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Harnessing the hypoxia-inducible factor in cancer and ischemic disease

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ABSTRACT

The α/β-heterodimeric transcription factor hypoxia-inducible factor (HIF) functions when the oxygen level in tissues is low, i.e. when the tissue microenvironment becomes hypoxic, and is non-functional when the level of oxygen is high. Certain pathophysiological conditions such as ischemic disorders and cancer encounter low levels of local tissue oxygenation due to a defective or insufficient vasculature. Highly proliferating tumour cells rapidly form into a mass that becomes located too far from the vasculature to be nourished and oxygenated. Under such conditions HIF activates or represses a vast array of genes that in particular, initiate the formation of new blood vessels and modify metabolism. In this way the tumour mass re-establishes conditions favourable for further proliferation. Interest is being expressed in the direct repression or stimulation of HIF activity, respectively, in the treatment of cancer and of ischemic disorders. The modulation of other HIF-target genes implicated, in particular, in tumour metabolism and intracellular pH control may also prove to be useful in cancer therapy. However, before going further a better understanding of the basics of the HIF signalling pathway is essential. This review will introduce the reader to the molecular mechanisms that regulate HIF and some of the biological consequences of its action, in particular in tumour metabolism, growth and invasion. Approaches to either enforce tumour regression or increase blood vessel formation through the targeting of HIF or its downstream effectors will also be discussed.

1. Introduction

An adequate supply of oxygen to tissues is essential in maintaining mammalian cell function and physiology. A deficiency in the oxygen supply to tissues is a characteristic of a number of pathophysiological situations in which there is insufficient blood flow to provide adequate oxygenation (Fig. 1). The hypoxic (low oxygen) environment of tissues activates a signalling cascade that drives the induction or repression of the transcription of a multitude of genes

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Abbreviations: bHLH, basic-helix-loop-helix; BNIP3, Bcl-2/adenovirus EIB 19kDa-interacting protein 3; CITED, CBP/p300 interacting transactivator with ED-rich tail 2; CBP, CREB binding protein; Epo, erythropoietin; FIH, factor inhibiting HIF-1; FH, fumarate hydratase; HIF, hypoxia-inducible factor; HO-1, haem oxygenase; HRE, hypoxia response element; iNO-2, inducible nitric-oxide synthase 2; IRES, internal ribosome entry site; mTOR, mammalian target of rapamycine; NHE1, Na^+/H^+ exchanger; 2-OG, 2-oxoglutarate; ODDD, oxygen degradation dependent domain; PAS, Per-Arnt-Sim; PGI, phosphoglucose isomerase; PTEN, phosphatase and tensin; PHD, prolyl hydroxylase domain; SDH, succinate dehydrogenase; TAD, transcriptional activation domain; TCA, tricarboxylic acid; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau

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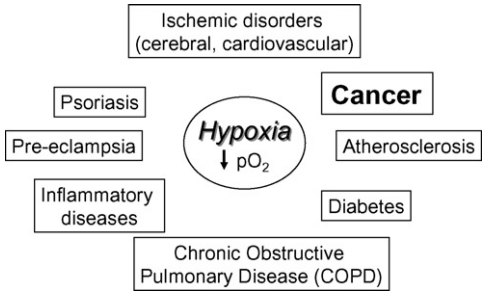

Fig. 1 – Pathophysiology of hypoxia. Hypoxia, a low level of oxygen in tissues or low oxygen partial pressure (pO_2), resulting from defective vascularisation is a characteristic of a number of diseases.

