Review Article

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Hypoxia Inducible Factor-1 (HIF-1) and Cancer Progression: A Comprehensive Review

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Abstract

Hypoxia is characterized as a decrease oxygen levels in tissue, represents a fundamental pathophysiological condition in the microenvironment of solid tumors. The key component of hypoxia sensing in the cell is the hypoxia-inducible factor (HIF), a transcriptional activator that mediates adaptive responses to hypoxia. HIF is a heterodimer comprising an oxygen-regulated α-subunit (HIF-1α) and a constitutively expressed β-subunit (HIF-1β). HIF-1 activity increases in the majority of human cancers and acts as a master transcription factor that has received the most intense attention in the field of cancer biology. The stability and activity of HIF-1 are regulated by its post-translational modifications such as hydroxylation, ubiquitination, acetylation, and phosphorylation. HIF-I induces a series of genes that participate in angiogenesis, iron metabolism, glucose metabolism, and cell proliferation/survival. Some novel agents have been shown to be targeted HIF-1 through a variety of molecular mechanisms and could represent a novel approach to cancer therapy.

Keywords: Hypoxia-Inducible Factor-1; Cancer Progression; Therapeutic Target.

Introduction

Oxygen is essential for eukaryotic life and is inextricably linked to the evolution of multicellular organisms. Hypoxia is characterized by decreased oxygen supply to the tissues as resultant cells are not able to carry out normal metabolic functions sufficiently. Several investigators define hypoxia as areas with O_2 tensions (pO_2 values) ≤ 2.5 mm Hg [1]. Hypoxia represents a fundamental pathophysiological condition in the microenvironment of solid tumors. Solid tumors comprise approximately 90% of all known cancers and develop from a single mutated cell [2] and it is very common in locally advanced solid tumors resulting from an imbalance between oxygen (O_2) supply and consumption [3]. Major causative factors of tumor hypoxia are abnormal structure and function of the microvessels supplying to the tumor, increased diffusion distances between the blood vessels and the tumor cells, and reduced O_2 transport capacity of the blood [3]. The process of tumor progression (proliferation, local invasion, and distant metastasis) is characterized by rapid cellular growth accompanied by alterations of the microenvironment of the tumor cells [4]. Protection against hypoxia in solid tumors is an important step in tumor development and progression [5].

Hypoxia induces the expression of transcription factor hypoxia-inducible factor-1 (HIF-1), a key regulator responsible for the induction of genes that facilitate adaptation, and survival of cells and the whole organism from normoxia (~21% O_2) to hypoxia (~1% O_2) [6,7]. Hypoxia inducible factors (HIFs) are a group of heterodimeric transcription factors that regulate transcription of thousand genes in response to hypoxia [8].

HIF activation results in up-regulation of erythropoietin, angiogenic factors, activation of glycolytic enzymes to carry out anaerobic metabolism and even a mitochondrial hibernation like phenomenon resulting in decreased oxygen demand [9,10]. Although, the identification of HIF was done in 3 decades ago, but our knowledge has grown exponentially about the mechanism of the

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HIF-1 pathway and its role in cancer progression. Therefore, this review will focus on hypoxia/HIF-1 regulated target genes related to metabolic regulation, tumor progression, invasion, and metastasis and further elucidate the implication of HIF-1 as a potential therapeutic target.

Historical Perspective

More than 90 years ago Krogh's [11] classical morphometric studies revealed that angiogenesis was related to some form of metabolic regulation which was maintained by muscle capillary density and the metabolic rate of different species. Intervention studies indicated that alteration of metabolic demand can change the capillary density. Later, it was observed that immobilization can reduce the muscle capillary density; whereas continuous neural stimulation can increase muscle capillary density [12,13]. Earlier studies had shown that damage of the capillaries at the wound site generates a hypoxic environment and starts restorative angiogenic response [14] in presence various factors like platelet-derived growth factor [15], vascular endothelial growth factor (VEGF) [16,17]. It had also indicated that circulating erythropoietin increases several hundred folds within hours of hypoxic stimulation. Subsequent studies established that expression of erythropoietin and angiogenic growth factors are mediated by a hypoxia induced transcriptional complex, HIF-1 [18,19]. HIF-1 was discovered by the identification of a hypoxia response element in the 3′ enhancer of the gene for erythropoietin (EPO) [20,21]. Development of a tumor arising from the increasing metabolic demands of the growing cell mass creates a severely hypoxic microenvironment [22]. Hypoxia inducible factor-1 (HIF-1) is a key regulator responsible for the induction of genes that facilitate adaptation and survival of cells in hypoxic condition [7].

Hypoxia Inducible Factors (HIFs)

There are three different types of HIF namely HIF-1, HIF-2, HIF-3. All HIFs are made up of one alpha-subunit and one beta-subunit. Researchers found that HIF-2α and HIF-3α are selectively expressed in certain tissues, including vascular endothelial cells, type-II pneumocytes, renal interstitial cells, liver parenchymal cells and cells of the myeloid lineage; however, HIF-1α ubiquitously expressed in all cells [23]. The adequate oxygen supply to the micro-environment of cells promotes oxygen dependent proteosomal degradation of HIF-1α; whereas, hypoxia prevents proteosomal degradation, rather HIF-1α is stabilized with HIF-1β and form a dimer. This heterodimer is ultimately translocated into nucleus, binds to HRE (hypoxia responsive element), starts expression of target genes such as EPO, GLUT, glycolytic enzymes, haemoxygenase-1, inducible nitric oxide synthase (iNOS), transferrin, VEGF [4,9,10,24].

