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Review Article

Targeted genes and interacting proteins of hypoxia inducible factor-1

Wei Liu[1], Shao-Ming Shen[1], Xu-Yun Zhao[1], Guo-Qiang Chen[1,2]

[1]Department of Pathophysiology, Shanghai Universities E-Institute for Chemical Biology, Key Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education, Shanghai Jiao Tong University School of Medicine (SJTU-SM), Shanghai 200025, CHINA; [2]Institute of Health Sciences, Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences & SJTU-SM, Shanghai 200025, CHINA

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Abstract: Heterodimeric transcription factor hypoxia inducible factor-1 (HIF-1) functions as a master regulator of oxygen homeostasis in almost all nucleated mammalian cells. The fundamental process adapted to cellular oxygen alteration largely depends on the refined regulation on its alpha subunit, HIF-1α. Recent studies have unraveled expanding and critical roles of HIF-1α, involving in a multitude of developmental, physiological, and pathophysiological processes. This review will focus on the current knowledge of HIF-1α-targeting genes and its interacting proteins, as well as the concomitant functional relationships between them.

Keywords: Hypoxia inducible factor-1alpha, targeting gene, interacting protein

Introduction

Cellular and systemic oxygen homeostasis is a finely regulated process which is essential for energy metabolism as well as the survival of mammalian cells. It has well established that hypoxia inducible factor-1 (HIF-1), a heterodimeric transcriptional factor composed of the constitutively expressed HIF-1β (also named aryl-hydrocarbon receptor nuclear transporter, ARNT) subunit and the highly regulated HIF-1α subunit, plays a critical role in cellular adaptation to changes in oxygen availability [1, 2]. Both HIF-1α and HIF-1β subunits are members of a subfamily of basic helix-loop-helix (bHLH) transcription factors containing periodic-aryl hydrocarbon receptor nuclear translocator-single-minded (Per–Arnt–Sim, PAS) domain, which required for heterodimerization and the binding of the subunits to DNA. The HIF-1α subunit also contains an oxygen-dependent degradation (ODD) domain and two transactivation domains (TAD), rendering it to proteasomal degradation and target gene regulation respectively.

Since HIF-1 was first identified as a key regulator required for hypoxia-induced transcription of the human erythropoietin (EPO) gene [3], the transcription factor has been attracting tremendous interests owing to its rapidly expanding and critical role involving in a multitude of developmental, physiological, and pathophysiological processes [4]. This review attempts to outline the current knowledge of HIF-1α-targeting genes and its interacting proteins, the functional relationships between them will also be discussed.

The target genes of HIF-1α

It has been unraveled that, HIF-1α is hydroxylated by oxygen-activated prolyl hydroxylases (PHDs) and rapidly degraded via ubiquitin–proteasome pathway under normoxic conditions. But under hypoxic or hypoxic-mimic conditions, PHDs activity is inhibited and HIF-1α accumulates, translocates to nucleus, and heterodimerize with HIF-1β [5-8]. As a transcriptional factor, the heterodimer HIF-1 recognizes and binds to the consensus sequence 5'-(A/G) CGTG-3' named hypoxia-responsive elements (HREs) to activate the transcriptional activity of target genes [9].

For the purpose of understanding the regulatory

network of HIF-1, a range of methods and techniques, such as luciferase reporter assay, electrophoretic mobility shift assay (EMSA) and chromatin immunoprecitation (ChIP), have been widely used to identify the downstream-target genes of HIF-1. Recently, Benita et al. [10] reported a new strategy to find HIF-1-target genes based on integrative genomic approach and computational strategies. Using this strategy and microarray analysis, they recovered 41% of the previously confirmed HIF-1-target genes and successfully identified ANKRD37 as a novel target gene of HIF-1α. Meanwhile, Barahona et al. described a powerful computational strategy based on the combination of phylogenetic footprinting and transcription profiling meta-analysis for the identification of HIF-target genes, which was validated by ChIP assay and disclosed several novel HIF targets, including RE1-silencing transcription factor co-repressor (RCOR2) [11]. Besides genome-wide analyses, proteomics strategies have also been applied to identify the direct targets of HIF-1. Our group performed two-dimensional electrophoresis (2DE) analysis to compare the protein expression profiles within hypoxia-treated human acute promonocytic leukemic U937 cells and untreated U937 cells. The differential expression proteins were identified by mass spectrometry, and among them, several potential target genes of hypoxia/HIF-1, such as N-myc downstream regulated gene 1 (NDRG1), were identified [12]. Moreover, by using differential gel electrophoresis (DIGE) based proteomics analysis, we further identified three novel direct targets of HIF-1α, which were confirmed by luciferase and ChIP assay [13]. To date, more than 100 direct target genes of HIF-1 have been uncovered, which have been shown to be functionally involved in tumor metastasis, angiogenesis, energy metabolism, cell differentiation and apoptosis [13, 14]