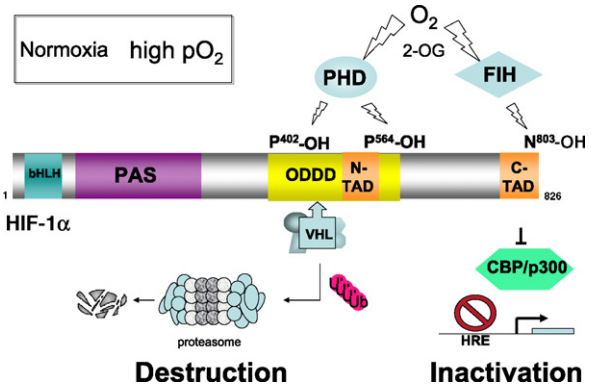

Fig. 2 – Hydroxylation the key to HIF-α regulation. Under normoxic (high pO_2 levels) conditions the oxygen sensor proteins prolyl hydroxylase domain (PHD) proteins and the factor inhibiting HIF-1 (FIH) are active. These dioxygenases use oxygen and 2-oxoglutarate (2-OG) in hydroxylating respectively, two proline residues in the oxygen-dependent degradation domain (ODDD) and an aparagine resuidue in the C-terminal transcriptional activation domain (C-TAD) of the alpha subunit of the hypoxia-inducible factor-1 (HIF-1). Prolyl hydroxylation signals the binding of a von Hippel-Lindau (VHL) protein-containing complex with E3 ubiquitin ligase activity that ubiquitinates HIF-1α earmarking it for destruction by the proteasome. Asparaginyl hydroxylation abrogates interaction with a co-activator CBP/p300 leading to inactivation of HIF-1.

implicated in events such as angiogenesis (neo-vascularisation), glucose metabolism and cell survival/death [1,2]. The key to this hypoxic transcriptional response lies in the transcription factor, the hypoxia-inducible factor (HIF). HIF is over-expressed in a vast array of cancers through hypoxia-dependent and -independent mechanisms and expression is associated with poor patient prognosis. A full understanding of the molecular mechanistics and role of this factor in hypoxic signalling should open up the way to the development of novel approaches to: (1) induce tumour cell death rather than survival and thus force tumours to regress and (2) encourage vessel formation in ischemic disorders.

2. Posttranslational switching of HIF

Posttranslational modification is the crux of HIF regulation through control of both stability and activity (for review see [3]).

2.1. *HIF prolyl hydroxylases destabilise HIF-α*

Two oxygen-dependent HIF hydroxylases, which hydroxylate the alpha subunit of the α/β HIF heterodimer, determine its stability and activity. The hydroxylation of prolyl residues in the oxygen-dependent degradation domain of HIF-α (prolines 402 and 564 of human HIF-1α) by prolyl hydroxylase domain (PHD) enzymes signals recruitment to HIF-α of an E3 ubiquitin ligase complex containing the von Hippel-Lindau (VHL) protein (Fig. 2). VHL is merciless, together with its companions elongin B, elongin C, Cul 2 and Rbx-1 it strikes down HIF-α giving it an amazingly short half-life of less than five minutes in well-oxygenated cells. This occurs through posttranslational ubiquitination of HIF-α and subsequent recognition by the destructive machinery of the proteasome. So in the presence of oxygen and within 5 min of synthesis HIF-α is hydoxylated, ubiquitinated and degraded by the proteasome. Mutations in the *vhl* gene that lead to loss of function are associated with renal cell carcinoma (RCC) and VHL disease, a familial syndrome. HIF-α is therefore stable and active in these cancers and HIF target genes activated, as reflected in an excess of vascularisation in tumours [4]. These observations provide a strong link between HIF and angiogenesis and tumourigenesis.

The PHDs of which there are threes isoforms are regulated at several levels. The oxygen concentration not only determines PHD activity but also expression, where the *phd*2 and *phd*3 but not *phd*1 genes are upregulated by hypoxia/HIF [5]. This feedback regulation assures rapid intervention if and when the oxygen concentration is re-established to a high level. As for HIF-α the PHDs are subjected to ubiquitin-proteasomal degradation but by different E3 ubiquitin ligases, Siah1a and Siah2 [6], which in turn are also HIF regulated. In addition to oxygen the PHD proteins require as co-substrate 2-oxoglutarate (2-OG), and as cofactor Fe^{2+} and ascorbate. Since 2-OG is a metabolite of the TCA cycle, activity will also be regulated by TCA cycle function. To add another level of complexity, the succinate produced by the hydroxylase reaction and by the TCA cycle acts as an inhibitor [5]. It is interesting to note that mutations in enzymes of the TCA cycle such as succinate dehydrogenase (SDH) and fumarate hydratase (FH) (also termed fumarase) are linked to tumourigenesis [7]. SDH and FH have been identified as tumour suppressors and loss of function mutations lead to accumulation of respectively, succinate or fumarate. Under these conditions HIFα would be stable and HIF would activate/repress downstream genes.

