REVIEW

An overview of biological research on hypoxia-inducible factors (HIFs)

Zhe Liu[1], Zixuan Wu[2], Yuxin Fan[1], Yudong Fang[1]

[1]*Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China*
[2]*Shanghai University of Finance and Economics, Shanghai, China*

Abstract

Hypoxia-inducible factors (HIFs), as a family of transcription factors involved in the cellular response to hypoxia, are key regulatory factors in the regulation mechanism of an organism's response to hypoxia. A large number of studies have shown that HIFs are closely related to the angiogenesis, erythropoiesis, cell metabolism, and autophagy of organisms, as well as the occurrence and development of tumours. Therefore, it is of great significance to further study HIFs to understand and treat tumours or other related diseases. This paper summarises the structure, oxygen-dependent degradation mechanism, non-oxygen-dependent degradation mechanism, transcriptional activation mechanism, relevant signalling pathways, and inhibitors of HIFs, in order to provide new clues for the treatment of tumour, vascular, and other related diseases. **(Endokrynol Pol 2020; 71 (5): 432–440)**

Key words: *HIFs; biological research*

Introduction

Oxygen content affects human health, and excessive oxygen content leads to increased body free radicals, while insufficient content causes a lack of oxygen. The body regulates the supply of oxygen to an appropriate physiological concentration range through a variety of mechanisms, including cell-level mechanisms, tissue-organ-level mechanisms, and system-level mechanisms [1–3]. In 1992, Semenza et al. [4] first discovered this transcription factor when studying the expression of the erythropoietin (EPO) gene in the oxygen-deficient hepatoma cell line 3b Hep3B. Later, it was found that this factor could regulate the transcription of various hypoxic response genes and participate in the signal transduction process of hypoxic reaction, so it was named hypoxia-inducible factor 1 (HIF-1) [5]. It was found that HIF-1 is a heterodimer composed of α subunits and β subunits. HIF-1β is stable in cytoplasm or cytoplasm and plays a structural role. An active subunit of HIF-1 in the cytoplasm is HIF-1α. It is also a key factor in response to hypoxia stress [6]. The hypoxia induction pathway of most hypoxia-inducible genes is achieved through HIF-1. As a result, HIF-1 is often referred to as a housekeeping transcription factor that regulates hypoxic adaptation [7]. In 1997 and 1998, HIF-2 and HIF-3 were successively discovered. It is thought that there may be a family of HIFs in the human body. Transcription, translation, and activity of HIFs can be induced by hypoxia. To mediate physiological or pathological effects, HIFs must combine with the hypoxia response elements (HRE) in the target gene to regulate its expression.

There are currently more than 60 genes known to be directly regulated by HIF-1 [8]. As a core transcription factor, HIF-1α mainly regulates the oxygen homeostasis of the body's environment and is widely involved in the adaptive response induced by hypoxia [9]. Vascular endothelial growth factor (VEGF), erythropoietin (EPO), and heat shock proteins (HSP) are downstream target genes of HIF-1, and these genes are closely related to angiogenesis, erythropoiesis, energy metabolism, cell proliferation, and apoptosis [10–13]. Hypoxia-inducible factors can regulate metabolic reprogramming of tumour cells, inhibit tumour cell apoptosis, and induce autophagy to promote tumour cell survival. It is also closely associated with neovascularisation, pH homeostasis, autocrine, maintenance of tumour stem cell (CSC), and tumour prognosis [14–17]. At present, research of HIF inhibitors is essential for targeting anticancer drugs. Most HIF inhibitors target HIF-1α and HIF-2α, and specific inhibitors for HIF-3α have not been developed [18].

✉ Yudong Fan, Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China;
e-mail: 13301901601@163.com

Structure of HIFs

Hypoxia-inducible factors are DNA-binding proteins and include two subunits, which are the hypoxia-regulated a subunit (HIF-1α, HIF-2α, HIF-3α) and the oxygen-insensitive b subunit, also called ARNT. Among the two subunits that make up HIF-1, the β subunit exists steadily in cells, while the α subunit is regulated by oxygen concentration, so the regulation of HIF-1 mainly lies in the α subunit. The *HIF-1α* gene is located on chromosome 14 (14q21-24), the *HIF-1β* gene is located on chromosome 1 (1q21). The biochemical properties of HIF-1α and HIF-2α are very similar, recognising the same DNA binding region, but each has unique biological effects. For example, during embryonic development, HIF-1α regulates the growth of blood vessels, and HIF-2α regulates the production of catecholamine. As a transcription activator, HIF acts on the enhancer sequence of the target gene and regulates the hypoxia-induced expression of various genes. These genes mainly include glucose metabolism, cell growth, oxygen transport and transmission, etc. [19]. The N-terminus of HIF-1α contains a basic bHLH configuration, which is necessary for binding to DNA. The downstream proline-serine-threonine (Pro/Ser/Thr) is a specific structure that forms a heterodimer and binds to the target gene [20]. The C-terminal contains three domains, one is transactivation domain-C terminal (TAD-C), which is functional in regulating transcription. The other is the transactivation domain-N terminal (TAD-N), which can activate transcription. There is also an oxygen-dependent degradation domain (ODDD) that is rich in Pro/Ser/Thr and can degrade HIF-1a protein through ubiquitination pathway [21]. There is also a nuclear localisation signal (NLS) at the C-terminus, which can help HIF-1α protein and nuclear pore protein bind to the nucleus. The N-terminal activation domain binds to HIF-1β to form the heterodimer HIF-1, and it binds to cis-acting elements of hypoxia response elements (HRE) for transcription.

