Histol Histopathol (2014) 29: 589-600

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Grow$_2$: The HIF system, energy homeostasis and the cell cycle

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Summary. Cell cycle progression is an energy demanding process and requires fine-tuned metabolic regulation. Cells must overcome an energy restriction checkpoint before becoming committed to progress through the cell cycle. Aerobic organisms need oxygen for the metabolic conversion of nutrients into energy. As such, environmental oxygen is a critical signalling molecule regulating cell fate. The Hypoxia Inducible Factors (HIFs) are a family of transcription factors that respond to changes in environmental oxygen and cell energy and coordinate a transcriptional program which forms an important part of the cellular response to a hostile environment. A significant proportion of HIF-dependent transcriptional target genes, code for proteins that are involved in energy homeostasis. In this review we discuss the role of the HIF system in the regulation of energy homeostasis in response to changes in environmental oxygen and the impact on cell cycle control, and address the implications of the deregulation of this effect in cancer.

Key words: Hypoxia, HIF-system, Energy homeostasis, Cell cycle, Cancer

Introduction

One of the main factors determining cell fate is its energy status. Cell growth and division is a highly energy demanding process and as such can only occur when the cellular energy balance is favourable. It has long been known that cells need to overcome an energy restriction checkpoint during G1, a few hours before entry into S-phase, after which they become committed to DNA replication and progress through the cell cycle (Pardee, 1974). Additionally, growing cell populations also need increased biomass production, which requires adequate oxygen and nutrient supply and the activation of biosynthetic pathways. Cancer cells are able to survive and acquire a growth advantage by enabling metabolic reprograming to adapt to harsh environments. This reprograming will, in turn, create conditions for genomic instability, which can enhance cancer cells ability to invade neighbouring tissues.

Cellular oxygen is both a nutrient and a signalling molecule. It is essential as a catalyst or co-factor for many biochemical reactions, as well as being the stimulus for a complex transcriptional program. Hypoxia (low oxygen tensions) occurs when the normal oxygen supply to a tissue is disturbed. Perturbations in environmental oxygen have important implications for many of the cellular functions required for energy homeostasis. Changes in environmental oxygen are sensed by a group of dioxygenases that control the activity of an essential transcription factor family known as the Hypoxia Inducible Factors (HIFs) (Kenneth and Rocha, 2008). The HIF system co-ordinates the cells transcriptional program in response to changes in environmental oxygen concentration triggering mechanisms to ensure cell survival and the reestablishment of oxygen supply. Here, we review the role of the HIF system in the control of energy homeostasis and the impact on cell cycle control, and discuss the implications of its deregulation in cancer.

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The HIF System

HIF1 was first identified in studies of erythropoietin gene expression (Semenza and Wang, 1992). HIF is a heterodimeric transcription factor that consists of a constitutively expressed HIF1ß subunit and an O_2-regulated HIF1α subunit (Wang et al., 1995). Three isoforms of HIF1α have been identified since these initial studies (HIF1α, 2α, and 3α). The HIF-α isoforms are all characterized by the presence of bHLH (basic helix–loop–helix)–PAS (Per/ARNT/Sim), and ODD (oxygen-dependent degradation) domains (Fig. 1). Both HIF1α and 2α have important cellular functions as transcription factors with some redundancy in their targets (Carroll and Ashcroft, 2006; Hu et al., 2006). HIF2α protein shares sequence similarity and functional overlap with HIF1α, but its distribution is restricted to certain cell types, and in some cases, it mediates distinct biological functions (Patel and Simon, 2008). HIF3α is the most recently discovered isoform. It is thought to operate as a dominant negative inhibitor of HIF1α and HIF2α since it lacks the C-terminal transactivation domain required to initiate transcription. The expression of HIF3α is largely restricted to tissue where control of neo-angiogenesis is tightly regulated (Makino et al., 2001). Several splice variants of HIF1ß [also known as ARNT (aryl hydrocarbon receptor nuclear translocator)] have been identified (Bardos and Ashcroft, 2005; Rocha, 2007). Thought their exact functions are not known, at least one splice variant has been associated with poor prognosis in estrogen receptor-negative breast cancer (Qin et al., 2001).

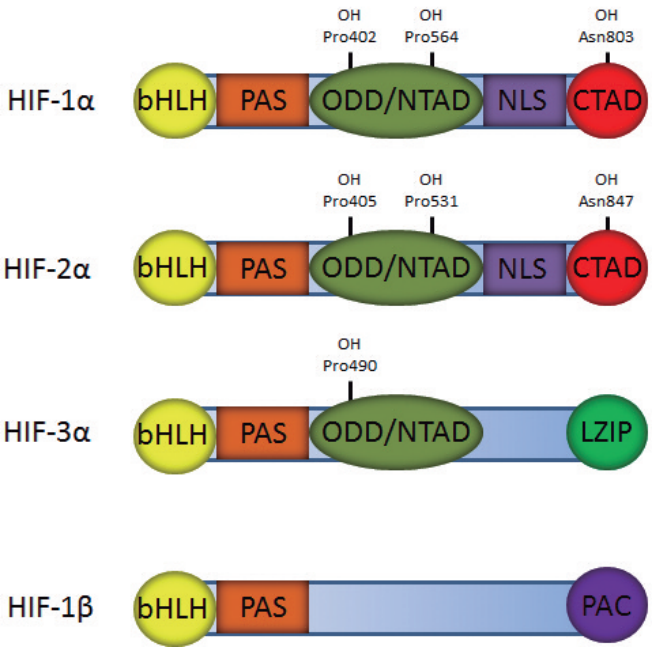

Fig. 1. The HIF isoforms and their functional domains: The HIF system consists of three -α and one -ß isoforms. All isoforms possess a basic helix-loop-helix (bHLH) domain used to bind DNA and a Per-ARNT-Sim (PAS) domain for heterodimerisation. The -α isoforms additionally possess hydroxylatable oxygen dependent degradation (ODD) and N-terminal transactivation (NTAD) domains that are dynamically prolyl hydroxylated in response to changes in environmental oxygen. Additional nuclear localisation (NLS) and aspartate hydroxylation domains (CTAD) also feature on both the HIF-1α and -2α which are important for nuclear translocation and the recruitment of transcriptional co-factors respectively. HIF-3α acts as a dominant negative isoform and possess a C-terminal leucine zipper (LZIP) rather than the CTAD of the active -α isoforms. HIF-1ß, of which there is one known isoform and several splice variants has a PAS associated C terminal Activation Domain (PAC).

