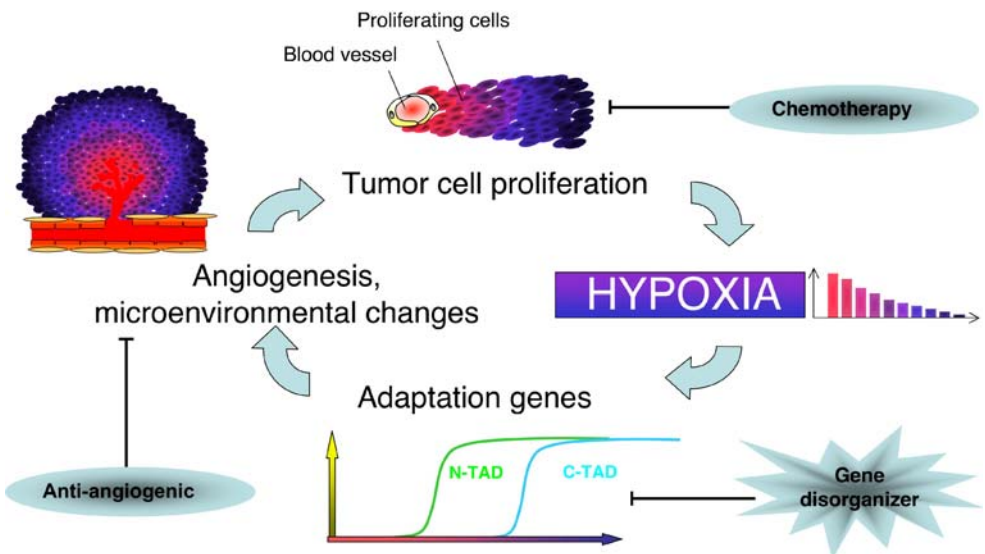

Fig. 8 Cyclic dynamics of tumor growth and putative anti-cancer approaches. Massive tumor cell proliferation leads to the formation of hypoxic zones at the periphery of blood vessels. The hypoxic signal stimulates a series of adaptation genes controlled by both the N-TAD and C-TAD of HIF-1α. Consequently, a set of adaptative changes (for instance involved in angiogenesis) make the microenvironment permissive for cell proliferation (for instance by reoxygenating hypoxic areas). From a therapeutic point of view, there are different ways to break this cycle. Classical chemotherapy inhibits cell proliferation but results in severe patient side-effects. Anti-angiogenics aim at blocking one feature of this adaptation phenomenon: the growth of new blood vessels. An alternative strategy consists in abolishing the overall adaptation mechanism by disorganizing the HIF-target genes. In addition, FIH inhibitors would represent a new class of anti-cancer drugs. Such candidate molecular targets may lead to innovative anti-microenvironmental adaptation approaches

toxic free radicals). Hypoxic cells are thus particularly strategic targets. Compounds such as tirapazamine (TPZ) [99–101] are pro-drugs that become activated by the reducing effect of a drop in oxygen tension. Therefore their toxicity is targeted to hypoxic areas in tumors. However, the question of diffusion of this kind of drug from blood vessels still needs to be addressed. Yet it is conceivable that their lower non-specific toxicity should allow high dosage. Future studies should evaluate the exact sensitivity and toxicity of this kind of treatment. Interestingly the activity of the HIF pathway has been shown to decrease TPZ activity probably due to the redistribution of intracellular oxygen following mitochondrial inhibition [47]. This recent data suggests that a combined anti-HIF and TPZ therapy should clearly improve treatment. Alternatively, the hypoxic characteristic of tumors can be exploited in therapy by using obligate anaerobic bacteria such as clostridia, which show lytic activity in tumor cells [102].

The hypoxic status of a tumor might also be considered as a key parameter in the choice of the anti-cancer arsenal of physicians. Susceptibility of a given drug could indeed be oxygen dependent [103]. For instance this seems to be the case for the proteasome inhibitor bortezomib [104] and for mTOR inhibitors [105]. Thus the hypoxic score might not only be predictive of the tumor aggressiveness but also guide the clinician in the therapeutic strategy to adopt.