Degradation mechanism of HIFs

Oxygen-dependent degradation

Under normoxic conditions, although the HIF-1α subunit is expressed, it degrades quickly, so there is no accumulation of HIF protein. Under hypoxic conditions, the degradation of the α subunit is blocked, resulting in the accumulation of HIF-1α in the nucleus, and after binding with the β subunit, it can identify the HIF response element (HRE) in the promoter of target gene of hypoxic response. The oxygen-sensitive region near the c-terminal of the PAS region (non-PAS region) of HIF-1α and HIF-2α is composed of about 200 amino acid residues, and is an important structure affecting degradation. When this region is lost, HIF-1α keeps not degraded under normoxic conditions, so it is called oxygen-dependent degradation domain (ODD). Under normoxic conditions, conserved proline residues within the polypeptide sequence of the ODD region of HIF-1α are hydroxylated by proline hydroxylase under aerobic conditions [22–23]. The hydroxylated proline residue is recognised by the Von Hippel-Lindau tumour suppressor protein (pVHL), a component of the ubiquitin ligase complex, which leads to the recognition and degradation of HIF-1α by the proteasome. A key regulator is catalysed by the iron-dependent prolyl hydroxylase family [24]. The interaction of HIF and pVHL depends on the hydroxylation of proline residues in the degradation domain of HIF protein. And under hypoxia conditions, this hydroxylation process is inhibited, and HIF-1α expression increased exponentially [25–26]. Hypoxia and iron ion chelating agents, such as deferoxamine, can block the effect of pVHL [27–28]. Regulation of protein stability is only one way for hypoxia to induce HIFs activity. Despite the ODD region, the three subunits also include two regions that are critical for gene expression and bind to common activation factors. One of them overlaps with ODD, and its regulation may be a minor part of protein stability [29]. The other is the hydroxyl terminal trans-transcription active region (C-TAD), which is independent of ODD and can bind to co-activating factor complexes such as P300/CBP under hypoxic conditions. The regulation of C-TAD activity is based on the hydroxylation process of conserved asparagine residues by oxygen-dependent asparagine hydroxylase [30].

Oxygen-independent degradation

Chaperone-mediated autophagy (CMA) leads to the non-oxygen-dependent degradation of HIFs that happens in lysosome. Its core components are constitutive heat shock cognate 70 (HSC70) and LAMP2A. In detail: CMA is a kind of selective autophagy, which is responsible for degrading nearly 30% of soluble proteins in the cytoplasm due to oxidative damage. And these proteins all contain KFERQ-like pentapeptide motifs [31]. In the pathway of CMA-mediated lysosomal degradation of HIF-1α subunits, the molecular chaperone HSPA8/HSC70 binds to it by recognising the KFERQ-like pentapeptide motif in HIF-1α. After stretching the HIF-1α subunit peptide chain, it is transported to the CMA receptor-lysosomal-associated membrane protein 2A (LAMP2A). This protein mediates the translocation of HIF-1α subunit into the lysosomal cavity, and HIF-1α is eventually degraded by acidic proteases in lysosome [32]. Adam et al. [33] found an E3 ubiquitin ligase SIAH 1/2 (seven-in-absentia homologue

1/2) in breast cancer cell line MCF-7 by reducing the stability of its substrate PHD3 in a manner that is not affected by the O_2 level, maintaining the level of HIF-1α subunit and promoting the metastasis and invasion of breast cancer cells. Activated protein kinase C1 receptor RACK1, spermidine/spermine N1-acetyltransferase SSAT1, calcineurin, hypoxia-associated factor (HAF), differentiated embryonic cartilage development gene SHARP1, and HSP70/CHIP (carboxy terminus of Hsp70 interacting protein) also regulate the proteasome degradation of the HIF-1α subunit in an oxygen-independent manner [30].

Transcription activation mechanism of HIFs

The transcription activity of the HIF-αs subunit is also regulated by oxygen concentration. Under normoxic conditions, the aspartic acid residue of HIF-1α is hydroxylated, which inhibits the binding of the transcription activation domain to co-activated molecules such as CBP and p300 and inhibits the transcription activity of HIF-1α. During hypoxia, hydroxylation is inhibited, HIF-1α dissociates from heat shock protein 90 (HSP90) into the nucleus and combines with the nuclear protein HIF-1β to form the HIF-1 complex. Under the action of co-activating molecules, HIF-1 is fully activated and combines with hypoxic response element (HRE) containing the 5'-RCGTG-3 sequence in the target gene to exert transcriptional activity [34–35]. Regulation of HIF-αs subunit transcriptional activity is often achieved through hydroxylation, phosphorylation, deacetylation, etc. These modifications affect the affinity of the HIF-αs subunit to p300/CBP, influence the polymerisation and interaction with pVHL, and thus have positive or negative effects on regulating the transcriptional activation of HIFs.