Tumor metastasis

Metastasis is a critical step in tumor progression and causes high mortality in human deaths. Increasing evidences implicate HIF-1 function in tumor cell metastasis. Immunohistochemistry analysis showed that HIF-1α was overexpressed in tumor types compared with the respective normal tissues, including colon, breast, gastric, lung, skin, ovarian, pancreatic, prostate, and renal carcinomas [15]. Target genes of HIF-1α have been further identified and suggested to be involved in tumor metastasis in several aspects. Among them, TWIST, a most important and crucial transcription factor directly regulated by HIF-1, is implicated to be essential to hypoxia mediated epithelial-mesenchymal transition (EMT) and cancer metastasis [16]. HIF-1α- and TWIST-null mice show similarities in their phenotypes and siRNA-mediated repression of TWIST in HIF-1α- overexpressing or hypoxic cells reversed metastastic phenotypes. Further reports showed that HIF-1α can regulate intercellular adhersion molecules, such as α5β3, α5β5, and β1 intergrins, expression in tumor matrix [17-19]. On the other hand, E-cadherin, a calcium-dependent cell adhesion protein, is also down-regulated by HIF-1α via its direct regulation of TCF3, ZFHX1A, ZFHX1B, which repress E-cadherin gene transcription [20]. Matrixmetalloproteinases, MMP2 and MMP9 have also been reported to be regulated by HIF-1α [21, 22]. The impact of hypoxia on MMP-9 expression as well as keratinocyte migration could be attenuated by inhibitors of protein kinase C (PKC), which indicated hypoxia-induced tumor cell migration is mediated by increased MMP-9 via the PKC pathway. Besides, urokinase-type plasminogen activator receptor (uPAR) and plasminogen activator inhibitor-1 (PAI-1), two major components of fibrinolysis system, have also been shown to be the target of HIF-1α and that is important for hypoxia-induced metastasis [23, 24]. Another mechanism of metastatic spread in various cancer types involves in chemokine/cytokine receptors. It has been reported that HIF-1α could regulate the expression of CXC chemokine receptor-4 (CXCR4), c-Met and CC chemokine receptor 7 (CCR7), which in turn play critical roles in cell chemotaxis, homing as well as hypoxia-induced invasive growth [25-27].

Tumor angiogenesis

Angiogenesis plays an important role in the pathogenesis of tumor progressing. HIF-1 is known to stimulate angiogenic response by activating transcription of the genes encoding several growth factors, including vascular endothelial growth factor (VEGF). The HIF-1α-knockout mice mainly showed abnormal vascular development [28]. In further research , Tsuzuki Y et al. reported that HRE-/- ES tumors produced the same level of VEGF as the $VEGF^{-/-}$ ES tumors indicating the role of HIF-1α/HRE in transcription regulation of VEGF production in tumor cell

[29]. Recently，calcitonin receptor-like receptor (CRLR), a G-protein coupled receptor shows a remarkable range of effects on promotion of angiogenesis, has been reported to be transcriptionally upregulated by HIF-1α [30]. It has also been showed that, the expression of Semaphorin 4D (Sema4D), one member of 20 semaphorins, which promotes angiogenesis, has a correlation with HIF-1 activity in HNSCC specimens [31]. In addition, other factors such as stem cell factor (SCF) and angiopoietin 2 (ANGPT2), which promote angiogenesis by binding to their cognating receptors, were further identified as the targets of HIF-1α [32, 33].