HIF-2α (also known as endothelial PAS protein-1, EPAS-1) has been associated with pluripotential cells [25] which facilitates oxygen delivery and cellular adaptation to hypoxia by stimulating erythropoiesis, angiogenesis, and anaerobic glucose metabolism [26] and also elicits response to increase synthesis of epidermal growth factor receptor (EGFR) protein that is required for tumor cell growth autonomy [27].

HIF-3 is homologous to HIF-1; it is expressed abundantly in lung epithelial cells in moderate hypoxic condition and may therefore contribute to protection during early intervals of hypoxia and/or moderate hypoxia, while, HIF-1α and HIF-2α may confer protection against severe and/or prolonged hypoxia [28].

Hypoxia and HIF-1

Hypoxic condition has two approaches, i) chronic that exerts anti-proliferative effects and induces apoptosis and necrosis, ii) acute which promotes aggressive phenotype of tumor and induces their invasiveness and metastasis. Dai et al [29]. reported that when PC-3 cells and prostate cancer cell lines were exposed to chronic hypoxia (1% oxygen for >24hrs), decreased cell proliferation and induce cell death. However, the prostate cancer cells exposed to acute hypoxia (<6hrs) displayed increased motility, clonogenic survival and invasive capacity. The up-regulation of HIF-1 is considered as the molecular "switch" or "event" that is turned on by hypoxia. However, stimulation of cells with a variety of growth factors and cytokines, including EGF, FGF-2, heregulin, insulin, IGF-1, IGF-2 and IL-1β also induces the expression of HIF-1α protein [30]. The growth factor mediated expression of HIF-1 was mediated by PI-3 Kinase and MAP-Kinase pathway [31]. During hypoxia, HIF-1 plays a central role as a transcription factor, upregulates the expression of many genes involved in cell metabolism, proliferation, apoptosis, and angiogenesis [32,33].

Biochemical Structure of HIF-1

HIF-1α is an 826-amino acid protein and contains

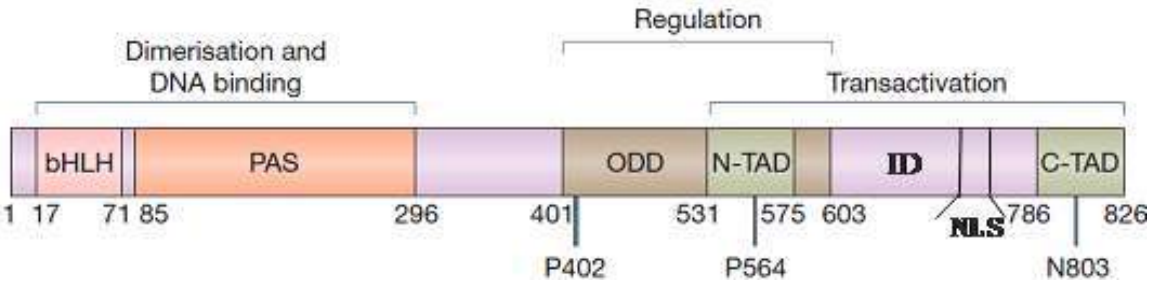

Fig. 1: Schematic representation of structure of the HIF-1 protein and its functional domains. The protein consists of basic helix-loop-helix (bHLH) and PER-ARNT-SIM (PAS) domain, oxygen-dependent degradation (ODD) domain, two transacting domains (TAD), an inhibitor domain (ID), and a nuclear localization signal (NLS). P402, 564, N803 are the hydroxylation site and K532 is the acetylation site. P- proline, N- asparagine, K- lysine [83]

several domains (Fig.1). The N-terminal half consists of i) basic domain (aa. 17-30), ii) a helix-loop-helix domain (aa. 31-71), iii) a PAS (PER-ARNT-SIM) domain (aa. 85-298), which is required for dimerization with HIF-1β and binding to the HRE DNA core recognition sequence (5′-RCGTG-3′). The PAS domain is also divided into two sub-domains i) PAS-A (aa.85-158) and ii) PAS-B (aa. 228-298) [6]. The C-terminal half of HIF-1 contains transactivation domains (TADs). They are present in between amino acids 531-575 (N-terminal TAD) and amino acids 786-826 (C-terminal TAD); these are separated by an inhibitory domain [34,35]. There are two Nuclear localization signals (NLSs) in HIF-1 i) N-terminal (aa.17-74) and ii) C-terminal (aa. 718-721) [36]. The C-terminal NLS is essential for nuclear import of HIF-1and it contains two PEST-like motifs at amino acids 499-518 and 581-600 [6]. The PEST motif contains a sequence rich in proline (P), glutamic acid (E), serine (S), and threonine (T) [10]. Basically, HIF-1 is a very unstable protein with a short half-life less than 10 min under normoxic conditions because in this state it is ubiquitinized, and then targeted by the proteasome [37]. The oxygen-dependent degradation of HIF-1 in normoxic condition is mediated by oxygen-dependent degradation (ODD) domain which is present at amino acids 401-603 before N-terminal TAD [38].