Acetylation, another form of posttranslational modification was also reported to destabilise HIF-α but as revealed more recently it may only be of minor consequence and even absent in human cells [8,9].

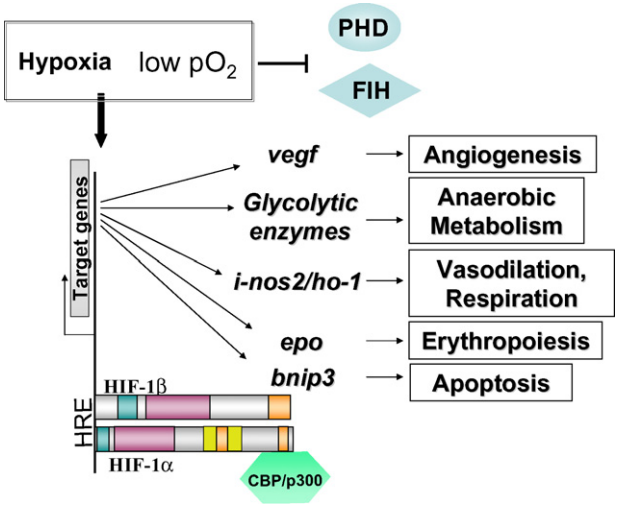

Fig. 3 – HIF-dependent gene induction. Under hypoxic (low pO_2) conditions the oxygen sensor proteins prolyl hydroxylase domain (PHD) proteins and the factor inhibiting HIF-1 (FIH) are inactive. The alpha subunit of the hypoxia-inducible factor is stable, heterodimerises with the beta subunit and binds to DNA at hypoxia-response elements of genes. The co-activator CBP/p300 binds to the C-terminal transcriptional activation domain of HIF-α and activates or represses the transcription of a vast array of genes including the vascular endothelial growth factor (*vegf*), glycolytic enzymes, haem oxygenase-1 (*ho-1*) and inducible nitric-oxide synthase (*i-nos2*), erythropoietin (*epo*) and Bcl-2/adenovirus EIB 19 kDa-interacting protein 3 (*bnip3*) that are involved in a variety of cell functions.

2.2. *HIF asparaginyl hydroxylase inactivates HIF-α*

If for some reason the flow of events leading to destruction of HIF-α is not complete or defective, resulting in the escape from degradation of some HIF-α protein, the HIF-α activity will nonetheless be inhibited by hydroxylation by another oxygen-dependent HIF hydroxylase termed factor inhibiting HIF-1 (FIH). Hydroxylation by this enzyme occurs on an asparagine residue in the C-terminal transcriptional activation domain (C-TAD) of HIF-α with the resulting inhibition of interaction with the transcriptional co-activators CREB binding protein (CBP) and p300. So cells have developed an additional locking mechanism in the event of malfunction of the PHDs, though as will be seen below this may also reflect a mechanism for selective gene induction. In the presence of low levels of oxygen (hypoxia), or under conditions of elevated reactive oxygen species [10], these enzymes are inoperably and HIF-α is stable and translocates to the nucleus where it interacts with its beta subunit (also called ARNT), which is constitutively expressed and not influenced by the oxygen concentration. The α/β HIF complex then binds to hypoxia-response elements (HRE) of target genes to regulate their transcription. More and more HIF target genes are being identified and at least 70 have been shown to be upregulated [2]. The most investigated genes to date include those involved in angiogenesis, vasodilation/respiration, erythropoiesis, anaerobic metabolism, tumour invasion and cell survival/death (Fig. 3) but more and more interest is being shown in genes involved in other functions such as in inflammation and in differentiation in embryonic development. Although the transcriptional activity of most genes is increased by HIF binding HIF can also repress transcription of genes, though the mechanisms still need to be investigated [11].