The expression of HIFα subunits is mainly regulated at the post-transcriptional level, by hydroxylation-dependent proteasomal degradation, but transcriptional and translational regulation mechanisms have also been described that involve NF-κB (Rius et al., 2008; van Uden et al., 2008, 2011) and the mammalian target of rapamycin (mTOR) (Linehan et al., 2010; Smolkova et al., 2011), respectively. We, and others, have shown that NF-κB is a direct modulator of HIF1α expression, in basal and hypoxic conditions as well as in response to inflammatory stimulus (Rius et al., 2008; van Uden et al., 2008), and also directly regulates HIF1ß mRNA and protein levels, resulting in modulation of HIF2α protein levels (van Uden et al., 2011). Additionally, HIF1α mRNA stability has been shown to be dependent on P-body function (Bett et al., 2013).

mTOR exists in two distinct complexes, mTORC1 and mTORC2. Knockout experiments showed that HIF1α translation is dependent on mTORC1 and mTORC2 signalling, while HIF2α translation relies mainly on mTORC2 activity (Toschi et al., 2008).

More recently, a lysosomal pathway for HIF1α degradation has been described, involving chaperone-mediated autophagy. This degradation mechanism seems to be independent of O_2. Instead, nutrient starvation lead to a decrease in HIF1α levels, suggesting this could be an additional mode of HIF1α regulation, by nutrient availability (Hubbi et al., 2013).

HIF regulation by oxygen

In well-oxygenated cells, HIF1α is hydroxylated in its ODD. For HIF1α this is at prolines (Pro) 402 and 564 (Kaelin and Ratcliffe, 2008), whereas HIF2α is hydroxylated at Pro 405 and 531 (Haase, 2012). This proline hydroxylation is catalysed by a class of dioxygenase enzymes called prolyl hydroxylases (PHDs) (Fig. 2). There are three known PHDs, 1-3, all of which have been shown to hydroxylate HIF1α. PHD2 has a higher affinity for HIF1α, whereas PHD1 and PHD3 have higher affinity for HIF2α (Berra et al., 2003; Appelhoff et al., 2004). All PHDs require Fe^{2+} and α-ketoglutarate (α-KG) as co-factors for their catalytic activity and have an absolute requirement for molecular oxygen as a co-substrate, making their activity reduced in hypoxia (Epstein et al., 2001; Fandrey et al., 2006, Frede et al., 2006; Bruegge et al., 2007). These

experiments were very useful since they identified mechanisms by which the PHDs are pharmacologically sensitive to the iron chelator desferrioxamine (DFX), and dimethyloxalylglycine (DMOG), an analogue and competitive antagonist of α-KG (Epstein et al., 2001). Prolyl-hydroxylation of HIF1α attracts the von Hippel-Lindau (vHL) tumor suppressor protein, which recruits the Elongin C-Elongin B-Cullin 2-E3-ubiquitin-ligase complex, leading to the Lys48-linked poly-ubiquitination and proteasomal degradation of HIF1α (Ivan et al., 2001; Jaakkola et al., 2001; Yu et al., 2001). Interestingly, PHDs have also been shown to be able to sense aminoacid availability through α-KG oscilations, indicating an additional function as nutrient sensors for these enzymes (Duran et al., 2013).

In hypoxia the PHDs are inactive since they require O_2 as a cofactor. Under these conditions HIF1α is stabilized, can form a heterodimer with HIF1ß in the nucleus and bind to the consensus cis-acting hypoxia response element (HRE) nucleotide sequence 5'-RCGTG-3', which is present within the enhancers and/or promoters of HIF target genes (Pugh et al., 1991; Semenza et al., 1996; Schodel et al., 2011). HIF1α stabilisation therefore allows the cell to enact a transcriptional programme that is appropriate to the hypoxic environment (Kaelin and Ratcliffe, 2008).

Further fine-tuning of the cellular response to hypoxia is provided by hydroxylation within the C-terminal transactivation domain of HIF1α and 2α by the Factor Inhibiting HIF (FIH). This ubiquitously expressed protein is an asparaginyl hydroxylase that can hydroxylate HIF1α at asparagine (Asn) 803 and HIF-2α at Asn847, preventing the recruitment of the transcriptional co-activators p300/CBP [CREB (cAMP-Response Element-Binding Protein) Binding Protein] and full target gene activation (Mahon et al., 2001; Lando et al., 2002a,b; Rocha, 2007; Lisy and Peet, 2008). FIH provides a degree of specificity to the HIF response. Several studies have shown that in the absence of FIH (Dayan et al., 2006), or p300 binding (Kasper et al., 2005), a number of target genes remain HIF-inducible. FIH, like the PHDs, requires Fe^{2+} and α-KG as co-factors and molecular oxygen as a co-substrate to function but has reduced sensitivity to molecular oxygen compared to the PHDs. Interestingly, FIH can also be specifically inhibited by peroxide in hypoxia (Masson et al., 2012).

HIF1 regulates the expression of hundreds of target genes involved in angiogenesis, erythropoiesis, metabolism, autophagy, apoptosis and other physiological responses to hypoxia (Semenza, 2009).

HIF regulation by energy and nutrient levels

HIF is induced by growth factors and hormones in normal cells, even in normoxic conditions (Fig. 3). This is most relevant to maintain cellular energy homeostasis and cell cycle control. This regulation is mainly mediated by mTOR-dependent translation (Knaup et al., 2009).

mTOR integrates the input from upstream pathways activated by growth factors, nutrient availability and oxygen and energy levels. mTOR is a serine/threonine kinase that confers catalytic activity to two distinct complexes named mTORC1 and 2. These differ not only in composition but also in target specificity and nutrient sensitivity. While mTORC1 signaling is nutrient-sensitive, can be inhibited by rapamycin and it's main downstream effectors are 4EBP1 and the p70 ribosomal S6 kinase, mTORC2 is not sensitive to nutrients, insensitive to rapamycin, and its known substrates include kinases such as Akt, SGK, and PKC family members (Shaw, 2009).