SUMO-specific protease 1 (SENP1) has been shown to be essential for the stability and activity of HIF-1α under hypoxia conditions [34]. Recently, the same group also reported that HIF-1α could direct regulate the transcriptional activity of SENP-1, which indicates a positive feedback loop mediated by SENP1 within hypoxic endothelial cells that is important for VEGF production and angiogenesis [35].

Tumor energy metabolism

The energy metabolism process in tumor presents a shift from glucose metabolism coupled with mitochondrial oxidative to anaerobic glycolysis, which is known as the Warburg effect and shows decreased mitochondrial respiration versus increased lactate production [36]. Glucose transporters GLUT1 and GLUT3, responsible for constitutive or basal glucose uptake, and 6-Phosphofructo-2-kinase (pfkfb3), a key regulator of fructose-2,6-bisphosphate (Fru-2,6-P2) which regulates glycolytic flux, have been confirmed as the targets of HIF-1α [37-40]. In this process, the transactivation of these genes regulated by HIF-1α reprograms the intracellular fate of glucose, resulting in increased flux of reducing equivalents from glucose to lactate [41]. Two glycolytic enzymes, namely phosphoglycerate kinase 1 (PGK 1) and pyruvate kinase M2 (PKM2) have been shown to be transcriptionally activated by HIF-1 [42, 43]. The monocarboxylate transporter MCT4, which mediates lactic acid efflux from most tissues, is upregulated by hypoxia through a HIF-1α - dependent mechanism [44]. HIF-1 also actively suppresses metabolism by directly transactivating the gene encoding pyruvate dehydrogenase kinase 1 (PDK1) [45]. As a kinase of pyruvate dehydrogenase (PDH), PDK1 prevents the pyruvate oxidative decarboxylation to acetyl-CoA and reduce pyruvate entry into the tricarboxylic acid (TCA) cycle. Forced PDK1 expression could rescue hypoxic HIF-1α null cells from apoptosis accompanied with increased ATP level and decreased ROS generation [46].

MXI1, a C-Myc antagonist, which protects against C-Myc-dependent sensitization to hypoxia-induced apoptosis, has been revealed as a transcriptional target of the HIF-1 [47, 48]. Furthermore, study shows that dysregulated activity of HIF-1 in VHL-deficient renal carcinoma cells leads to the inhibition of C-Myc transcriptional activity by MXI-1 expression and increased C-Myc degradation by the proteasome, then the resulting loss of C-Myc-dependent PGC-1β expression is responsible for the reduction in mitochondrial biogenesis and energy metabolism reprogramming [47, 48].

Cell differentiation

Self-renewal and the capacity to differentiate into specialized cell types are two essential properties of stem cells. It is well documented that hypoxia and HIF-1α can promote the undifferentiated state of various stem and precursor cell populations. Recently, it is reported that normal hematopoietic stem cells (HSCs) maintain intracellular hypoxia and stabilize HIF-1α protein, and HSCs keep cell cycle quiescence through the precise regulation of HIF-1α level [49]. Hypoxic culture appears to be necessary to maintain full pluripotency of human embryonic stem (hES) cells, as the appearance of differentiated regions of cultured hES cells, such as the production of human chorionic gonadotropin and progesterone, the loss of stage-specific embryonic antigen-4 and Oct-4 in early-stage mammalian embryos, is markedly reduced under hypoxic conditions [50]. Further study showed that HIF-2α but not HIF-1α regulated Oct-4 by binding its promoter and inducing expression and transcriptional activity of Oct-4 [51]. Besides, evidence suggests that hypoxia can prevent the differentiation of progenitor cells as well as promote de-differentiation state of cancer cells [52-54]. It has been shown that hypoxia blocks differentiation of neuronal and myogenic progenitors via HIF-1-Notch signaling, whereby HIF-1α interacts with Notch intracellular domain, be recruited to Notch-responsive promoter, and elevate expression of Notch downstream genes which involve in stem cell