A Non-oxygen Centered Anti-microenvironmental Strategy

Clinical evidence suggests that HIF-1 overexpression is associated with higher cancer mortality or treatment resistance and thus inhibition of HIF activity has been proposed [5]. A number of HIF modulators that target different steps in HIF function are currently under evaluation [5, 103, 106]. However, the benefit of such a therapeutic approach remains speculative since HIF is a pleiotropic factor, able to promote survival, proliferation, and cell death, thus modulating its action might have drastic consequences on general physiology. Thus, instead of targeting HIF itself, targeting a particular set of HIF downstream products may show potential. However, this strategy might suffer from the complexity and potential redundancy of the HIF pathway. Even if a precise adaptation gene is inactivated, the relay may be taken up by one of the myriad of HIF downstream targets. The expected cytotoxic effect could possibly be circumvented thanks to another microenvironmental adaptation phenomenon. Interrupting the dialogue between HIF and the microenvironment certainly appears to be complex and requires an in-depth understanding of the mechanisms involved in regulation.

Agents such as echinomycin impair HIF-1 association with its target DNA [107] while growth factor directed therapies such as traztuzumab (Herceptin), which inhibits HER2/Neu, indirectly decrease HIF-1α protein levels [35, 106]. Benzoquinone ansamycin drugs like geldanamycin, also decrease the HIF-1α protein level by favoring its proteasomal degradation [108]. Alternately, interest in developing HIF activating drugs in the treatment of ischemic disorders has led to research into agents such as dimethyloxaloylglycine which inhibit the 2-OG dependent dioxygenase activity resulting in stabilization and activation of HIFα [109].

As mentioned, selective inhibition of the appropriate HIF-downstream gene product may be preferable. VEGF and VEGF-R are such targets and although precursors in this approach they may not be the only HIF downstream targets of interest. The combination of the anti-angiogenic with anti-HIF metabolic adaptation agents could be envisaged by interfering with key glycolytic enzymes such as LDH-A. Another strategy would consist in impairing the HIF-induced pHi regulating system [43]. In this case, the natural propensity of cancer cells to switch to pyruvate metabolism to lactic acid and consequently to acidosis would impact on proliferation. As a proof of principle, genetically altered cell lines that are not able to regulate their pHi do not proliferate normally under acidic conditions and show a very poor tumorigenic potential in xenograft experiments [110]. From a therapeutic point of view, the expected cytotoxicity would result from necrotic cell death sensitized by intracellular acidosis.

A third approach, which is a compromise between the previous ones, would be to exploit the HIF pathway by destabilizing its global pro- and anti-survival balance in favor of tumor regression. This would consist in targeting a qualitative modulator of the HIF activity. In this context, FIH would be an interesting candidate. Indeed, we discussed above the potential role of FIH as a discriminator between two subtypes of genes and as a determinant of the gene localization in a tumor hypoxic gradient [20]. The FIH activity would direct the expression of a precise gene at a precise localization in the tumor, associated with a given oxygen tension. It is likely that genes associated with cell death and anti-proliferation are activated in severe hypoxic regions whereas survival genes are induced in milder hypoxic zones. Displacing anti-survival genes to oxygenated regions might be of great interest. By targeting FIH, our working model predicts such a disorganization of the whole gene pattern that we believe should impair the general microenvironmental adaptation process.

Conclusions

The microenvironment dictates its constraints on HIF mainly *via* the oxygen level, and in turn HIF responds by

pleiotropic and sometimes apparently contradictory effects. The prospect of altering this signaling network in developing tumor specific anti-environmental adaptation therapies holds promise. However, efficacious strategies that target microenvironmental adaptation will only emerge from a full understanding of the whole adaptation process. Success will depend on: (1) the identification of the most pertinent HIF downstream gene product to be targeted and (2) the elucidation of the global orchestration of adaptation genes.