Regulation of mTOR by nutrient levels is dependent on the 5' AMP-activated protein kinase (AMPK). AMPK is activated under conditions of energy stress, when intracellular ATP levels decline and intracellular AMP increases, as occurs during nutrient deprivation or hypoxia (Kahn et al., 2005). AMP directly binds to AMPK and this is thought to prevent dephosphorylation of the critical activation loop threonine, which is absolutely required for AMPK activation (Sanders et al., 2007). The serine/threonine kinase LKB1 is the major kinase phosphorylating the AMPK activation loop under conditions of energy stress (Hardie, 2007). Active AMPK can in turn phosphorylate Tuberous Sclerosis Complex 2 (TSC2) and also the mTORC1 subunit raptor which both contribute to inhibit mTOR (Shaw, 2009).

mTOR regulation by growth factor and hormone

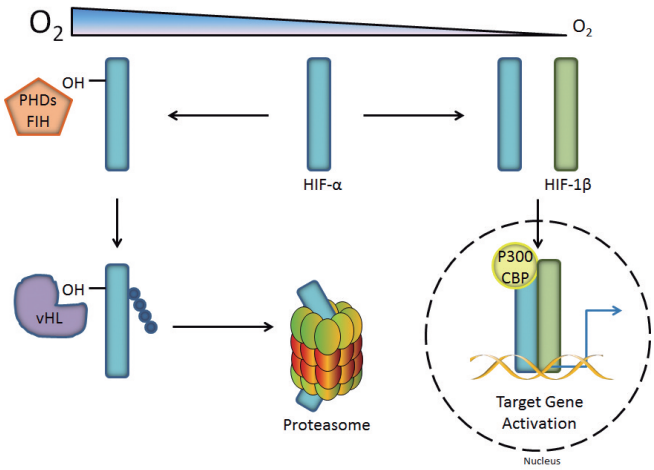

Fig. 2. Regulation of the HIF system by oxygen: The HIF-α isoforms are dynamically regulated in response to changes in environmental oxygen. In normoxia, the dioxygenase enzymes (PHDs and FIH) hydroxylate HIF-α at specific residues which attracts the von Hippel-Lindau (vHL) protein and results in their poly-ubiquitination and subsequent degradation by the proteasome. The dioxygenase enzymes require molecular oxygen as a co-factor and have reduced activity in hypoxia, HIF-α is stabilised, can form a heterodimer with HIF-1ß and translocate to the nucleus where it can recruit co-activators, activating the transcription of its target genes that allow the cell to respond to its hostile environment.

signalling is mainly mediated by the PI3K/Akt pathway. Akt phosphorylates mTOR and also directly phosphorylates TSC2, resulting in the impairment of TSC1/TSC2 inhibition of mTOR (Wullschleger et al., 2006). Interestingly, HIF induces the expression of growth factors such as VEGF, IGF, FGF and of matrix metalloproteinases (MMPs), which can in turn activate Akt, creating a regulatory positive feedback loop (Deudero et al., 2008).

In normoxia, mTOR induced HIF activation is most likely transient since the increase in HIF activity will lead to an increase in PHD levels, which will be active to promote HIF degradation (Demidenko and Blagosklonny, 2011). Interestingly, it was recently demonstrated that mTOR activation by amino acids requires αKG-dependent PHD activitiy (Duran et al., 2013). Thus, an original growth factor stimulus may, through these mechanisms, originate another positive feedback loop, in situations where nutrient availability is not limited, allowing for growth and cell cycle