maintenance [52-54]. Erythropoietin (EPO), a glycoprotein hormone that is secreted into the blood and binds to its cognate receptor on erythroid progenitor cells, stimulates red blood cells survival and differentiation has been identified as a direct target of HIF-1α in the past few years [55]. More interestingly, HIF-1α-stabilized insults such as hypoxia, cobalt chloride, desferrioxamine and tiron, 4,5-dihydroxy-1,3-benzene disulfonic acid can induce differentiation of acute myeloid leukemia [8, 56, 57]. Also, all-trans retinoic acid (ATAR), a clinically effective differentiation-inducing drug for acute promyelocytic leukemia (APL) rapidly increases endogenous and inducible expressed HIF-1α protein in leukemic cells [58]. Especially, intermittent hypoxia induces tumor arrest and differentiation with prolonged survival in a mouse model of acute myeloid leukemia [59]. Our results suggest that hypoxia-induced AML cell differentiation involves in interaction of HIF-1α with C/EBPα and Runx 1 proteins (see below) [60]. In addition, galectin-1, a member of the galectin family and has a high affinity for galactose and N-acetylglucosamine moieties of glycoproteins, is shown to be direct target of HIF-1 and C/EBP, and its synergistic induction by these two proteins partially contributes to AML cell differentiation [61, 62].

By the way, HIF-1α plays an important role in the genetic program that regulates chondrogenesis. In hypoxic prechondrogenic cells, the expression of Sox9, a DNA-binding protein of the high mobility group (HMG) family as well as a key regulator of chondrocyte differentiation, is regulated and increased by HIF-1α which has been shown can directly bind to the promoter to Sox9 gene, in turn, resulting in the changing cellular landscape in endochondrial bone formation [63].

Cell apoptosis

It has been shown that hypoxia, the most physiologically relevant stress, induces apoptosis by leading to DNA damage at a significant high level [64]. Graeber TG et al. reported that hypoxia induced apoptosis in oncogenically transformed cells, whereas loss of p53 tumor-suppressor gene reduced hypoxia-induced cell death. Accordingly, highly apoptotic regions strongly correlate with hypoxic regions in transplanted tumors expressing wild-type p53 but not p53-deficient tumors [65]. p53 has been suggested to regulate cell programmed death and growth arrest through transcriptional activation of its downstream molecules, like Puma, Bax and p21 [66-68]. Studies also indicate that wild-type p53 can be stabilized by HIF-1α, and the interaction between HIF-1 and p53 can be either direct [69]or indirect [70], the later is probably mediated by binding of HIF-1α to p53 ubiquitin ligase Mdm2 [71]. Further results show that another direct target of HIF-1α, named NPM, a multifunctional protein which is overexpressed in actively proliferating cells and cancer cells, is associated with P53. Suppression of NPM expression by shRNA increases hypoxia-induced apoptosis [72]. In addition, HIF-1α has been shown to induce the expression of human urocortin 2 (hUcn2) via a specific HRE in the 3'FLR of hUcn2 gene, which may help to preserve cardiac function and prevent apoptosis in ischaemic conditions in the heart [73].

BNIP3, a member of the Bcl-2 family that is expressed in mitochondria and induces apoptosis, has been reported directly regulated by HIF-1α, play a dedicated role in the pathological progression of hypoxia-mediated apoptosis, necrosis and autophagy [74, 75]. Besides, BH3-only Bcl-2 family protein Noxa, a molecule mediating p53-induced apoptosis, has also been identified as a target of HIF-1α. Noxa-mediated hypoxic cell death relates to reactive oxygen species and resultant cytochrome c release [76].

The interacting proteins of HIF-1α

The mechanism of signal transduction by HIF-1α is a complex multistep process, while the ultimate goal is to execute its role as a transcription factor. After synthesis in the cytosol, HIF-1α needs to undergo nuclear translocation to access target promoters and to become transcriptionally active. The whole process is tightly regulated by direct interaction with other proteins, which have been identified in large numbers of studies by employing Co-Immunoprecipitation (Co-IP), glutathione S-transferase (GST)-pull down, immuno-colocalization and yeast two-hybrid assays. Functionally, HIF-1α interacting proteins can mainly be categorized into following classes.