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References

1. Hockel M, Vaupel P (2001) Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 93:266–276
2. Padhani AR, Krohn KA, Lewis JS et al (2007) Imaging oxygenation of human tumours. Eur Radiol 17:861–872
3. Baish JW, Jain RK (2000) Fractals and cancer. Cancer Res 60:3683–3688
4. Grizzi F, Russo C, Colombo P et al (2005) Quantitative evaluation and modeling of two-dimensional neovascular network complexity: the surface fractal dimension. BMC Cancer 5:14
5. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732
6. Hunter A, Hendrikse A, Renan M et al (2006) Does the tumor microenvironment influence radiation-induced apoptosis? Apoptosis 11:1727–1735
7. Brahimi-Horn MC, Pouyssegur J (2007) Harnessing the hypoxia-inducible factor in cancer and ischemic disease. Biochem Pharmacol 73:450–457
8. Mazure NM, Brahimi-Horn MC, Berta MA et al (2004) HIF-1: master and commander of the hypoxic world. A pharmacological approach to its regulation by siRNAs. Biochem Pharmacol 68:971–980
9. Manalo DJ, Rowan A, Lavoie T et al (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 105:659–669
10. Schofield CJ, Ratcliffe PJ (2004) Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5:343–354
11. Lando D, Peet DJ, Gorman JJ et al (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 16:1466–1471
12. Jaakkola P, Mole DR, Tian YM et al (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292:468–472
13. Koivunen P, Tiainen P, Hyvarinen J et al (2007) An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor alpha. J Biol Chem 282:30544–30552
14. Schofield CJ, Ratcliffe PJ (2005) Signalling hypoxia by HIF hydroxylases. Biochem Biophys Res Commun 338:617–626
15. Hewitson KS, McNeill LA, Riordan MV et al (2002) Hypoxia-inducible factor (HIF) asparagine hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. J Biol Chem 277:26351–26355
16. Lee C, Kim SJ, Jeong DG et al (2003) Structure of human FIH-1 reveals a unique active site pocket and interaction sites for HIF-1 and von Hippel-Lindau. J Biol Chem 278:7558–7563
17. Bilton R, Trottier E, Pouyssegur J et al (2006) ARDent about acetylation and deacetylation in hypoxia signalling. Trends Cell Biol 16:616–621
18. Brahimi-Horn C, Mazure N, Pouyssegur J (2005) Signalling via the hypoxia-inducible factor-1alpha requires multiple posttranslational modifications. Cell Signal 17:1–9
19. Hu CJ, Iyer S, Sataur A et al (2006) Differential regulation of the transcriptional activities of hypoxia-inducible factor 1 alpha (HIF-1alpha) and HIF-2alpha in stem cells. Mol Cell Biol 26:3514–3526
20. Dayan F, Roux D, Brahimi-Horn MC et al (2006) The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res 66:3688–3698
21. Koivunen P, Hirsila M, Gunzler V et al (2004) Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. J Biol Chem 279:9899–9904
22. Stolze IP, Tian YM, Appelhoff RJ et al (2004) Genetic analysis of the role of the asparaginyl hydroxylase factor inhibiting hypoxia-inducible factor (HIF) in regulating HIF transcriptional target genes. J Biol Chem 279:42719–42725
23. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
24. Gerhardt H, Golding M, Fruttiger M et al (2003) VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 161:1163–1177
25. Tang N, Wang L, Esko J et al (2004) Loss of HIF-1alpha in endothelial cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. Cancer Cell 6:485–495
26. Maisonpierre PC, Suri C, Jones PF et al (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277:55–60
27. Noseda M, Chang L, McLean G et al (2004) Notch activation induces endothelial cell cycle arrest and participates in contact inhibition: role of p21Cip1 repression. Mol Cell Biol 24:8813–8822
28. Seagroves TN, Ryan HE, Lu H et al (2001) Transcription factor HIF-1 is a necessary mediator of the pasteur effect in mammalian cells. Mol Cell Biol 21:3436–3444
29. Mans AM, DeJoseph MR, Hawkins RA (1994) Metabolic abnormalities and grade of encephalopathy in acute hepatic failure. J Neurochem 63:1829–1838
30. Hirsila M, Koivunen P, Xu L et al (2005) Effect of desferrioxamine and metals on the hydroxylases in the oxygen sensing pathway. Faseb J 19:1308–1310
31. Vordermark D, Kraft P, Katzer A et al (2005) Glucose requirement for hypoxic accumulation of hypoxia-inducible factor-1alpha (HIF-1alpha). Cancer Lett 230:122–133
32. Natsuizaka M, Ozasa M, Darmanin S et al (2007) Synergistic up-regulation of Hexokinase-2, glucose transporters and angiogenic factors in pancreatic cancer cells by glucose deprivation and hypoxia. Exp Cell Res 313:3337–3348
33. Shaw RJ, Kosmatka M, Bardeesy N et al (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proc Natl Acad Sci U S A 101:3329–3335
34. Bernardi R, Guernah I, Jin D et al (2006) PML inhibits HIF-1alpha translation and neoangiogenesis through repression of mTOR. Nature 442:779–785