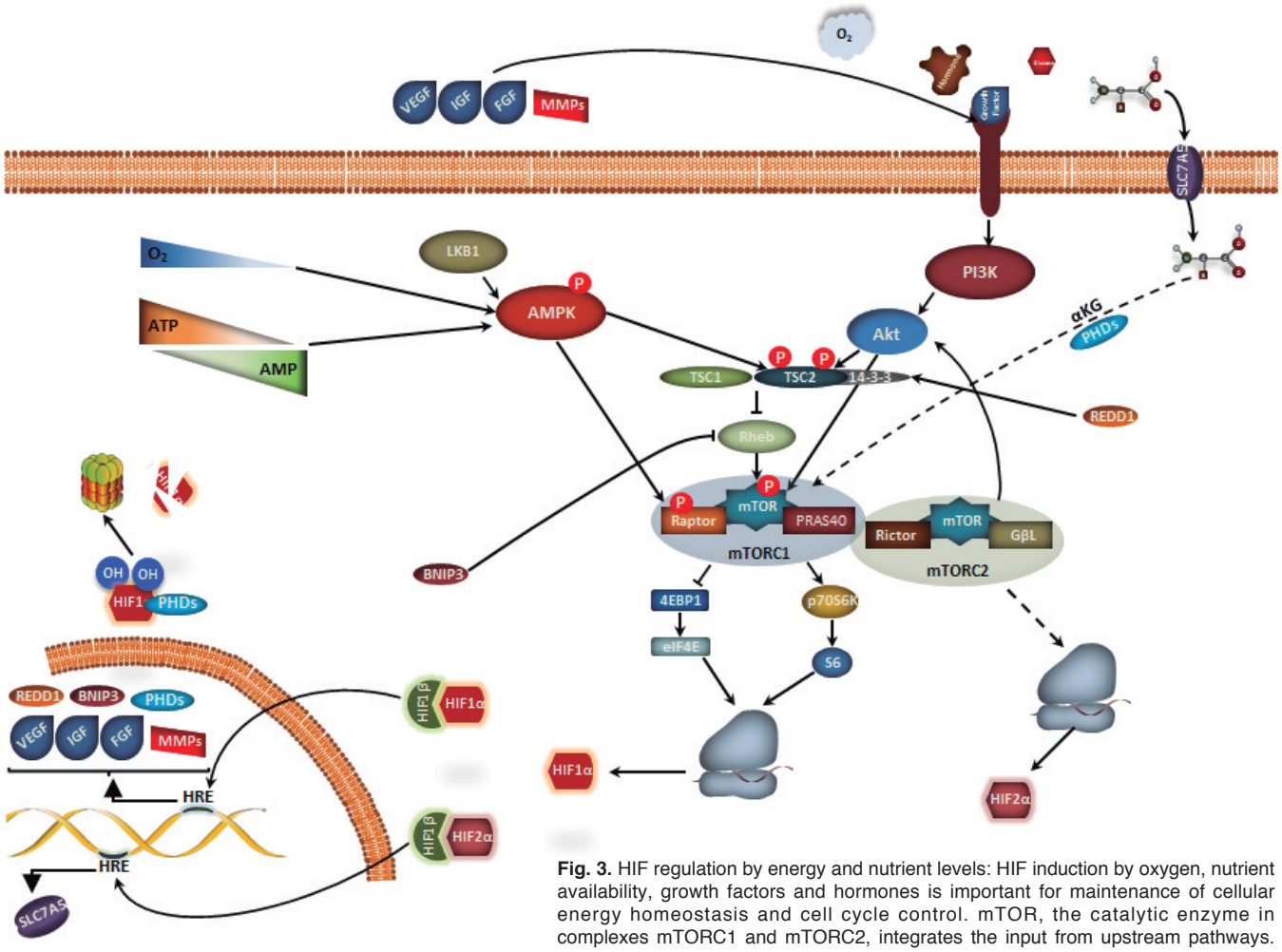

Fig. 3. HIF regulation by energy and nutrient levels: HIF induction by oxygen, nutrient availability, growth factors and hormones is important for maintenance of cellular energy homeostasis and cell cycle control. mTOR, the catalytic enzyme in complexes mTORC1 and mTORC2, integrates the input from upstream pathways. mTORC1 is sensitive to nutrient availability and the main downstream effectors are 4EBP1 and the p70 ribosomal S6 kinase (p70S6K). mTORC2 is not sensitive to nutrients and its known substrates include kinases such as Akt. Regulation of mTOR by nutrient levels is dependent on the 5' AMP-activated protein kinase (AMPK). AMPK is activated under conditions of energy stress, when intracellular ATP levels decline and intracellular AMP increases. Active AMPK phosphorylates Tuberous Sclerosis Complex 2 (TSC2) and also Raptor, both contributing to inhibit mTOR. mTOR regulation by growth factor and hormone signalling is mediated by the PI3K/Akt pathway. Akt phosphorylates mTOR and TSC2, resulting in the impairment of TSC1/TSC2 inhibition of mTOR. HIF induces the expression of growth factors such as VEGF, IGF, FGF and of matrix metalloproteinases (MMPs), which can activate Akt, creating a regulatory positive feedback loop. mTOR induced HIF activation leads to an increase in PHD levels, which can work to promote HIF degradation. mTOR activation by amino acids requires αKG-dependent PHD activitiy, which means that a growth factor stimulus may originate a positive feedback loop, in situations where nutrient availability is not limited. Hypoxia and HIF1α can inhibit the mTOR pathway. HIF induction by low oxygen levels up-regulates the expression of REDD1, which directly binds to and sequesters 14-3-3 proteins from TSC2, resulting in TSC activation and mTORC1 inhibition. HIF1α-induced BNIP3, has also been implicated in hypoxia-dependent mTORC1 inhibition by decreasing the activity of the small GTPase Rheb. HIF2α induces mTORC1 activity by upregulating the expression levels of amino acid transporter SLC7A5. Downregulation of mTOR signalling leads to an impairment of protein synthesis and translation, the major energy consuming processes in the cell.

progression.

Hypoxia and HIF1α can inhibit the mTOR pathway however the mechanisms involved are still poorly understood. HIF induction by low oxygen levels up-regulates the expression of REDD1. It has been proposed that REDD1 directly binds to and sequesters 14-3-3 proteins from TSC2, resulting in TSC activation and subsequent mTORC1 inhibition (Brugarolas et al., 2004, DeYoung et al., 2008). REDD1 induction was also shown to reduce mithochondrial reactive oxygen species (ROS) production (Horak et al., 2010). Additionally, HIF1α-induced BNIP3, has also been implicated in hypoxia-dependent mTORC1 inhibition by decreasing the activity of the small GTPase Rheb, which is required for mTORC1 activity (Li et al., 2007). Together, these negative feedback loop mechanisms provide further fine-tunning of HIF1α expression.

It has also been suggested that mTOR can regulate HIF1 activity by direct phosphorylation of HIF1α. HIF1α interacts with raptor through an mTOR signaling motif making it thus possible that mTOR directly phosphorylates HIF1α. This phosphorylation was shown to promote HIF1α ability to function as a transcription factor (Knaup et al., 2009).

While HIF1α-driven mTORC1 inhibition works on attenuating cell proliferation, HIF2α has been shown to promote tumourigenesis even in conditions of hypoxia and HIF1α expression (Raval et al., 2005). It was recently reported that HIF2α specifically induces mTORC1 activity by upregulating the expression levels of the amino acid carrier SLC7A5, which was shown to be a HIF2α target (Elorza et al., 2012). Since amino acid carriers SLC1A5 and SLC7A5 are required to sustain mTORC1 activity (Nicklin et al., 2009), HIF2α induction seems to play a critical role in the regulation of mTORC1 activity when amino acid availability is limited.

Downregulation of mTOR signalling leads to subsequent suppression of protein synthesis and translation, the major energy consuming processes in the cell.