Regulation of HIF-1 Activity

HIF-1 is a uniquely identified protein, associated with the transcription of the hypoxia-inducible genes. Wang et al [6]. stated that all the dimeric HIFs including HIF-1 belong to a family of structurally related basic helix-loop-helix–Per-ARNT-Sim (bHLH–PAS) protein. Subsequent studies have revealed that heterodimeric HIF-1 consists of HIF-1α and HIF-1β [32,39,40]. Hypoxia induces the expression of HIF-1α subunit; whereas HIF-1β is a constitutively expressed subunit in cell [6]. Basically, HIF-1β had previously been identified as the aryl hydrocarbon nuclear receptor translocator (ARNT), which is dimerized with the aryl hydrocarbon receptor [41]. HIF-1α has four functional domains: bHLH, PAS, ODD, TAD (N-TAD and C-TAD) [40,42] and HIF-1β contains 3 domains: bHLH, PAS, and transactivation domain (N-TAD and C-TAD) [43]. In normoxic condition (21% oxygen level) HIF-1α protein is rapidly and continuously expressed and degraded. HIF-1α protein degradation is controlled by ODD domain (Fig. 2) and deletion of entire ODD region renders HIF-1α stable even in the absence of hypoxia signaling [38]. Hydroxylation of proline residue at 402 and 564 within the ODD domain of HIF-1α mediates it's interaction with the von Hippel-Lindau tumor suppressor protein (pVHL), which is recognized as a component of an E3 ubiquitin ligase, leading to ubiquitination of HIF-1α protein and subsequently degraded in 26S proteosome. These proline residues are embedded within the amino acid motif LXXLAP, which is conserved in the HIF-1 proteins of other species and HIF-2 [10]. The hydroxylation process is governed by three evolutionary conserved HIF prolyl hydoxylase (PHD1-3) [44]. PHD-1 and PHD-2 hydroxylate 402 and 564 proline residues whereas, PHD-3 hydroxylates only 564 proline residue [9]. All three PHDs contain ferrous iron (Fe^{2+}) in their active site and L-ascorbate acts as cofactor during hydroxylation [45]. However, hydroxylation reaction is coupled with the conversion of 2-oxoglutarate (2-OG) into succinate. Actually after hydroxylation, the enzymes are inactivated (Fe^{3+} state) and to carry further hydroxylation reaction, the enzymes must have to be activated; the latter is governed by L-ascorbate that donates electron to Fe^{3+} center of PHDs [42,44,46,47].