2.3. *Kinases activate HIF-α*

Although it is not altogether clear which kinases lead to activation of HIF it is clear that HIF-1α and -2α are phosphorylated and that this parallels an increase in their transcriptional activity. Phosphorylation does not influence the stability of HIFα or its DNA binding capability but may favour heterodimerization with HIF-1β or interaction with co-activators [3]. The Ras-Raf-ERK/MAPK pathway is implicated in activation possibly by favouring interaction between HIF and its co-activators and/or by modulating co-activator activity ([12] and reviewed in [13]).

3. Selectivity of HIF-dependent gene induction

It is becoming apparent that HIF can selectively induce different genes through at least two different mechanisms that are either isoform or transcriptional activation domain (TAD) dependent.

3.1. *Isoform selectivity*

The HIF-α subunit exists in human cells as three isoforms expressed by individual loci (Fig. 4). There is considerable similarity in the protein sequences of HIF-1α and -2α (overall, 48%; bHLH, 85%; PAS-A, 68%; PAS-B, 73%) and both are

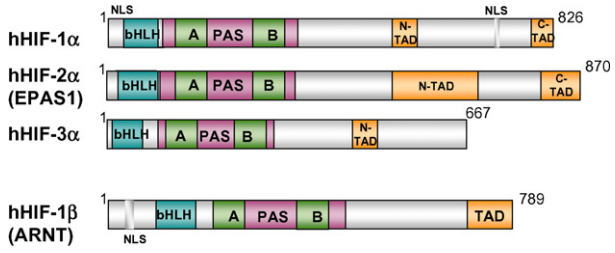

Fig. 4 – Domain structure of the HIF bHLH-PAS proteins involved in the hypoxic response. Three isoforms of the human (h) α subunit of the hypoxia-inducible factor have been described to be involved in the hypoxic response. HIF-2α has also been termed EPAS1 (endothelial PAS domain protein 1). Three isoforms of the β subunit have also been identified but HIF-1β, also termed ARNT (aryl hydrocarbon receptor nuclear translocator), is the form the most studied in the hypoxic response. The basic-helix-loop-helix (bHLH) domains and the conserved PAS (Per-Arnt-Sim)-A and -B domains are shown in the N-terminal part of the proteins while one or two trancriptional activation domains (TAD) are shown in the C-terminal part at either the extremity (C-TAD) or slightly toward the N-terminus (N-TAD). Nuclear localisation signals (NLS) have been identified on certain isoforms.

subjected to the same posttranslational regulation. The little studied HIF-3α may exist as no less than six splice variants [14] and may play a dominant negative role in the hypoxic response [15]. Data to suggest that HIF-1α and -2α may be selective in the genes they induce or repress has been provided (for review [16]). For example, the gene carbonic anhydrase 9 (*ca9*) is predominantly HIF-1 induced [17] while HIF prolyl hydroxylase 3 (*phd3*) is HIF-1 and -2 induced [18]. The extent of overlap; certain genes induced by one or the other or both, or cell type specificity with dominant expression of one or the other is still to be clarified.

3.2. *Transcriptional activation domain selectivity*

Indication of TAD selectivity in gene targeting comes from a study, published by our laboratory, which investigated the respective roles of the two TAD of HIF-1α [19]. Both HIF-1α and -2α posses two TAD (Fig. 4), this bicephalous nature is unusual for transcription factors; most transcription factors have only one TAD [20]. The NH_2- and COOH-terminal-TAD, respectively N-TAD and C-TAD of human HIF-1α have no more than 20% protein sequence similarity while the human N-TAD protein sequence of HIF-2α and -3α share 65% and 60% identity with human HIF-1α, respectively. The human HIF-1α C-TAD is also well conserved when compared to the human HIF-2α C-TAD (more than 70%). In addition, both the N-TAD and C-TAD show high interspecies protein sequence conservation (more than 90%). As mentioned above the transcriptional activity of HIF is inhibited by asparaginyl hydroxylation of the C-TAD by FIH. Thus the modulation, up or down, of the level of expression of FIH leads respectively to clamping or release of the C-TAD activity. Using this approach we were able to demonstrate the existence of two groups of genes, FIH-inhibited or non-FIH inhibited and thus driven respectively by the C-TAD (with or without the N-TAD) or N-TAD. Given that the two oxygen sensors PHDs and FIH have a differential K_m for oxygen [21], where the PHDs require a higher level than FIH for activity, we postulate that in areas close to blood vessels, where cells are oxygenated, the PHDs will be active leading to complete degradation of HIF-1α. A drop in the oxygen level will first inactivate the PHDs resulting in stabilization of the HIF-1α protein, however with maintenance of a clamp on the C-TAD activity since FIH is still active (Fig. 5). With a further drop in oxygen, total inhibition of the activity of both sensors will lead to complete stabilization of HIF-1α and a total release of the C-TAD, by enabling interaction with cofactors. Thus, liberation of the C-TAD will occur only under severe hypoxic conditions when FIH is totally inhibited [22]. So N-TAD and C-TAD or N/C-TAD-dependent gene expression will be mediated by the oxygen gradient in tissues. Where under mild hypoxic conditions genes dependent on only the N-TAD will be expressed. In contrast, severe hypoxia will lead to full activation of a family of C-TAD or N/C-TAD-sensitive genes.