HIF system in energy homeostasis

HIF-1 can activate a wide range of target genes, many of which are important components in cellular

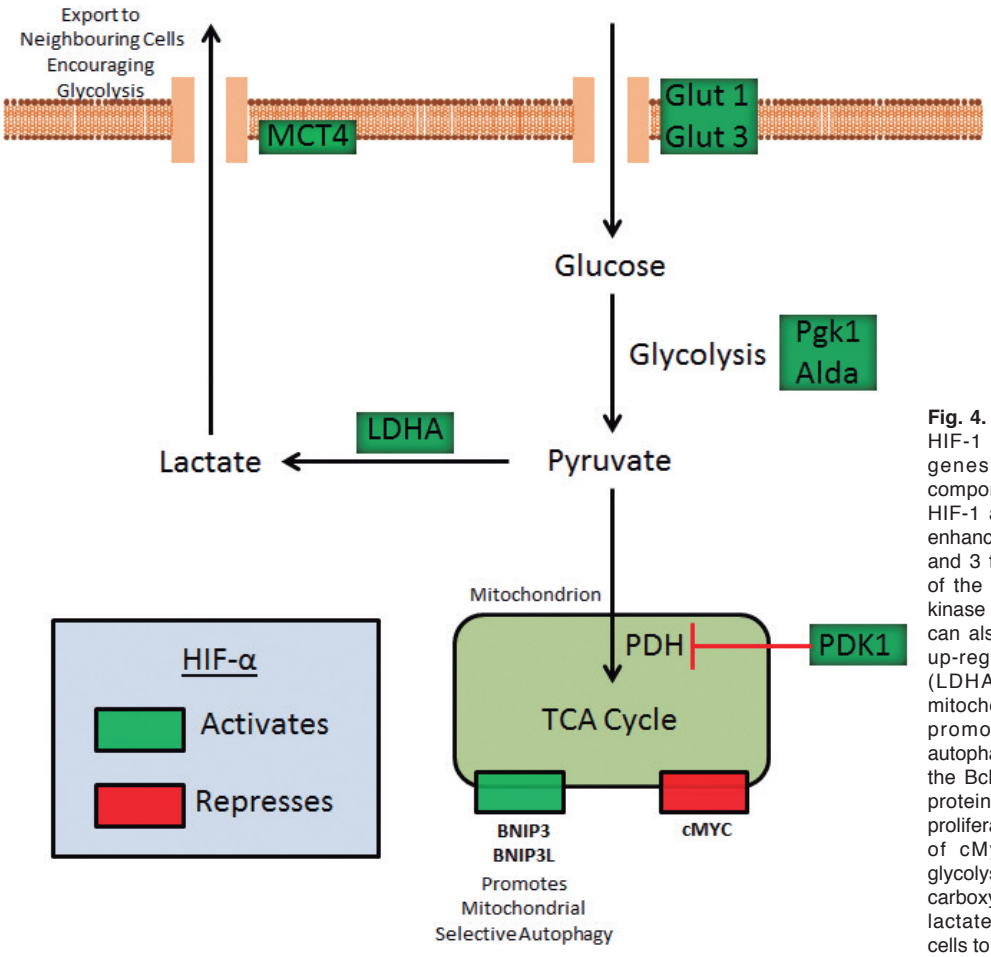

Fig. 4. The HIF system in energy metabolism: HIF-1 can activate a wide range of target genes, many of which are important components in cellular energy homeostasis. HIF-1 activation can increase glycolysis by enhancing glucose uptake through the GLUT1 and 3 transporters and through up-regulation of the glycolytic enzymes phosphoglycerate kinase 1 (Pgk1) and aldolase A (Alda). HIF-1 can also enhance lactate production through up-regulation of Lactate dehydrogenase A (LDHA). Oxidation of pyruvate in the mitochondria is further reduced through the promotion of selective mitochondrial autophagy as a direct result of up-regulation of the Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3 and BNIP3L) and reduced proliferative demand through indirect inhibition of cMyc. HIF can also stimulate local glycolysis through up-regulation of the mono-carboxylate transporter 4 (MCT-4) dependent lactate efflux that encourages neighboring cells to use this pathway.

energy homeostasis (Fig. 4). An essential adaptation to hypoxia is mediated through increased glycolysis and lactate production and decreased oxidation of pyruvate in mitochondria, reducing oxidative phosphorylation and hence O_2 consumption. However, cancer cells can maintain this phenotype even at normal O_2 pressures, a phenomenon known as the Warburg effect (Gatenby and Gillies, 2004). Activation of HIF1, which commonly occurs in human cancers either as a result of hypoxia or genetic alterations, can drive this change (Harris, 2002; Semenza, 2010), leading to a switch from oxidative to glycolytic metabolism through activation of its energy-specific target genes (Seagroves et al., 2001; Wheaton and Chandel, 2011). HIF1 target genes include those encoding: the glucose transporters GLUT1 and GLUT3, which increase glucose uptake; lactate dehydrogenase A (LDHA), which converts pyruvate to lactate; and pyruvate dehydrogenase kinase 1 (PDK1), which inactivates pyruvate dehydrogenase, thereby shunting pyruvate away from the mitochondria and inhibiting O_2 consumption (Ke and Costa, 2006). In addition, HIF1α exclusively induces the hypoxic transcription of glycolytic genes such as phosphoglycerate kinase 1 (Pgk1) and aldolase A (Alda) (Hu et al., 2003; Wang et al., 2005).

LDHA activity is required to maintain the levels of NAD^+ to enable glycolysis when oxidative phosphorylation is decreased (Fantin et al., 2006). Excess lactate can be exported via specific mono-carboxylate transporters (MCTs), to neighbouring cells and be up taken by these to fuel energy metabolism. MCT4, the lactate exporter is induced by hypoxia and oxidative stress and is a known HIF1α target gene (Ullah et al., 2006). In fact, this is one of the mechanisms by which the malignant tumour and its microenvironment influence each other's metabolic profile. In a co-culture model of a breast cancer cell line and normal fibroblasts, the oxidative stress generated by the cancer cells induced MCT4 expression on the adjacent tumour associated fibroblasts (Whitaker-Menezes et al., 2011). Interestingly, activation of HIF1α and HIF2α were described to have distinct consequences if they occurred in the tumour or the surrounding microenvironment in another breast cancer model. In mouse tumour xenografts, activation of HIF1α in the stroma fibroblasts promoted increased glycolysis and lactate production, with increased MCT4 and decreased MCT1 expressions, and tumour growth. HIF1α activation in breast cancer cells reduced tumour volume, though it also induced a shift towards aerobic glycolysis and lactate extrusion. Conversely, HIF2α activation in the stromal cells did not alter their metabolic profile while activation in the tumour cells resulted in increased tumour volume and the expression of genes important for cell cycle progression (Chiavarina et al., 2012). Interestingly, HIF2α does not seem to activate genes related with a glycolytic switch but rather regulates the induction of genes related with cell proliferation and stem cell fate (Hu et al., 2003, 2006; Chiavarina et al., 2012).