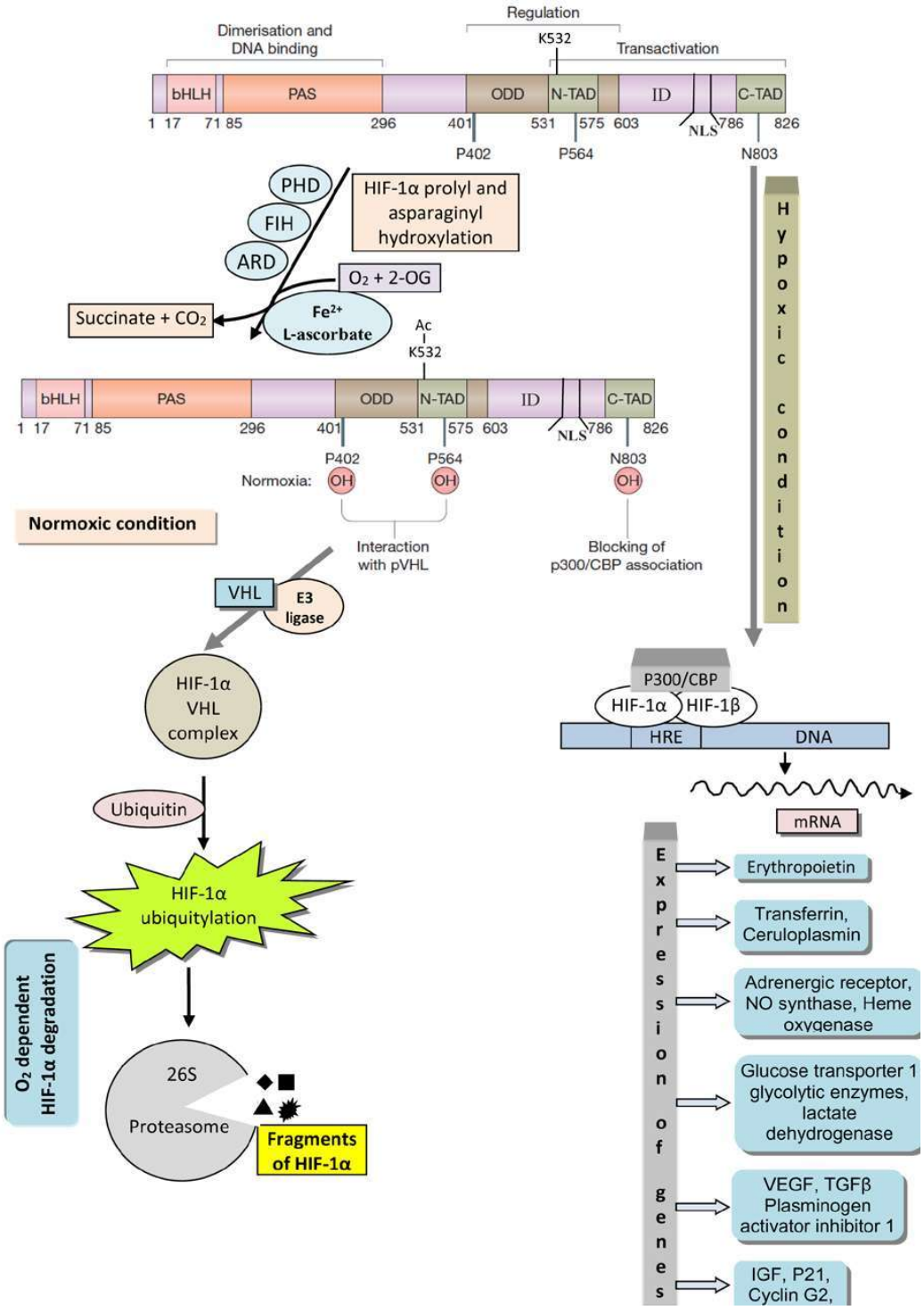

Fig. 2: Represent the regulation of HIF-1á in normoxic and hypoxic conditions. HIF-1á contains two proline residues (P402 at N-terminal, P564 at C-terminal of O_2-dependent degradation domain) and asparagine (N803) at C-terminal end. The oxygen dependent prolyl and asparaginyl hydroxylation of HIF-1á are the target point for its stability. P402 and P564 are hydroxylated by the prolyl hydroxylase domain (PHD) enzymes (PHD3 can only hydroxylate P564), and N803 by factor inhibiting HIF (FIH) in presence of O_2, 2-OG, and cofactors (Fe^{2+} and L-ascorbate). Acetylation of lysine (K532) is done by ARD and favours the interaction of HIF-1á with VHL. In normoxic condition, hydroxylated HIF-1á is recognized by the von Hippel-Lindau tumor suppressor (pVHL) E3 ubiquitin ligase complex, leading to degradation of HIF-1 in 26S proteasome. Hydroxylated N803 blocks the recruitment of transcriptional coactivator CBP/p300. Hypoxia mediated inhibition of prolyl hydroxylation is sufficient to allow HIF-1á to escape from pVHL E3-dependent proteolytic destruction and form an active transcriptional complex with HIF-â (lower right). Nonhydroxylated N803 of HIF-1_ allows CBP/p300 recruitment to the target genes, resulting in various gene expressions. [P - proline, N – arparagine, K-lysine] [9, 42,83]

Under hypoxic conditions, prolyl hydroxylation within the ODD domain is inhibited and the interaction of HIF-1α with PVHL is also prevented; the result is blocking of ubiquitination and degradation of HIF-1α and subsequent amplification of this protein (Figure 2). The accumulated HIF-1α translocates to the nucleus where it dimerizes with HIF-1β via the bHLH and part of the PAS domain to form the HIF-1 complex [32]. HIF-1 recruits transcriptional co-activators such as P300/CBP and binds with the hypoxia response element (HRE) within the promoter region of HIF-1 responsive target genes, including IGF-2, VEGF, TGF-α, MDR-1, transglutaminase2, hexokinase (HK) 1 and 2, Phosphofructokinase L (PFKL) and so on [24,48-50]. Hypoxia-response element (HRE) contain the cis-acting element 5´-RCGTG-3´ (R=purine; mostly adenine) in their core of HRE [51]. Functionally active HREs had been identified in the promoter region of more than hundred mammalian genes involved in erythropoiesis, glycolysis, angiogenesis, carcinogenesis, and other biological activities [50,52]. The transcriptional activity of HIF-1 is negatively regulated by an asparagine hydroxylase, also known as FIH (factor inhibiting HIF-1) which is able to interact with pVHL and modulates the stabilization of HIF-1α. In normoxia, hydroxylation of an asparagine residue (N803) in the transactivation domain (C-TAD) of HIF-1α blocks its association with the co-activators CBP and p300 [53-55].

HIF-1 Mediated Gene Expression and Metabolic Control

Recently, a large-scale microarray technique revealed that HIF-1 activates hundreds of target genes [56] and it facilitates the survivality of cells in the tumor microenvironment in hypoxic condition. HIF-1 regulates expression of genes for cell proliferation (IGF-2, WAF-1, TGF-α etc), survival (ADM, EPO, VEGF, NOS2, etc), motility (AMF/GPI, c-MET, LRP-1, TGF-α), apoptosis (NIX, NIP-3, RTP801), cytoskeletal structure (KRT14, VIM, KRT18), cell adhesion (MIC2), erythropoiesis (Epo), angiogenesis (ENG, LEP, VEGF, TGF-β3), vascular tone (ADM, NOS-2, heme oxygenase-1), nucleotide metabolism (adenylate kinase), iron metabolism (transferrin, ceruloplasmin), glucose metabolism (HK-1, GLUT-1, ENO-1, AMF/GPI, PFK-1), amino acid metabolism (transglutaminase-2), energy metabolism (LEP) and so on [52].

The metabolism of cancer cells is profoundly different from that of normal cells: Cancer cells manifest an increased rate of glycolysis coupled with a decreased rate of oxidative metabolism. Cancer cells, especially metastatic cells show high glucose uptake and anaerobic glycolysis. The universality of this finding was demonstrated by using ^{18}F-fluorodeoxyglucose PET scanning (FDGPET) [57]. Tumor cells are able to survive in hypoxic condition with the help of HIF-1 which alters the metabolic processes, primarily carbohydrate metabolism (glucose uptake, anaerobic glycolysis, glycogen metabolism, pentose phosphate pathway) and also lipid metabolism. It was observed that HIF-1 induced the expression of genes of specific glucose transporters for initial glucose internalization, and monocarboxylic acid transporters to promote lactate efflux. HIF-1 dependent transcription is strikingly isoform or isoenzyme specific. For instance, hypoxia up-regulates lactate dehydrogenase A (LDH-A) and monocarboxylic transporter 4 for conversion of pyruvate to lactate and lactate efflux from the cell, but down-regulates monocarboxylate transporter 1 and LDH B which act to promote lactate uptake and conversion of lactate into pyruvate [58].