35. Laughner E, Taghavi P, Chiles K et al (2001) HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 21:3995–4004
36. Hudson CC, Liu M, Chiang GG et al (2002) Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol 22:7004–7014
37. Lang KJ, Kappel A, Goodall GJ (2002) Hypoxia-inducible factor-1alpha mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia. Mol Biol Cell 13:1792–1801
38. Pore N, Jiang Z, Shu HK et al (2006) Akt1 activation can augment hypoxia-inducible factor-1alpha expression by increasing protein translation through a mammalian target of rapamycin-independent pathway. Mol Cancer Res 4:471–479
39. Brugarolas J, Lei K, Hurley RL et al (2004) Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev 18:2893–2904
40. Mathew R, Karantza-Wadsworth V, White E (2007) Role of autophagy in cancer. Nat Rev Cancer 7:961–967
41. Lee H, Paik SG (2006) Regulation of BNIP3 in normal and cancer cells. Mol Cells 21:1–6
42. Webster KA, Graham RM, Bishopric NH (2005) BNip3 and signal-specific programmed death in the heart. J Mol Cell Cardiol 38:35–45
43. Pouyssegur J, Dayan F, Mazure NM (2006) Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 441:437–443
44. Mazure NMBG, Garcia-Medina R, Gounon P, Roux D, Pouyssegur J (2007) Hypoxia-induced Autophagy is mediated through the HIF-dependent induction of BNIP3 and BNIP3L. Bull Cancer 94:534
45. Fantin VR, St-Pierre J, Leder P (2006) Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. Cancer Cell 9:425–434
46. Kim JW, Tchernyshyov I, Semenza GL et al (2006) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177–185
47. Papandreou I, Cairns RA, Fontana L et al (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3:187–197
48. Fukuda R, Zhang H, Kim JW et al (2007) HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell 129:111–122
49. Tsukihara T, Aoyama H, Yamashita E et al (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. Science 272:1136–1144
50. Brahimi-Horn MC, Chiche J, Pouyssegur J (2007) Hypoxia signalling controls metabolic demand. Curr Opin Cell Biol 19:223–229
51. Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nat Rev Cancer 4:891–899
52. Mekhail K, Gunaratnam L, Bonicalzi ME et al (2004) HIF activation by pH-dependent nucleolar sequestration of VHL. Nat Cell Biol 6:642–647
53. Willam C, Warnecke C, Schefold JC et al (2006) Inconsistent effects of acidosis on HIF-alpha protein and its target genes. Pflugers Arch 451:534–543
54. Fukumura D, Xu L, Chen Y et al (2001) Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors in vivo. Cancer Res 61:6020–6024
55. Xu L, Fukumura D, Jain RK (2002) Acidic extracellular pH induces vascular endothelial growth factor (VEGF) in human glioblastoma cells via ERK1/2 MAPK signaling pathway: mechanism of low pH-induced VEGF. J Biol Chem 277:11368–11374
56. Kubasiak LA, Hernandez OM, Bishopric NH et al (2002) Hypoxia and acidosis activate cardiac myocyte death through the Bcl-2 family protein BNIP3. Proc Natl Acad Sci U S A 99:12825–12830
57. Papandreou I, Krishna C, Kaper F et al (2005) Anoxia is necessary for tumor cell toxicity caused by a low-oxygen environment. Cancer Res 65:3171–3178
58. Ullah MS, Davies AJ, Halestrap AP (2006) The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J Biol Chem 281:9030–9037
59. Shimoda LA, Fallon M, Pisarcik S et al (2006) HIF-1 regulates hypoxic induction of NHE1 expression and alkalinization of intracellular pH in pulmonary arterial myocytes. Am J Physiol Lung Cell Mol Physiol 291:L941–L949
60. Cardone RA, Bellizzi A, Busco G et al (2007) The NHERF1 PDZ2 domain regulates PKA-RhoA-p38-mediated NHE1 activation and invasion in breast tumor cells. Mol Biol Cell 18:1768–1780
61. Bergersen LH (2007) Is lactate food for neurons? Comparison of monocarboxylate transporter subtypes in brain and muscle. Neuroscience 145:11–19
62. Koukourakis MI, Giatromanolaki A, Sivridis E et al (2005) Pyruvate dehydrogenase and pyruvate dehydrogenase kinase expression in non small cell lung cancer and tumor-associated stroma. Neoplasia 7:1–6
63. Hosoya K, Kondo T, Tomi M et al (2001) MCT1-mediated transport of L-lactic acid at the inner blood-retinal barrier: a possible route for delivery of monocarboxylic acid drugs to the retina. Pharm Res 18:1669–1676
64. Hunt TK, Aslam RS, Beckert S et al (2007) Aerobically derived lactate stimulates revascularization and tissue repair via redox mechanisms. Antioxid Redox Signal 9:1115–1124
65. Brahimi-Horn MC, Pouyssegur J (2007) Oxygen, a source of life and stress. FEBS Lett 581:3582–3591
66. Fukuda R, Hirota K, Fan F et al (2002) Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 277:38205–38211
67. Zhong H, Chiles K, Feldser D et al (2000) Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 60:1541–1545
68. Richard DE, Berra E, Gothie E et al (1999) p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. J Biol Chem 274:32631–32637
69. Sang N, Stiehl DP, Bohensky J et al (2003) MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300. J Biol Chem 278:14013–14019
70. Mylonis I, Chachami G, Samiotaki M et al (2006) Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha. J Biol Chem 281:33095–33106
71. Page EL, Robitaille GA, Pouyssegur J et al (2002) Induction of hypoxia-inducible factor-1alpha by transcriptional and translational mechanisms. J Biol Chem 277:48403–48409
72. Erler JT, Bennewith KL, Nicolau M et al (2006) Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 440:1222–1226
73. Lucero HA, Kagan HM (2006) Lysyl oxidase: an oxidative enzyme and effector of cell function. Cell Mol Life Sci 63:2304–2316