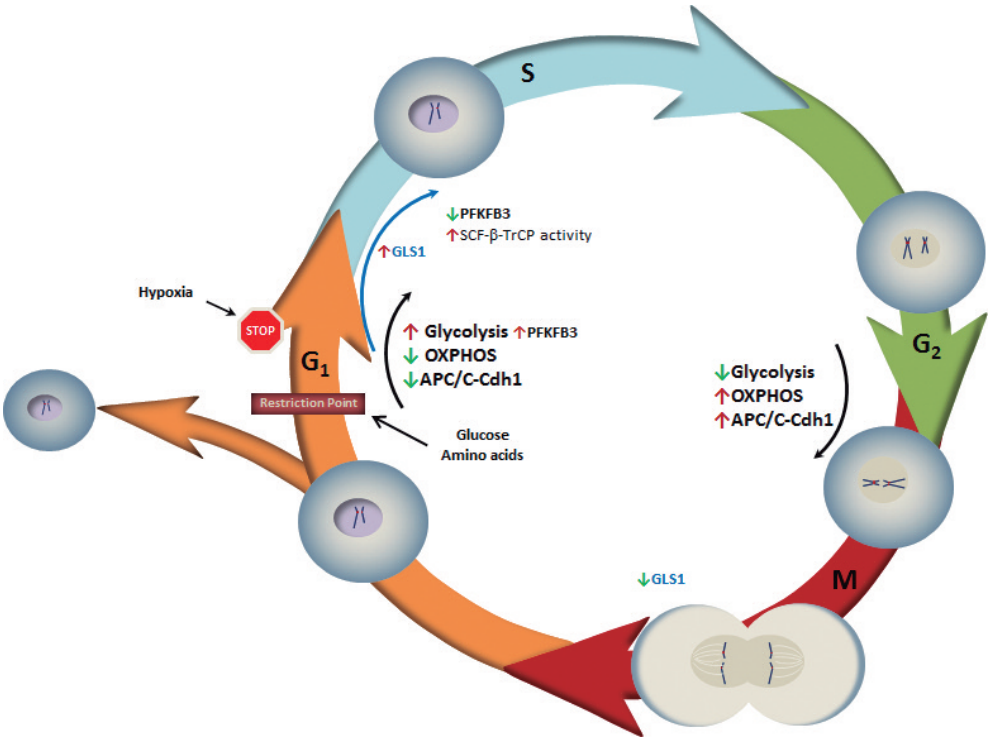

Fig. 5. Regulation of the cell cycle by oxygen and energy levels: Hypoxia and induction of HIF1α lead to G_1-phase cell cycle arrest. Cells have to overcome an energy restriction checkpoint in late G_1-phase before entering the cell cycle. Glucose and amino acid availability are important regulators of the progression from G_1 to S-phase. Oxidative phosphorylation and glycolysis are tightly regulated as a function of cell cycle phase. An increase in glycolysis in the late G_1 phase of the cell cycle was shown to be required for progression into S-phase, whereas G_2-M transition seems to be highly dependent on oxidative phosphorylation. The levels of phosphofrutokinase isoform 3 (PFKFB3) are enhanced in glycolysis and are critical for cell cycle progression. PFKFB3 is increased in late G_1 following the decrease in the ubiquitin ligase APC/C-Cdh1. The decrease in PFKFB3 as cells enter the S-phase, results from increased activity of the SCF-ß-TrCP ubiquitin ligase complex. Glutaminase 1 (GSL1) is regulated by APC/C-Cdh1, which mediates degradation of GLS1 as cells exit mitosis and during the G_1-phase.

The fact that tumours must sustain a high rate of proliferation results in higher metabolism, in which energy needs exceed the energy supplied by nutrients from blood, inevitably resulting in aglycemia. Consequently, new metabolic reprograming must be initiated to ensure tumour cell survival where glutamine is used as an energy source, and for the synthesis of biomolecules, a process called glutaminolysis, which also generates lactate (Deberardinis et al., 2008; Mullen et al., 2012). Most catalytic steps in glutaminolysis occur in the mitochondria and require a partial tricarboxylic acid cycle (TCA cycle) activity involving one of two possible mechanisms. The first involves glutamine conversion into to α-KG and then citrate using the forward order of the TCA cycle, where partial oxidative phosphorylation is restored. Alternatively, after generation of α-KG this is converted to isocitrate and then citrate with the reactions in the reverse order of the TCA cycle. This mechanism is independent of oxidative phosphorylation and can be active even in anoxia. This later form may even increase the tumour's malignancy but it does not produce ATP, so hypothetically anoxic glutaminolysis could be followed by intermittent glycolytic periods, sustaining tumour survival and growth (Smolkova et al., 2011).

Mitochondrial metabolism provides precursors to build macromolecules in growing cancer cells. In functional tumour cell mitochondria, oxidative metabolism of glucose- and glutamine-derived carbon produces citrate and acetyl-coenzyme A for lipid synthesis, which is required for tumorigenesis (Deberardinis et al., 2008; Mullen et al., 2012). HIF1α activation results in a decrease in mitochondrial activity and biogenesis through c-myc inhibition (Zhang et al., 2007; O'Hagan et al., 2009). Additionally, the HIF1α target gene BNIP3, is involved in the regulation of mitochondrial autophagy (Zhang et al., 2008). However, HIF2α activation has been shown to increase mitochondrial activity and oxidative phosphorylation in breast cancer cells (Chiavarina et al., 2012). HIF2α also regulates cellular lipid metabolism by suppressing fatty acid ß-oxidation and increasing lipid storage capacity in vHL-deficient hepatocytes (Rankin et al., 2009). Furthermore, HIF2α activation has been shown to be sufficient to stimulate glutaminolysis and decrease glycolysis in renal cell carcinoma (Gameiro et al., 2013).