Interacting proteins regulating HIF-1α stability

As documented that HIF-1α protein is rapidly degraded by the proteasome in normoxia, but

stabilized under hypoxia. The most recognized HIF-1α degradation pathway is mediated by its direct binding with Hippel-Lindau (VHL) tumor suppressor protein, pVHL. pVHL is part of a multiprotein complex that includes elongin C, Rbx1 and Cul2 [77-79]. This complex functions as an E3 ubiquitin ligase that directly binds to and targets HIF-1α for ubiquitination and proteasomal degradation.

The physical interaction of HIF-1α and pVHL is critically controlled by prolyl hydroxylation and acetylation of HIF-1α protein [6, 80, 81]. O_2-dependent hydroxylation of two proline residues (P402 and P564) in ODD domain of HIF-1α by PHDs is required for binding of pVHL [82, 83]. In mammalian cells, three isoforms of PHDs, PHD1, PHD2 and PHD3, have been identified and shown to hydroxylate HIF-1α *in vitro* [84, 85]. However, only PHD2 was proved to control the steady-state levels of HIF-1α by experiments carried out in human cells [86]. Although HIF-1α and PHD2 bind to each other, efficient PHD2 activity is dependent on OS-9, a protein that binds to both HIF-1α and PHD2, thereby ensuring stable complex formation [87]. In the same degradation domain of HIF-1α protein, acetylation of Lys532 by ARD1 also enhances interaction of HIF-1α with pVHL and subsequent HIF-1α degradation [81]. Metastasis-associated protein 1 (MTA1) binds to HIF-1α and counteracts the activity of ARD1 [88]. Interestingly, most of these proteins can be regulated by hypoxia. PHD2 and MTA1 are hypoxia inducible while OS-9 and ARD1 expression decrease under hypoxic conditions.

The interaction between HIF-1α and pVHL is also regulated by other HIF-1α interacting proteins. The protein SSAT2 interacts with HIF-1α, pVHL and elongin C, stabilizes the interaction of pVHL and elongin C, and promotes ubiquitination of HIF-1α [89]. Ubiquitination of HIF-1α can also be reversed by its interaction with pVHL-interacting deubiquitinating enzyme 2 (VDU2), which stabilizes HIF-1α through deubiquitination [90, 91]. ATP6V0C competes with pVHL in HIF-1α binding by directly interacting with HIF-1α under the stimulation of bafilomycin [92]. Hepatitis B virus X protein (HBx), which is closely involved in the development of hepatocellular carcinoma, directly interacts with the bHLH/PAS domain of HIF-1α and decreases the binding of pVHL to HIF-1α [93].

Despite the pivotal role of pVHL complex in cellular oxygen homeostasis, it is not the only E3 ligase that mediates HIF-1a degradation. In renal carcinoma cell lines that lack functional pVHL and express stable HIF-1α protein in normoxia, the HSP90 antagonist geldanamycin promotes ubiquitination and proteasomal degradation of HIF-1α by disrupting its association with HSP90, indicating that HIF-1α/ HSP90 association protects HIF-1α from degradation via an O_2/PHD2/pVHL-independent pathway [94-96]. The receptor of activated protein kinase C (RACK1) can compete with HSP90 for binding to HIF-1α and subsequently subject HIF-1α for O_2/ PHD2/pVHL-independent proteasomal degradation by recruiting elongin C, the same E3 ubiquitin ligase component that is bound by pVHL [97, 98]. SSAT1, a protein bears 46% amino acid identity to SSAT2, also binds to HIF-1α and triggers its ubiquitination and degradation [99]. However, in contrast to SSAT2, which plays a role in the O_2/PHD2/pVHL-dependent degradation of HIF-1α, SSAT1 executes its role by stabilizing the interaction of HIF-1α with RACK1 [99]. Thus, the paralogs SSAT1 and SSAT2 play complementary roles in promoting O_2-independent and O_2-dependent degradation of HIF-1α. A mammalian septin family member, SEPT9_v1, interacts with HIF-1α and protects it from O_2-independent proteasomal degradation by preventing the interaction of HIF-1α with RACK1 [100]. It is also reported that upon HSP90 inhibition, a protein complex consisting of HSP70 and HIF-1α is formed to facilitate 20S proteasomal degradation in an ubiquitin-independent manner [101-103]. The copper metabolism MURR1 domain containing 1 protein (COMMD1) has been shown can compete with HSP90 and form a tripartite complex with HSP70/ HIF-1α, thus facilitates HIF-1α proteolysis [104]. Hypoxia-associated factor (HAF) has also been identified as an E3 ligase for HIF-1α that mediates the degradation of HIF-1α through a novel pVHL and O_2-independent pathway [105, 106]. As reported by Ravi R et al., Mdm2 is another E3 ligase that is suggested to bind HIF-1α and mediate its ubiquitination/proteasomal degradation in the presence of p53 which acts as a molecular chaperone [107]. However, contradictory results suggest that HIF-1α degradation is not affected by Mdm2 or p53 status of the cells in hypoxia, Mdm2 promotes HIF-1α activation rather than its degradation [108].