An additional mechanism for selectivity may arise from the choice of the dimer partner of the different alpha and beta subunit combinations, but this has not been significantly investigated [13].

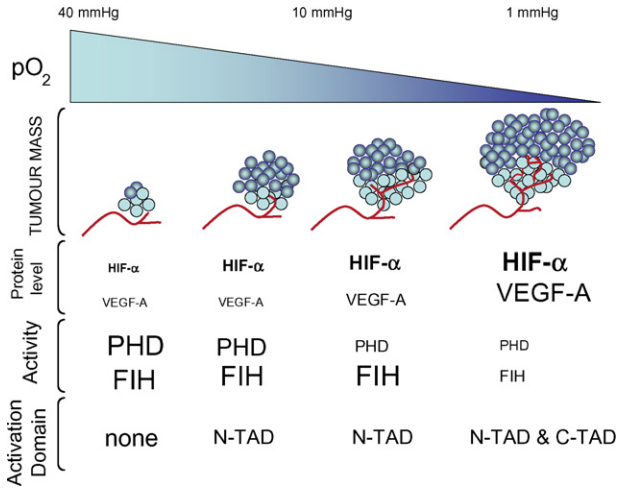

Fig. 5 – Transcriptional activation domain selectivity of HIF-dependent gene transcription. The pO_2 drops progressively as tumour cells are distanced from blood vessels and the tumour mass expands. A parallel increase in the level of stable HIF-1α and the downstream effector VEGF-A is detectable. The prolyl hydroxylase domain (PHD) proteins are more sensitive to a drop in oxygen levels than factor inhibiting HIF-1 (FIH), as suggested by the determination of the *in vitro* determined K_m. Thus the PHDs are more rapidly inhibited than is FIH when the oxygen concentration drops. So at moderate oxygen concentrations some stable HIF-1α will accumulate but genes dependent on its C-TAD will not be fully induced due to the restriction imposed by the FIH activity. However, genes requiring only the N-TAD will be induced. A further drop in the pO_2 will prevent FIH hydroxylation and remove inhibition on the C-TAD allowing binding of the transcriptional co-activator CBP/p300. Conditions under which HIF-1 will attain full transcriptional activity.

4. Tumour metabolism in cell survival/death and metastasis

Stabilization and activation of HIF has profound effects on metabolism, in particular on glucose utilization (glycolysis) and proteins synthesis [23]. This in turn has repercussions on cell fate leading to either cell survival or death and possibly metastasis.