Hypoxia adaptation causes altered glycogen metabolism consistent with an acute induction of glycogen synthesis catalyzed by glycogen synthase, followed by a subsequent induction of glycogen phosphorylase-dependent breakdown of glycogen (Favaro et al., 2012).

Hypoxia will also increase the production of reactive oxygen species, namely by inducing the activity of NADPH oxidases (Ushio-Fukai and Nakamura, 2008). This will in turn potentiate DNA damage and the damaging oxidation of several macromolecules. However, under oxidative stress conditions, excessive

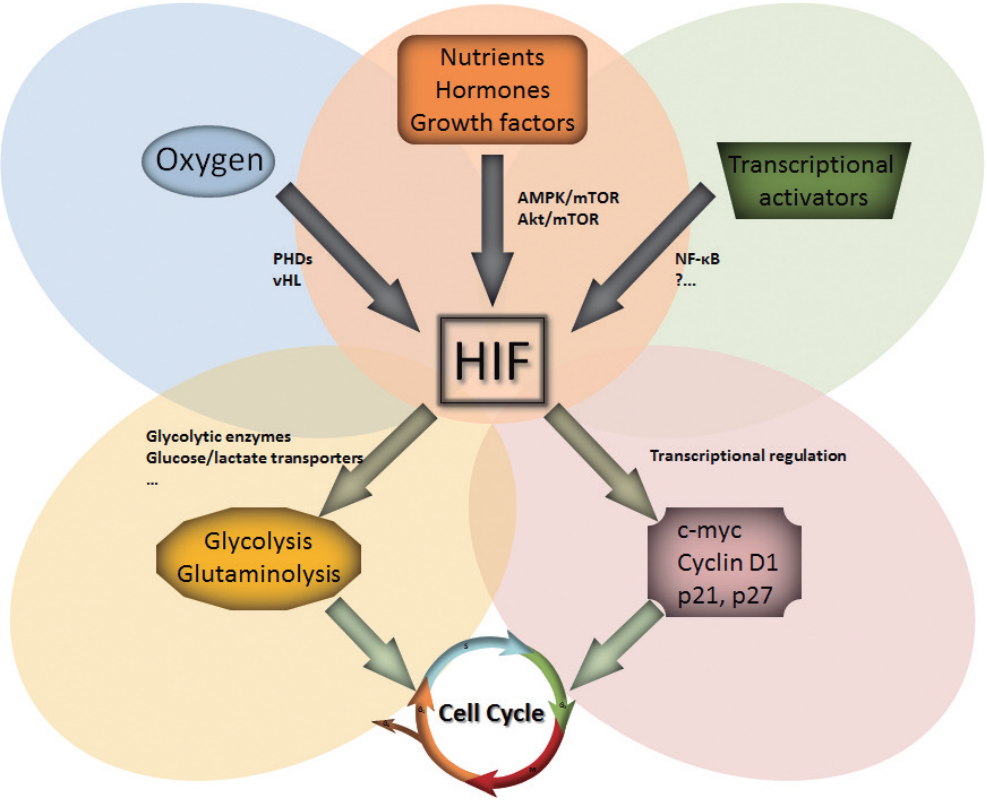

Fig. 6. The HIF system is a key regulator translating environmental cues into metabolic adaptation: Environmental stimuli such as oxygen and nutrient availability as well as additional stresses like inflammation all contribute to the fine-tuned regulation of the HIF system. HIFα-dependent transcription impacts mainly on metabolic adaptation and on the expression levels of key cell cycle regulators, both contributing to the cell cycle progression.

ROS can damage cellular proteins, lipids and DNA, leading to fatal lesions in cell that contribute to carcinogenesis.

As tumours grow, most will have hypoxic areas, due to high rates of cell proliferation and insufficient blood supply. Hypoxia and HIF deregulation have been associated with radiotherapy resistance and increased risk of mortality in diverse tumour types, such as, bladder, brain, breast, colon, cervix, endometrium, head/neck, lung, ovary, pancreas, prostate, rectum, and stomach (Semenza, 2012). Elevated cell proliferation, requires adequate levels of energy and nutrient supply to allow for doubling of biomass during the cell cycle. As a master regulator of cell metabolic programing, the HIF system is of particular interest in the regulation of cell cycle progression.

Regulation of the cell cycle by oxygen and energy levels

Induction of HIF1α by hypoxia leads to G1-phase cell cycle arrest in multiple cell types including various cancer cell lines (Box and Demetrick, 2004; Koshiji et al., 2004; Gordan et al., 2007; Hubbi et al., 2011), and forced overexpression of HIF1α is sufficient to inhibit cell proliferation (Hackenbeck et al., 2009). The HIFs can alter cell cycle progression through transcriptional targets such as Cyclin D1 (Baba et al., 2003) and indirect modulation of the cyclin dependant kinase (CDK) inhibitors p21 and p27 (Gardner et al., 2001; Green et al., 2001; Goda et al., 2003; Koshiji et al., 2004; Gordan et al., 2007). Additionally, HIF1α downregulates c-myc induced transcription and induces G1 cell cycle arrest by upregulating CDK inhibitors p21 and p27 (Goda et al., 2003). Conversely, HIF2α has been shown to promote hypoxic cell proliferation by enhancing c-myc transcriptional activity (Gordan et al., 2007).

Cells have to overcome an energy restriction checkpoint before entering the cell cycle. It was shown, that there is a period in cell division during G1 in which cells become committed to DNA replication, and no longer require the presence of mitogenic signals to progress through the cell cycle (Pardee, 1974). This is the stage in which energy and nutrient sensors regulate cell growth and proliferation, however, which metabolites are important for this regulation and what are the mechanisms involved is still largely unknown (Fig. 5).