Glycolytic Pathway

Glycolytic pathway is the prime metabolic route for glucose utilization, energy production and integration of metabolism; three enzymes, namely phosphofructokinase-1 (PFK-1), pyruvate kinase (PK) and hexokinase (HK) regulate the pathway. The prime regulatory enzyme PFK-1 exists as 3 isoforms (PFK-L, PFK-P, and PFK-M) which differ in their sensitivity to ATP and Krebs cycle intermediate citrate. The principal isoform PFK-L, least sensitive to inhibitors is up-regulated by HIF-1 but its activity remains allosterically controlled by fructose-2,6-bisphosphate that promotes PFK tetramer formation and increases catalytic activity. Interestingly, the expression of enzyme that catalyzes the formation of fructose-2,6-bisphosphate is also controlled by HIF-1 [59,60].

The second regulatory enzyme pyruvate kinase catalyzes the terminal step of glycolysis and able to alter the metabolic fate of glucose: either to maximize ATP generation or to slow down the process; resulting, accumulation of glycolytic intermediates for biosynthetic pathways. HIF-1 induces transcription of PKM (pyruvate kinase isoform M) gene in cancer cells, specifically a less active embryonic form PKM-2 instead of normal form the of pyruvate kinase (PKM-1) through alternative splicing [61]. The switch to PKM-2 also facilitates the catalysis of PEP dependent histidine phosphorylation of upstream enzyme Phosphoglycerate mutase (PGAM-1), which increases the activity of PGAM-1 and is again proposed to redirect glycolytic flux away from ATP synthesis and into the production of biosynthetic

intermediates [62]. However, contradictory results had also been found in xenograft tumor models [63,64]; PKM-2 isoform specific deletion enhances tumerogenesis in mice [65]. Recent report had proposed the non-glycolytic role of PKM-2 in transcriptional co-activation of HIF-1α through specific interaction with the HIF system [61]. The isoenzyme specific targeting by HIF-1 in the glycolytic pathway strongly suggests that HIF contributes to up-regulate parallel pathways of glycolysis in cancer to overcome the action of tumor suppression genes [9].

Glycogen Metabolism

Dysregulation of glycogen metabolism appears with glycolysis and enzymes catalyzing multiple steps in glycogen biosynthesis have been identified as HIF-1 target genes, including phosphoglucomutase-1, UDP-glucose phosphorylase 2, glycogen synthase and glucan, branching enzyme-1 Actually, hypoxia induced energy storage appears paradoxical and it is an adaptive response for the future threat of energy starvation. This response does indeed survival during adverse growth condition [66,67]. However, glycogenolytic enzyme glycogen phosphorylase (PYGL) is also induced by hypoxia, but over a longer time scale than the synthetic enzymes [68].

Lipid Metabolism

Increase in lipid biosynthesis and also glycogen accumulation are the common feature of cancer cells; for instance, both of these were familiar in renal carcinoma cells. Up-regulation of FAS (fatty acid synthase) correlates strongly with aggressive malignancy and inhibition of FAS rapidly inhibits cancer cell proliferation, including cell cycle arrest and apoptosis [69]. Hypoxia promotes many synthetic pathways as well as cellular lipid uptake and interactions between lipid and hypoxia signaling pathways occur at multiple levels. Actually, this lipid synthesis potentially provides a resource for the production of new membrane and lipid signaling molecules that are important for cell proliferation [70,71]. Experimentally, it was observed that tissue culture in hypoxic condition promotes the induction of both the cytosolic form of Acetyl-CoA-synthetase and FAS gene [72].

M*itochondrial Activity*

HIF down-regulates mitochondrial oxidative phosphorylation through a range of actions on mitochondrial metabolism and biosynthesis. Pyruvate dehydrogenase complex (PDH), is the key regulatory enzyme for TCA cycle. The activity of this enzyme is regulated by covalent modification; phosphorylation and dephosphorylation of the PDH are regulated by pyruvate dehydrogenase kinase (PDK) isoforms 1-4 and pyruvate dehydrogenase phosphatase (PDP) isoforms 1-2 respectively. HIF-1 dependent induction of PDK-1 leads to PDH inhibition, disconnecting the TCA cycle from glycolysis [73,74].

Mitochondrial function can also be attenuated by the HIF-dependent down-regulation of several components of electron transport chain including Complex-I, Succinate dehydrogenase (Complex II), Cytochrome c-oxidase (COX). The activity of complex-I is inhibited by HIF-1-dependent activation of NADH dehydrogenase ubiquinone 1 alpha sub-complex 4-like 2, NDUA4L2 [75]. Succinate dehydrogenase (SDH) complex (SDHA, B, C and D) is down regulated by HIF-1 dependent reduction in SDHB protein levels through a post-transcriptional mechanism [76]. Cytochrome c oxidase (COX), the last enzyme in the electron transport chain has two HIF-dependent regulatory subunits: COX4-2 is a HIF transcriptional target and is up-regulated in hypoxia; whereas the COX4-1 subunit is down-regulated through an indirect mechanism by activating the HIF-dependent mitochondrial LON protease that degrades COX4-1 [77].