Jun activation domain-binding protein-1 (Jab1), a coactivator of AP-1 transcription factor, has been reported to directly bind to HIF-1α, inter-

fere with HIF-1α-p53 interaction, and lead to stabilization of HIF-1α protein under hypoxia [109].

HIF-1α stability is also modulated by the covalent attachment of small ubiquitin-related modifier (SUMO). Hypoxia induces SUMOylation of HIF-1α, which promotes its binding to pVHL-E3 complex for degradation [34]. PIASy has been identified as the E3 ligase for hypoxia-induced HIF-1α SUMOylation. As a result, PIASy negatively regulates HIF-1α stability and activity [110]. However, different SUMOylation inducers may have distinct efficiencies, as HIF-1α has also been reported to be stabilized with increased SUMOylation by RSUME, an enhancer of SUMO conjugation, during hypoxia [111].

Other proteins, such as MSF-A, PSMA7 and LANA were also reported to interact with HIF-1α and regulate its degradation and activation, but the molecular mechanisms are relatively obscure [112-114].

Interacting proteins regulating transcriptional activity of HIF-1α

To exert its role as transcription factor, stabilized HIF-1α need to form a heterodimeric complex with HIF-1β and bind to HREs located within the promoters of target genes [9]. Besides its role in HIF-1α degradation, recent study has shown that COMMD1 can also prevent the dimerization of HIF-1α and HIF-1β and subsequently inhibit HIF-1-mediated gene expression [115]. Fatyol K et al. reported that the $p14^{ARF}$ tumor suppressor protein could directly inhibit the transcriptional activity of HIF-1 by sequenstering HIF-1α from nucleoplasm to the nucleolus [116]. In spermatozoa, the testis specific gene antigen 10 (TSGA10) co-localizes with HIF-1α and consequently prevents the nuclear localization and transcriptional activity of HIF-1α [117].

Besides HIF-1β, HIF-1α can also dimerize with other PAS family members. HIF-1α has been shown to interact and form transcriptionally active heterodimers with MOP3 and MOP9 respectively, although their targets remain unclear [118-120]. The circadian factor PER2, another PAS protein, has been reported to bind to HIF-1α, prevent HIF-1α/HIF-1β mediated transcription, and regulate the circadian rhythm of VEGF production in hypoxic tumor cells [121]. However, the interaction of HIF-1α with PER1 is thought to alter the stability of PER1 by protecting it from proteolytical degradation [122].