Glucose and amino acid availability are thought to be main determinants in progression from G1 to S-phase, the latter being the period in which most biosynthetic reactions occur. As a main sensor for oxygen and nutrients such as amino acids and glucose, the AMPK-mTOR pathway plays an important role linking mTOR-dependent translation of specific cell growth regulators, including cyclins D1, E and c-myc to nutritional sensing (Guertin and Sabatini, 2007; Shaw, 2009; Foster et al., 2010).

Oxidative phosphorylation and glycolysis seem to be coupled with each other and tightly regulated as a function of cell cycle phase. An increase in glycolysis in the late G1 phase of the cell cycle was shown to be required for progression into S-phase, whereas G2-M transition seems to be highly dependent on oxidative phosphorylation (Moncada et al., 2012). Cells in S-phase display an intermediary energy metabolism between that of G1 and G2-M phases (Bao et al., 2013). However, little is known about the mechanisms and regulators of the phase-specific metabolic program.

A key regulator of cell cycle progression is the expression level of phosphofrutokinase isoform 3 (PFKFB3), whose activity is enhanced in glycolysis (Duan and Pagano, 2011; Tudzarova et al., 2011; Moncada et al., 2012). Expression of this key glycolytic regulatory enzyme is tightly controlled and coupled with the cell cycle profile (Moncada et al., 2012). An increase in PFKFB3 is observed in late G1, consistent with increased glycolysis, followed by a rapid decrease as cells enter the S-phase. It was shown that this follows an opposite pattern as the activity of the ubiquitin ligase APC/C-Cdh1, known to be responsible for the degradation of PFKFB3 via the KEN box destruction motif (Tudzarova et al., 2011). This may be the mechanism that co-ordinates the provision of glucose

Table 1. Clinical significance HIF system subunit and dioxygenase deletion in mice.

Subunit	Phenotype	References
HIF-1α	Developmental arrest and embryological lethality by E11. Cardiovascular and neural tube defects	Iyer et al., 1998
HIF-2α	Embryonic lethality between E9.5 and E13.5 associated with multiple phenotypes affecting the sympatho-adrenal axis, angiogenesis and surfactant production	Tian et al., 1998, Peng et al., 2000, Compernolle et al., 2002, Scortegagna et al., 2003, Gruber et al., 2007
HIF-1ß	Embryologically lethal D10.5. Defective yolk sac, placental and branchial arch angiogenesis	Maltepe et al., 1997
PHD-1	Viable, moderate erythrocytosis	Takeda et al., 2008
PHD-2	Double knock out = Embryonic lethality d12.5-14.5; Conditional = erythrocytosis and premature death; Point mutations in active site cause familial erythrocytosis in humans	Percy et al., 2006, Takeda et al., 2006, 2008
PHD-3	Viable, moderate erythrocytosis and abnormal sympatho-adrenal development	Bishop et al., 2008, Takeda et al., 2008
FIH	Viable, Reduced body weight and elevated metabolic rate	Zhang et al., 2010

with the progression into S-phase. PFKFB3 was also shown to possess a DSGXXS motif known to be the substrate for SCF-ß-TrCP ubiquitin ligase complex. SCF-ß-TrCP specifically targets PFKFB3 during S phase, leading to a concomitant decrease in glycolysis during this stage of the cell cycle (Tudzarova et al., 2011).

Interestingly, an amino acid deficiency can also lead to PFKFB inhibition by uncharged tRNAs. Charged tRNAs have been shown to be sequestered within the cellular protein synthetic machinery. When the tRNA of mammalian cells is incompletely charged due to amino acid deficiency many metabolic events become limited and this appears to be because of the inhibition of phosphofructokinase by uncharged tRNAs (Rabinovitz, 1995).

Glutaminase 1 (GSL1), the first enzyme in the glutaminolysis pathway, is also regulated by ubiquitin ligases through the cell cycle via interaction with a KEN box destruction motif (Colombo et al., 2010). APC/C-Cdh1 mediates the degradation of GLS1 as cells exit mitosis and during the G1-phase. The peak of glutaminolysis activation overlaps only partially with that of glycolysis as GLS1, but not PFKFB3, is required to complete S phase (Duan and Pagano, 2011; Moncada et al., 2012).

Although hypoxia induces cell cycle arrest in the G1-phase of the cell cycle, it is conceivable that HIF can play a role in the activation of the metabolic pathways required for overcoming the energy restriction checkpoint and progress through the cell cycle, as HIF1α subunits are also up-regulated in response to growth factors and cytokines independently of hypoxia.

Conclusions

The HIF system and its regulatory pathways, as described above, are clinically significant for many disease processes. Loss of any subunit or regulatory enzyme results in embryological lethality or a clinically significant phenotype; a summary is shown in table 1. Furthermore, failure to adequately degrade HIF1α due to a defect in vHL results in Von Hippel Lindau disease. Individuals who are heterozygous for the vHL gene and develop a further mutation in their functional copy develop this disease which is characterised by an over-activation of the HIF system and a predisposition to form multiple benign and malignant tumours (Haase, 2012). Commonly associated tumours include central nervous system and spinal haemangioblastomas, retinal haemangioblastomas, clear cell renal carcinomas, phaeochromocytomas, pancreatic neuroendocrine tumours, pancreatic serous cystadenomas, endolymphatic sac tumours and epidermal papillary cystadenomas.

As a key regulator, translating environmental cues into metabolic adaptation, HIF has been associated with the onset of deregulated metabolism in tumour progression but the relative contribution of these mechanisms for cell cycle regulation has scarcely been studied. Further research is necessary to understand which metabolites and pathways are involved in energy and nutrient-dependent restrictions to cell cycle progression and what is the impact of the HIF system on these (Fig. 6).

It is conceivable that further elucidating these processes could highlight new potential therapeutic targets and target pathways to help prevent for example chemo- and radiotherapy resistance and delay tumour progression and make current available therapies more efficient.

Acknowledgements. This work was funded by a CR-UK Senior Research Fellowship (C99667/A12918) to SR, and a Wellcome Trust strategic award (083524/Z/07/Z). SM and JB are funded by Cancer Research UK.

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Accepted January 10, 2014