HIF-dependent transcriptional activation of the microRNA miR-210 down-regulates multiple targets, important for mitochondrial functions including NDUFA4, SDH, the iron-sulfur cluster assembly proteins ISCU1/2 and the COX assembly protein COX10 [78,79]. Studies with pVHL-defective renal carcinoma cell line RCC4 had revealed that HIF can exert negative regulation on c-MYC and lowers mitochondrial biogenesis and mitochondrial mass [80]. In addition, the HIF-1 target gene BNIP-3 contributes to reduced mitochondrial number by activating mitochondrial autophagy [81].

HIF and Cancer Progression

HIF-1α over expression has been demonstrated in the majority of human cancers [24,82]. Immunohistochemical analyses of patient's biopsy samples have shown that HIF-1 is over expressed in many tumor types including pancreatic, head and neck, oropharyngeal, breast, renal, ovarian, urothelial, bladder brain colorectal and prostate. Several studies had shown that there is a strong correlation between HIF-1 over expression and tumor progression with an increased aggressiveness, angiogenesis and metastasis [83]. There were several mechanisms to

Table 1: Represent the alteration of gene expression that enhances the activity of HIF-1

Alteration in tumor	Mechanism of HIF 1α induction	References
VHL loss of function	Decreased ubiquitylation	89
p53 loss of function	Decreased ubiquitylation	90
PTEN loss of function	Increased synthesis	86; 88
PI3K–AKT–mTOR signalling*	Increased synthesis	91]; 86
MEK–ERK signalling*	Increased synthesis	31
ERBB2 gain of function	Increased synthesis	91
EGFR signalling*	Increased synthesis	86
IGF1R signalling*	Increased synthesis	31
PGE2 signalling*	Increased synthesis	92; 93
ARF loss of function	Increased synthesis	94
SRC gain of function	Decreased nucleolar sequestration	95
BCL2 overexpression	Not determined	96

*Increased signalling could be due to genetic alteration in a component of the pathway or an upstream activator.

promote the over expression and induction of HIF-1 activity (Table-1). Tumor cells with constitutive activation of the Ras-MAP-Kinase pathway, Src or the PI3K-Akt (PKB) mTOR pathway have elevated expression of HIF-1α protein [84-86]. HIF-1 activity increases due to loss of function of tumor suppressor proteins such as p53 and PTEN that promotes constitutive activation of Akt [87,88].

HIF-1 and Angiogenesis

In a rapidly growing tumor, oxygen demand increases and oxygen delivery decrease due to insufficient blood flow and increasing diffusion distance between the blood vessels and the oxygen consuming cells [97]. These lead to hypoxia in expanding tumor mass, triggering events that stimulate angiogenesis in an effort to ameliorate the hypoxic condition. One of the potent stimulator of proliferation and migration of vascular endothelial cells (angiogenesis) is VEGF; production is induced by HIF-1 [4].

Vascular endothelial growth factor (VEGF) has been shown to stimulate migration of macrophages by activation of the VEGF-receptor (Flt-1) which ultimately produces several angiogenic factors, including VEGF and tumor necrosis factor alpha TNF-α.[98,99] Beside these, HIF-1 induces the expression of several angiogenesis-related gene products and receptors, including PDGF-B, VEGF-R-1, endothelin-1, inducible nitric oxide synthase (iNOS), monocyte chemotactic factor, adrenomodulin and EGF [100].

HIF-1 and Metastasis

Hypoxia is an important micro-environmental factor that induces cancer metastasis. There are different steps for conversion of a tumor cell to become metastatic including epithelial-mesenchymal transition (EMT), extracellular matrix modulation, intravasation, circulation, extravasation, homing at the premetastatic niche, and organotropic colonization [101,102].

EMT is one of the crucial mechanisms to cause early stage of tumor metastasis and the cells lose E-cadherin, an epithelial marker. It has been assumed that hypoxia may be an important factor contributing to the loss of E-cadherin in solid tumors [103]. HIF-1 shows metastatic effect by regulating expression of several numbers of genes that are categorized into different classes, including transcription factors, histone/chromatin modifiers, enzymes, receptors, kinases, small GTPases, transporters, adhesion molecules, surface molecules, membrane proteins, and microRNAs [104].

• *Transcription Factor and Histone/Chromatin Modifiers Associated to Metastasis*

Transcription factor and histone/chromatin modifiers have different roles in relation to hypoxia-induced metastasis. The EMT regulators, such as Twist1, Snail, Slug, ZEB1, ZEB2, and E12/E47 have been shown to be either directly or indirectly regulated by HIF-1α. These EMT regulators subsequently bind to the promoters of EMT marker genes, like E-cadherin, vimentin, and N-cadherin to mediate EMT [105]. In addition to transcriptional factors, chromatin modifiers could also be regulated by hypoxia. Induction of histone lysine-specific demethylase 4B (KDM4B, JMJD2B) correlates with invasion and advanced clinical stage in colorectal cancers, gastric cancer and lung metastasis and breast cancer [106-108].