4.1. *Glycolysis instead of oxidative phosphoryation*

Glucose is metabolised by a chain of events that go in the order of; glucose transport-glycolysis-tricarboxylic acid (TCA) cycle-oxidative phosphorylation (Fig. 6). The last step of oxidative phosphorylation in mitochondria is the cell's major ATP producing route, however it is oxygen dependent. So hypoxic cells must find an alternative way of obtaining enough ATP for survival. Glycolysis occurring in the cytoplasm also produces ATP but 19-fold less. By increasing the rate of glucose uptake and glycolysis through HIF-dependent up-regulation of the level of expression of glucose transporters and enzymes of the glycolytic pathway hypoxic cells increase their supply of ATP. Thus glycolysis in hypoxic cells compensates for diminished ATP production due to reduced oxygen-dependent oxidative

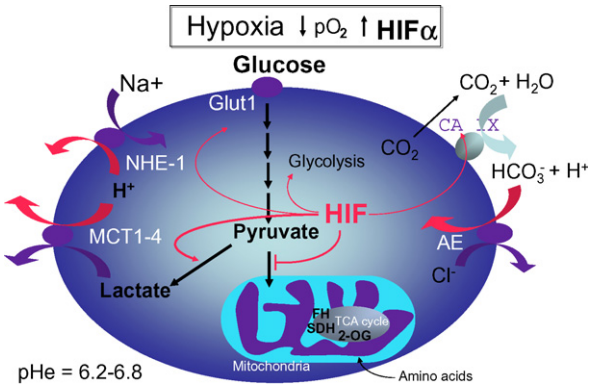

Fig. 6 – Tumour metabolism in hypoxia. Tumour cells respond to a hypoxic environment by increasing their expression of the glucose transporter Glut1 and enzymes of glycolysis. In normal cells the pyruvate generated is metabolized through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, which is efficient in energy production. However, under hypoxic conditions pyruvate is converted into lactate because oxidative phosphorylation is limiting. Since this option is less efficient in producing energy the tumour cells compensate by increasing glucose uptake and metabolism. An overload in lactate contributes to acidosis, which is a common feature of tumours. To maintain a balance between the intracellular and extracellular pH (pHe) lactate is extruded from cells *via* the H^+/lactate monocarboxylate transporter (MCT1-4) family while H^+ are extruded primarily by the growth factor-activatable and amiloride-sensitive Na^+/H^+ exchanger (NHE-1). The CO_2 generated is converted to carbonic acid by the membrane-bound ectoenzyme carbonic anhydrase (CA) IX or XII and HCO_3^-, a weak base, is taken up by members of the Na^+-dependent and -independent HCO_3^- transporters thereby increasing the intracellular pH. The co-substrate 2-oxoglutarate (2-OG) (α-ketoglutarate) required for the activity of the PHD and FIH hydroxylases is generated by the TCA cycle. Catabolism of amino acids is also a source of 2-OG. The production of succinate or fumarate by enzymes of the TCA cycle, respectively succinate dehydrogenase (SDH) and fumarate hydratase (FH) leads to feedback inhibition of these hydroxylases.

phosphorylation in mitochondria. The high rate of glucose uptake by tumours can be visualized using positron emission tomography (PET) after injection to patients with non-metabolisable, radioactive glucose [fluorine-18] 2-deoxy-2-fluoro-D-glucose (FDG) and its accumulation in solid tumours is predictive of aggressive tumours [24]. It has been know for quite some time that tumours have an increased rate of glycolysis, even when the oxygen level is favourable for oxidative phosphoryation. Termed the "Warburg effect" after Otto Warburg who described this phenomenon in the 1920s this rerouting of glucose metabolism by cancer cells is still poorly understood but may be explained by exposure of cells to cycles of hypoxia and re-oxygenation leading to adaptation and/or genetic modification.

However, oncogenes such as *c-myc* and *Akt* that are generally considered to be involved in cancer cell proliferation and survival have also been shown to activate glucose metabolism and may act in concert with HIF [24]. Another interesting link between cancer and mitochondrial dysfunction was recently uncovered through the demonstration that the last step of the mitochondrial electron chain, concerning cytochrome c oxidase, is p53-dependent [25].