74. Payne SL, Fogelgren B, Hess AR et al (2005) Lysyl oxidase regulates breast cancer cell migration and adhesion through a hydrogen peroxide-mediated mechanism. Cancer Res 65:11429–11436
75. Bouez C, Reynaud C, Noblesse E et al (2006) The lysyl oxidase LOX is absent in basal and squamous cell carcinomas and its knockdown induces an invading phenotype in a skin equivalent model. Clin Cancer Res 12:1463–1469
76. Ren C, Yang G, Timme TL et al (1998) Reduced lysyl oxidase messenger RNA levels in experimental and human prostate cancer. Cancer Res 58:1285–1290
77. Petrella BL, Lohi J, Brinckerhoff CE (2005) Identification of membrane type-1 matrix metalloproteinase as a target of hypoxia-inducible factor-2 alpha in von Hippel-Lindau renal cell carcinoma. Oncogene 24:1043–1052
78. Munoz-Najar UM, Neurath KM, Vumbaca F et al (2006) Hypoxia stimulates breast carcinoma cell invasion through MT1-MMP and MMP-2 activation. Oncogene 25:2379–2392
79. Koike T, Kimura N, Miyazaki K et al (2004) Hypoxia induces adhesion molecules on cancer cells: A missing link between Warburg effect and induction of selectin-ligand carbohydrates. Proc Natl Acad Sci U S A 101:8132–8137
80. Esteban MA, Tran MG, Harten SK et al (2006) Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. Cancer Res 66:3567–3575
81. Evans AJ, Russell RC, Roche O et al (2007) VHL promotes E2 box-dependent E-cadherin transcription by HIF-mediated regulation of SIP1 and snail. Mol Cell Biol 27:157–169
82. Krishnamachary B, Zagzag D, Nagasawa H et al (2006) Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. Cancer Res 66:2725–2731
83. Funasaka T, Yanagawa T, Hogan V et al (2005) Regulation of phosphoglucose isomerase/autocrine motility factor expression by hypoxia. Faseb J 19:1422–1430
84. Pennacchietti S, Michieli P, Galluzzo M et al (2003) Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 3:347–361
85. Pan J, Mestas J, Burdick MD et al (2006) Stromal derived factor-1 (SDF-1/CXCL12) and CXCR4 in renal cell carcinoma metastasis. Mol Cancer 5:56
86. Staller P, Sulitkova J, Lisztwan J et al (2003) Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature 425:307–311
87. Fischer K, Hoffmann P, Voelkl S et al (2007) Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 109:3812–3819
88. Lardner A (2001) The effects of extracellular pH on immune function. J Leukoc Biol 69:522–530
89. Cramer T, Yamanishi Y, Clausen BE et al (2003) HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 112:645–657
90. De Palma M, Murdoch C, Venneri MA et al (2007) Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. Trends Immunol 28:519–524
91. Condeelis J, Pollard JW (2006) Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124:263–266
92. Mantovani A, Schioppa T, Porta C et al (2006) Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev 25:315–322