• *Enzymes Related to Metastasis*

The most significant enzymes that are regulated by hypoxia to cause metastasis are metalloproteases including matrix metalloprotease-1 (MMP1) and MMP3 to induce metastasis [109], lysyl oxidase (LOX) essential for collagen metabolism [110], angiotensin converting enzyme (ACE) related to lung cancer [111], Sulfatase 1 (Hsulf-1) modulates the sulfation state of heparin sulfate proteoglycans [112].

• *Receptors, Kinases, Small GTPases, and Transporters Associated to Metastasis*

Various receptors, receptor-activated kinases, small GTPases, and transporters are regulated by hypoxia/HIF-1α, play the significant role in cancer metastasis. Chemokine receptor 4 (CXCR4) [113] urokinase-type plasminogen activator receptor (uPAR), Toll-like receptor 4 (TLR4) [114] RON tyrosine kinase [115] are activated by HIF-1α, play a crucial role for hypoxia-induced tumor cell growth and metastasis. HIF-1α-regulated small GTPases Cdc42 and Rac1 regulate nitric oxide-induced macrophage migration and metastasis [116]. Different transporters including glucose transporter type 1 (GULT-1) and multidrug resistance protein 1 (MDR1) are regulated by hypoxia/HIF-1α and implicated in the metastatic processes [117,118] . A truncated form of the voltage-dependent anion channel 1 (VDAC1) is induced by HIF-1α to promote cancer cell survival [119].

• *Adhesion Molecules, Membrane Proteins, and Various Proteins Involve in Metastasis*

Different adhesion or surface molecules including, angiopoietin-like 4 (ANGPTL4), L1 cell adhesion molecule (L1CAM) and CD151 (tetraspanin family) are important for cell adhesion, motility vascular metastasis [120,121]; membrane proteins like CD24, CD147, Galectin-1, MUC1 (O-glycoprotein membrane-bound mucin), Semaphorin 4D, Caveolin-1 are related to carcinogenesis in various organs like colon, breast, lung and kidney [122-127]; other proteins such as Liprin-α 4 (cytoplasmic protein) matricellular proteins [CYR61 (CCN1) and NOV (CCN3)], S100A4, CapG are involved in migration and invasion of cancer cells [128,129].

• *MicroRNAs and Metastasis*

A range of microRNAs (miRNAs) is shown to be regulated by hypoxia/HIF-1α. Hypoxia/HIF-1α mediated various microRNAs have a concise role in metabolism, DNA damage response, and angiogenesis [130]. The critical microRNA miR-210 involves in tumor initiation and metastasis [131,132]. Hypoxia induces miR-15b/16, miR-21, miR-372/373 and miR-103/107 to promote the tumor progression and metastasis [133]. However, hypoxia/HIF-α down regulates miR-34a and miR-17/20a that target signaling pathway [134,135].

Hypoxia Induced Genomic Changes and Clonal Selection

Hypoxia, with or without reoxygenation, promotes genomic instability through point mutations, gene amplification and chromosomal rearrangement [136]. Point mutation may develop in tumor cells exposed to hypoxia and reoxygenation through several mechanisms, including insufficient DNA repair, errors in DNA replication or both [137,138]. Metabolic damage to DNA bases may also play a role in point mutations, since a hypoxia-reoxygenation sequence may cause oxidative damage. Such damage has the potential to lead various pyrimidine-purine-derived lesions in DNA. The most abundant form of these effects are generation of 8-hydroxyguanine, which mispaired with adenine [139,140]; the ultimate results are point mutations, chromosomal rearrangements and gene amplification which promote development of metastatic disease by inactivation of metastasis suppressor gene or increased expression of oncogenes involved in angiogenesis and growth factors [4].

Hypoxia exerts a strong selection pressure on malignant cells [4]. The proteomic or genomic adaptive changes in malignant cells favor their survivality under hypoxic conditions that lead to advantageous for selection over non-adapted cells. The progeny of the adapted cells will increase at a greater rate than those of the non-adapted cells and eventually will become the dominant cell subpopulation within the tumor. The selected cells show more favorable character, including apoptotic insensitivity, invasion, metastasis capability, aggressiveness, treatment resistance and increased angiogenic potential which further aggravates tumor hypoxia and establishes a vicious circle of hypoxia and malignant progression in advanced stage of disease [141].

Reynolds et al. [138] had discussed about pattern of mutation frequency in hypoxically cultured cells; the rate of mutation frequency continued to rise with repeated exposure to hypoxia followed by reoxygenation and starts impairment of cellular repair capabilities [138]. The cycle of repeated hypoxia-reoxygenation may function as a mutagenic force by

increasing the levels of superoxides and other O_2 radicals [142]. However, hypoxia-reoxygenation cycle increases ROS production, which activate stress response genes, such as HSP-70 (an effective inhibitor of apoptosis) or stress-response transcription factor, such as NF-κβ (regulates numerous genes including VEGF) and finally promotes malignant progression [4].