4.2. *Acidosis in tumours*

Another characteristic of tumours that has been recognized for some time is their low interstitial pH. Tumour cells produce two major acids, lactic and carbonic acid resulting from increased metabolism particularly through glycolysis. Instead of entering into the TCA cycle the pyruvate produced through glycolysis in hypoxic cells is metabolised to lactate. This reaction is also driven by HIF through the up-regulation of the gene *lactate dehydrogenase A* [26] and through HIF-dependent restriction on the pyruvate dehydrogenase activity [27,28], two interesting effectors that lead to a decrease in cell respiration. The number of H^+ produced per ATP molecule is six-fold higher for glycolysis alone compared to glycolysis-TCA cycle-oxidative phosphorylation. Subsequently the tissue CO_2 concentration increases as the hydrogen ions generated are buffered by bicarbonate. To maintain pH homeostasis cells engage the action of a number of pumps, exchangers and transporters (Fig. 6). The monocarboxylate transporter (MCT) family of proteins excrete lactate and H^+ while the growth factor-activatable and amiloride-sensitive Na^+/H^+ exchanger (NHE-1) exchanges intracellular H^+ for Na^+ [29]. Hypoxia-induced membrane bound carbonic anhydrases (CA), such as CA IX and CA XII having ecto-activity, will rapidly transform the membrane diffusible CO_2 for reversible conversion to carbonic acid [30]. Exchangers such as the Na^+-dependent and -independent Cl^-/HCO_3^- exchanger (AE) will contribute to alkalization of the intracellular pH. Thus, an overload in carbonic and lactic acid leads to a lowering of the extracellular pH, i.e. acidosis. The modulation of tumour acidosis in Ras transformed fibroblasts by deletion of respectively the genes *nhe1* or *pgi* (phosphoglucose isomerase, an enzyme of the glycolytic pathway) has shown potential for inhibiting tumourigenesis [23,31]. Both the expression and activity of NHE-1 [32] and the expression of the MCT4 isoform [33] are regulated by hypoxia and CA IX is one of the most highly induced HIF target gene product [34].

4.3. *Protein synthesis*

Oxygen and nutrient supply go hand in hand since both are carried by the vasculature into tissues and limitations in one (hypoxia) or the other (nutrient depletion) result in inhibition of the mammalian target of rapamycin (mTOR) pathway that controls protein synthesis and growth [23,35]. Activation of mTOR occurs in the presence of growth factors, hormones, amino acids and extracellular components *via* signalling pathways such as the Ras/ERK and PI3K/Akt pathways that converge on the TCS1/2 complex upstream of mTOR. Repression of mTOR in hypoxia, on the other hand, occurs directly through activation of the TSC1/2 complex by the up-regulation

of the HIF-dependent REDDI/RTP801 protein [36]. Interestingly, mutations in the tumour suppressor TSC1/2 lead to tuberous sclerosis complex (TSC) a syndrome characterized by the formation of benign tumours termed hamartomas. TSC1/2 is also regulated by the tumour suppressor *phosphatase* and *tensin* (*pten*) gene where loss of function mutations in *pten* result in accumulation of HIF-1α possibly *via* activation of mTOR. In addition, an auto-regulatory loop linking hypoxia to tumour metabolism may exist through mTOR-induced HIF-1α stabilisation [37]. Hypoxia and nutrient depletion inhibit classic cap-dependent translation but mRNA containing internal ribosome entry sites (IRES) may still be translated. Both HIF-1α and its downstream target gene *vegf-A* contain such sequences thus under stress conditions induction angiogenesis is maintained [38].

4.4. *Cell survival/death*

The metabolic response of cells to hypoxia and nutrient depletion is a strategy that allows cells to adapt and survive but when conditions become drastic other tactics must be sought. The ultimate in survival is macroautophagy, a last chance mechanism by which cells feed on themselves in the face of nutrient depletion. Macroautophagy comprises bulk degradation of cytoplasmic proteins and organelles within a lysosomal/vacuolar system. It comes as no surprise that macroautophagy is regulated by the PI3K/Akt/mTOR pathway [39]. This process may be of significant importance to tumourigenesis as suggested by the implication of the pro-apoptotic HIF target gene *Bcl-2/adenovirus EIB 19 kDa-interacting protein 3* (*bnip3*). In addition, cell death mediated by bnip3 may require growth factor removal or acidosis or glucose deprivation [40]. So, depending on the intensity and duration of the exposure of cells to hypoxic stress, and their metabolic response, cells may follow the path of survival or death.