93. Hoekstra CJ, Stroobants SG, Smit EF et al (2005) Prognostic relevance of response evaluation using [18F]-2-fluoro-2-deoxy-D-glucose positron emission tomography in patients with locally advanced non-small-cell lung cancer. J Clin Oncol 23:8362–8370
94. Daruwalla J, Christophi C (2006) The effect of hyperbaric oxygen therapy on tumour growth in a mouse model of colorectal cancer liver metastases. Eur J Cancer 42:3304–3311
95. Hurwitz H, Fehrenbacher L, Novotny W et al (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–2342
96. Beebe JS, Jani JP, Knauth E et al (2003) Pharmacological characterization of CP-547,632, a novel vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for cancer therapy. Cancer Res 63:7301–7309
97. Rugo HS, Herbst RS, Liu G et al (2005) Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. J Clin Oncol 23:5474–5483
98. Wedge SR, Ogilvie DJ, Dukes M et al (2002) ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res 62:4645–4655
99. Peters KB, Brown JM (2002) Tirapazamine: a hypoxia-activated topoisomerase II poison. Cancer Res 62:5248–5253
100. Le QT, Taira A, Budenz S et al (2006) Mature results from a randomized Phase II trial of cisplatin plus 5-fluorouracil and radiotherapy with or without tirapazamine in patients with resectable Stage IV head and neck squamous cell carcinomas. Cancer 106:1940–1949
101. Smith HO, Jiang CS, Weiss GR et al (2006) Tirapazamine plus cisplatin in advanced or recurrent carcinoma of the uterine cervix: a Southwest Oncology Group study. Int J Gynecol Cancer 16:298–305
102. Groot AJ, Mengesha A, van der Wall E et al (2007) Functional antibodies produced by oncolytic clostridia. Biochem Biophys Res Commun 364:985–989
103. Magagnin MG, Koritzinsky M, Wouters BG (2006) Patterns of tumor oxygenation and their influence on the cellular hypoxic response and hypoxia-directed therapies. Drug Resist Updat 9:185–197
104. Veschini L, Belloni D, Foglieni C et al (2007) Hypoxia-inducible transcription factor-1 alpha determines sensitivity of endothelial cells to the proteosome inhibitor bortezomib. Blood 109:2565–2570
105. Thomas GV, Tran C, Mellinghoff IK et al (2006) Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. Nat Med 12:122–127
106. Lee K, Roth RA, LaPres JJ (2007) Hypoxia, drug therapy and toxicity. Pharmacol Ther 113:229–246
107. Kong D, Park EJ, Stephen AG et al (2005) Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 65:9047–9055
108. Mabjeesh NJ, Post DE, Willard MT et al (2002) Geldanamycin induces degradation of hypoxia-inducible factor 1alpha protein via the proteosome pathway in prostate cancer cells. Cancer Res 62:2478–2482
109. Epstein AC, Gleadle JM, McNeill LA et al (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 107:43–54
110. Pouyssegur J, Franchi A, Pages G (2001) pHi, aerobic glycolysis and vascular endothelial growth factor in tumour growth. Novartis Found Symp 240:186–196 discussion 196–188