Although PHD2-mediated hydroxylation within ODD domain of HIF-1α has been well documented, it is unraveled that HIF-1α can also be hydroxylated in its C-terminal TAD domain (N803 in human HIF-1α) by factor inhibiting HIF-1 (FIH-1) under normoxic conditions [123, 124]. The hydroxylation results in suppression of HIF-1α transcriptional activity via blocking the association of HIF-1α with its coactivators [123]. These coactivators, including p300/CBP, SRC-1 and TIF2, play two critical roles: bind to and stabilize the transcription initiation complex containing RNA polymerase II and also possess histone acetyltransferase activity that is required for the polymerase to access DNA within chromatin and transcribe it into RNA [125-128]. Recruitment of coactivators to HIF-1α is regulated by other interacting proteins. Thiol-redox regulator Ref-1 interacts with HIF-1α and modifies its C-terminal TAD domain, which facilitates the binding of coactivators [125, 126]. Histone deacetylase 7 (HDAC7) co-translocates to the nucleus with HIF-1α under hypoxic conditions and increases the transcriptional activity of HIF-1α through the formation of a complex with HIF-1α and p300 [129]. Regulatory associated protein of mTOR (Raptor) interacts with HIF-1α and promotes its activity by enhancing its association with cofactors p300/ CBP, which may be a link between the mTOR and HIF-1α signaling [130]. The orphan nuclear receptor estrogen-related receptors (ERRs) physically interact with HIF-1α and also serve as essential cofactors of HIF-1α in mediating hypoxic responses [131]. Recently, the SIRT1 deacetylase was found to bind and deacetylate HIF-1α at Lys674, which is acetylated by PCAF [132]. Thereby, SIRT1 inactivated HIF-1α by blocking p300 recruitment and consequently repressed HIF-1 target genes [132]. Moon HE et al. found that Necdin, a growth suppressor, directly associates with HIF-1α at ODD domain and inhibits the transcriptional activity of HIF-1 as well as angiogenic activity under hypoxia [133]. Budde A et al. applied T7 phage display system and identified a domain inherent in the retinoblastoma protein (pRB). Further study confirmed the physical and functional interaction between HIF-1α and pRB, as a result, causes HIF-1α transcriptional activation and reverses the transcriptional repressor function of pRB [134].

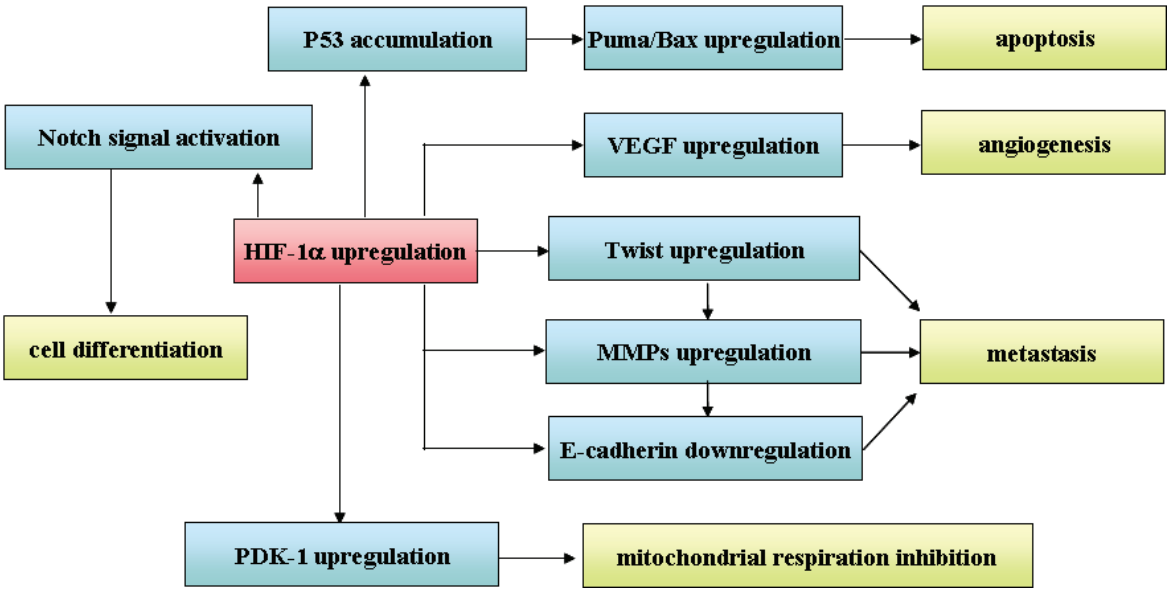

Figure 1. Representative target genes of HIF-1α and their functions.