4.5. *Metastasis*

Hypoxia and the resulting tumour acidosis may influence not only tumourigenesis but also metastasis. Metastasis implies disruption of cell–cell and cell–extracellular matrix contacts that promote cell migration through basement membranes and stromal tissue into the blood circulation and lymphatic system. A substantial number of proteins involved in metastasis are HIF-induced, including: vimentin, fibronectin, keratins 14, 18, 19, matrix metalloproteinase 2, cathepsin D and urokinase plasminogen activator receptor [2]. The acidic microenvironment of tumours can modulate the activity of proteases and high lactate concentrations were found to correlate with the incidence of metastasis and poor prognosis [41]. In addition, E-cadherin the key player in cell adhesion and epithelium mesenchyme transition is repressed by HIF activation in renal cancer cells [42]. Repression occurs through stabilisation and activation of the nuclear factor Snail. Since the product of the HIF target gene lysyl oxidase-like 2 induces a conformational change in Snail leading to its partial stability the repression of E-cadherin expression and thus invasion is linked to hypoxia/HIF in renal cell carcinoma. In addition, certain factors that promote cell migration are also HIF target genes such as *autocrine motility factor* (coding for the glycolytic enzyme phosphoglucose isomerase), the proto-oncogene receptor tyrosine kinase c-MET and the cytokine receptor CXCR4 [23].

5. HIF targeting in cancer and ischemia therapy

Considerable interest is being shown in the inhibition of angiogenesis (anti-angiogenic) for treatment of cancer and conversely in activation of angiogenesis (pro-angiogenic) in treatment of ischemic disorders. Approaches to inhibiting angiogenesis as a way of cutting off the vascular lifeline of tumours are now showing encouraging results in the clinic. However, the majority of these anti-angiogenic drugs target the downstream HIF-induced gene, the *vascular endothelial growth factor* (*vegf*) rather than HIF itself [43]. Given the plethora of genes induced or repressed by HIF it might be wise to attack downstream HIF targets rather than HIF itself, although this is open for discussion [44]. Since HIF plays a beneficial and protective role in the inflammatory response [45] its inhibition might lead to undesirable effects. Even the targeting of downstream genes involved in angiogenesis might enhance tumour hypoxia and increase metastasis [46]. However, the targeting of a downstream gene such as *vegf* might also lead to beneficial modulation of erythropoietin production and erythropoiesis [47]. Nonetheless, a substantial number of HIF small molecule inhibitors or activators are being investigated for treatment respectively, of cancer or ischemic disorder [2,48–51] and their auspicious/deleterious effects await clinical confirmation [44]. Inhibition may be targeted at different levels of the HIF signalling pathway including blocking: signalling pathways that favour accumulation of HIF, the synthesis of HIF, binding of HIF to HRE sequences on endogenous promoters/enhancers, heterodimerisation with HIF-β, interaction with co-activators and stimulating VHL-mediated degradation. Thus the most efficient way in which inhibition is obtained is still to be defined [44]. Alternative, cancer treatments may also arise from manipulation of the microenvironmental consequences of Hypoxia/HIF induction through for example exacerbating metabolic induced acidosis or necrotic cell death [23]. Cytotoxic drugs, depending on their dose, and vascular disrupting agents can independently mobilize bone marrow-derived circulating endothelial progenitor cells to tumours, which might contribute to tumour re-growth, which however can be repressed by anti-angiogenic treatment [52]. Combination therapies using classical cytotoxic drugs and anti-angiogenic or vascular disruptive agents might prevail where the timing of administration and dose will probably be critical to avoid adverse or ineffectual responses [52,53]. Thus, novel delivery strategies such as nanoscale systems, allowing temporal release of combination therapy, hold promise for effective targeting of the tumour vasculature [53]. The treatment of ischemic diseases such as stroke and myocardial infarction may lie in the inhibition of HIF degradation through hydroxylation by the oxygen sensors PHD and FIH or through activation of certain signalling pathways leading to stable and active HIF, which would in turn favour angiogenesis [49,50].

Substantial knowledge into the mechanistics of Hypoxia signalling through HIF has been gained in this last decade and proof-of-principle studies have shown promise for the treatment of cancer and ischemic disease by modulation of the HIF signalling pathway but as one says, the proof of the pudding is in the eating.

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