HIF-1α as a Therapeutic Target

The expression of HIF-1α occurs in the majority

Table 2: Represent the effects different agents that are responsible to decrease both level and activity of HIF-1α

Strategies	Agents	Mechanism	Refs.
HIF-1α DNA binding inhibition	Doxorubicin	Inhibits cellular defensive mechanisms and angiogenesis	151
HIF-1α mRNA expression inhibition	EZN-2968 (RNA antagonist)	Third generation oligonucleotide that specifically binds and inhibits the expression of HIF-1α mRNA.	152
	Aminoflavone (ligand of the aryl hydrocarbon receptor)	Disrupts HIF-1α mRNA expression.	153
HIF-1α protein degradation	Gefitinib (EGFR tyrosine kinase inhibitor)	Reduced protein stability without any change in the level of HIF-1α mRNA.	154
Inhibition of HIF-1α transcriptional activity	Topotecan (Hycamtin	Topoisomerase-1 inhibitor	155
	Chetomin (dithiodiketopiperazine metabolite of the fungus *Chaetomium* species	Inhibit transcription of HIF	156
	IC50	Inhibit HIF-1α expression as well as the phosphatidylinositol-3-kinase/Akt pathway	157
HSP90 inhibitors,	Geldanamycin and 17-allylaminogeldanamycin (17-AAG),	Block the binding of HSP90 to HIF-1α	158
Inhibition of HIF-1α translational activity	NSC-134754	Translation inhibitor	155
	103D5R	Translation inhibitor	159
	HIF-1α targeted SiRNA treatment	Inhibition of HIF-1 activation	160
Inhibitors of signal transduction pathways	Rapamycin	Reduce mTOR activity and thereby inhibit HIF-1α expression, HIF-1-dependent VEGF expression, and VEGF-driven angiogenesis.	161
	Genistein (natural products)	Receptor tyrosine kinases inhibitor	162
	Calphostin C	Inhibitor a protein kinase C	163
	Wortmannin and LY294002	Inhibitors PI3K–AKT pathway	
	PD98095	Inhibitor of Ras–MAPK pathway	
	Diphenylene iodonium and	A redox signaling blocker	
Inhibition of dimerization	Eolitetracycline (a semisynthetic pyrrolidnomethyltetracycline)	Block HIF- 1α–HIF-1β dimerization by targeting the PAS Domain and inhibits formation of the HIF-1 complex (Ref.).	164
Cell-based (HRE reporter)	Echinomycin	Inhibits DNA binding	165
	DJ12	DNA binding/transactivation	166
	Anthracycline chemotherapeutic Agents	DNA binding agent	151
	Trichostatin A and FK228 [histone deacetylase (HDAC) inhibitors]	Inhibit HIF-1α induction and HIF-1 activity	167
	Pleurotin	Thioredoxin redox system inhibitor	168
	TAS106 (ECyd)	RNA polymerase inhibitor	169
Protein–protein interaction	Chetomin	p300–HIF-1α interaction inhibitor	170
	Rolitetracycline	HIF-1α–HIF-1β (ARNT interaction inhibitor)	171; 164
Others	KRH102053	PHD2 activator	172
	HIF oligonucleotide decoy	Binds to and inactivates HIF-1α	173
	Digoxin	Potent inhibitor of HIF-1α synthesis	174
	RITA	Inhibtors of p53–HDM2 interaction	175
	Prolyl-hydroxylase inhibitors (FG-2216 and FG-4592)	Inhibtors of proline hydroxylation	176

of human cancers, plays a pivotal role in its progression by making therapeutic resistance. The low efficacy of several cytotoxic drugs, like cyclophosphamide, carboplatin (ParaplatinR; Bristol-Myers Squibb; Princeton, NJ), carmustine (BiCNUR; Bristol-Myers Squibb), and melphalan (AlkeranR; Celgene Corporation; Warren, NJ) appears in hypoxia mediated tumorogenesis [143,144]. Hypoxic cells are approximately three fold more resistant than well-oxygenated cells [145]. Hypoxia mediated therapeutic resistance occurs through 1) direct effects due to lack of O_2 which require to some drugs and radiation for maximum cytotoxic effects; 2) indirect effects via altered cellular metabolism that decreases drug cytotoxicity, and; 3) enhanced genetic instability may lead to more rapid development of drug resistant tumor cells [146]. In respect to therapeutic resistance, HIF-1 inhibitors can inhibit tumor growth and angiogenesis [147], and may have therapeutic utility. Several novel anti-cancer agents had been identified to inhibit HIF-1 activity (Table -2) [82,148]. They preferentially form cytotoxic and DNA-damaging free radicals under hypoxia, thus selectively eradicating hypoxic cells [149]. According to their putative mechanism of action, HIF-1 inhibitors could be tentatively divided into agents that modulate: 1) HIF-1α DNA binding; 2) HIF-1α mRNA expression; 3) HIF-1α protein degradation; 4) HIF-1α transcriptional activity; and 5) HIF-1α protein translation [150]. Beside these, several other approaches are also being applied: blocking of HIF-1α protein–protein interactions; inhibition of signal transduction pathways; inhibition of cell-based activity; blocking of dimerization [83].

Conclusion

Hypoxia is a common feature in growing tumor and has an important mechanism of HIF-1 activation. HIF-1 is a fundamental regulator of oxygen homeostasis and to control the physiological and pathological progression by targeting several genes related to metabolism, angiogenesis, metastasis, inhibition of apoptosis, inactivation of tumor suppression. The activity of HIF-1 is tightly regulated by hydroxylation of proline, asparagine and proteosomal degradation which are determined by cellular oxygen tension. The invention of HIF-1 has increased interest in the development of therapies against cancer cells in hypoxic microenvironment. Finally, HIF-inhibitors combined with existing treatments (radiotherapy and chemotherapy) will open a new era in the development of therapeutic strategies for the treatment of solid tumors.

Conflict of Interest

The authors declare that they have no conflict of interest.

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