HIF-1α interacting transcription factors

As a master transcription factor responsible for cellular oxygen homeostasis, HIF-1 regulates the expression of dozens of genes containing HRE motif. Nevertheless, HIF-1α has been found to interact with a variety of transcription factors and interfere with their transcriptional activities. Since these HIF-1α-interacting transcription factors are modulators for various cellular activities, the functional range of HIF-1α has been greatly extended.

As discussed above, direct association of HIF-1α and p53 not only induces HIF-1α degradation via recruiting the mdm2 E3 Ubiquitin ligase, but may be also necessary for stabilization-dependent accumulation of wild-type p53 [71, 107]. HIF-1α competes with T-cell factor-4 (TCF-4) for direct binding to β-catenin, thereby inhibits β-catenin-TCF-4 transcriptional activity but enhances HIF-1α-mediated transcription [135]. The dynamic role of β-catenin is thought to be a way of tumors' adaptation to hypoxia by constraining cell growth [135]. Study also shows that HIF-1α and Smad3 associate with each other and cooperatively activate VEGF promoter, suggesting a synergic activity between hypoxia and TGF-β [136]. HIF-1α has been shown to form a weak complex with Myc *in vivo* and functionally antagonizes its activity, resulting in derepression of $p21^{cip1}$ along with cell cycle arrest [137]. Tendler DS et al. suggested that the direct interaction of HIF-1α and IFN regulatory factor-1 (IRF-1) might be the underlying mechanism by which macrophages infiltrating into tumors, being activated to express nitric oxide synthase (NOS2) and to produce NO, a mediator of tumor apoptosis [138]. Under hypoxia, Sp1 serves as a molecular switch by recruiting HIF-1α to the promoter of MSH2 and MSH6 genes, thereby decreases levels of MutSα, a complex of MSH2 and MSH6 protein which recognizes base mismatches, leading to hypoxia-induced genetic instability [139]. The EPO 3'-enhancer contains both HIF-1α and HNF-4 binding sites, hypoxia induces the interaction between HNF-4 and HIF-1α, which results in augmentation of gene expression by the EPO enhancer [140].

Results from our group have revealed that HIF-1α contributes to the differentiation of acute myeloid leukemia cells via transcriptional activity-independent mechanisms [7]. HIF-1α can interact with hematopoiesis-related transcription factors C/EBPα and Runx1 so as to increase their transcriptional activities [56, 141, 142]. Meanwhile, the dimerization of HIF-1α and HIF-β as well as HIF-1 transcriptional activity is inhibited [141, 142]. The functional switch of HIF-1α may present dual effects. On the one hand, inhibition of HIF-1 transcriptional activity

by C/EBPα and Runx1 helps to produce low-oxygen microenvironment of the bone marrow due to inhibition of angiogenesis. On the other hand, increased transcriptional activity of C/EBPα and Runx1 mediated by HIF-1α promotes differentiation of leukemic cells [141].

Perspective

Functional activities of HIF-1α along with underlying mechanisms have been greatly expanded in the past decades (**Figure 1**). It has been revealed that HIF-1α participates in a variety of physiological and pathophysiological processes, including but not limited in tumor metastasis, angiogenesis, energy metabolism, cell differentiation and apoptosis, which could be either dependent or independent on its transcriptional activity. The discoveries of HIF-1α target genes as well as its interacting proteins not only extend our knowledge for hypoxia adaptation, but also provide potential drug targets that are more controlled and/or specific, and eventually offer the opportunities for therapeutic intervention.

Address correspondence to: Dr. Guo-Qiang Chen, Department of Pathophysiology, Shanghai Universities E-Institute for Chemical Biology, Key Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education, Shanghai Jiao Tong University School of Medicine (SJTU-SM), Shanghai 200025, CHINA; Institute of Health Sciences, Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences & SJTU-SM, Shanghai 200025, CHINA E-mail: chengq@shsmu.edu.cn or gqchen@sibs.ac.cn

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