Hydroxylation
The transcriptional activity of HIF-1α and HIF-2α subunits is regulated by aspartate hydroxylase (FIH-1). Aspartate hydroxylase, also known as HIF-1α inhibitor (factor-inhibiting HIF-1α), has a catalytic function that depends on the participation of O_2, α-ketoglutarate, and Fe^{2+} [36]. Under normoxia, FIH-1 can hydroxylate Asn803 residue in hHIF-1α subunit C-TAD domain and Asn851 residue in hHIF-2α subunit C-TAD domain, respectively (Fig. 4A), and can block the binding of HIF-1/2α to p300/CBP, thereby inhibiting the transcription activation function of HIF-1 and HIF-2. While, due to the lack of C-TAD domain in HIF-3α subunit, FIH-1 cannot regulate its transcription activity through hydroxylation modification [37, 38]. In hypoxia conditions or in the presence of CoCl2, DMOG, iron ion chelating agents, etc., FIH-1 activity is inhibited, and HIF-1/2α

and HIF-1β subunits without hydroxylation modification successfully enrich p300/CBP to activate the target gene transcription [39].

Phosphorylation
In mitogen-activated protein kinase (MAPK) pathway, phosphorylation of Thr796 residue of HIF-1α subunit and Thr844 residue of HIF-2α subunit by mitogen protein kinase p42 / p44 can enhance the interaction of C-TAD domain with CBP/p300, and significantly increase the transcription activity of HIF-1 and HIF-2. p42/p44 can also inhibit the interaction between HIF-1α and nucleoprotein CRM1 by phosphorylating HIF-1α subunit S641 and S643 residues, and promote the accumulation of HIF-1α in the nucleus, hence increasing the protein level of HIF-1 [40, 41]. The casein kinase 1 (CK1) phosphorylation of Ser247 residue in the PAS-B domain of HIF-1α subunit can inhibit the binding of HIF-1α to HIF-1β subunit and reduce HIF-1α target gene expression [35].

Deacetylation
The regulation of HAD-1α subunit activity by NAD+-dependent histone deacetylase Sirtuins1 (Sirt1) is inconclusive [42]. Under normoxia, Sirt1 prevents the enrichment of p300 by removing the acetyl group on the Lys674 residue of HIF-1α subunit, thereby inhibiting the transcription activity of HIF-1α subunit [43]. During hypoxia, the NAD+ produced by the redox reaction in the cell is reduced, the activity of Sirt1 deacetylase is reduced, and the inhibitory effect on HIF-1α is relieved. The acetylation of Lys674 residues in HIF-1α subunit is catalysed by p300/CBP-associated factor (PCAF), which has the ability to antagonise Sirt1 deacetylase activity [44]. Sirt1 in hepatocellular carcinoma cell line (HCC) can promote the accumulation of HIF-1α subunit and positively regulate its transcription activity [45]. Sirt7 can interact with HIF-1α and HIF-2α subunits at the protein level to negatively regulate their oxygen-independence [46].

Other transcriptional activation mechanisms
The activity of HIF-1α and HIF-2α subunits in cancer stem cells (CSC) is also regulated by the phosphatidylinositol 3-kinase signalling pathway (PI3K-AKT pathway). This pathway activates CSC survival-related genes (such as glycolytic enzyme genes) through the positive regulation of HIF-1α subunit, and at the same time inhibits the activity of tumour suppressor gene p53, hence promoting the survival of CSC. This pathway can also increase the expression level of downstream CSC stem-related genes Oct-4, Sox-2, etc. by activating HIF-2α subunit, and promote the stemness maintenance of CSC [47].

Signalling pathway of HIF-1α

PI-3K/Akt/HIF-1α pathway

The phosphatidylinositol-3kinase (PI-3K) signalling pathway works on cell proliferation and apoptosis. Under hypoxic conditions, PI-3K is activated and binds to downstream Akt to phosphorylate Akt, enhance HIF-1α activity, and initiate transcription of downstream target genes, resulting in increased cell proliferation and decreased apoptosis [48]. This pathway is related to the level of cellular glycolysis. Hexokinase II (HKII)/ /glucose transporter 1 (GLUT1) and lactated dehydrogenase (LDHA) may be the site of action downstream of this pathway. Under hypoxia conditions, epidermal growth factor (EGF) activates the PI3K/Akt pathway and participates in the regulation of glycolysis through HIF-1α; inhabiting the PI3K/Akt-HIF-1α pathway can significantly reduce glycolysis in a variety of cells, and this mechanism has potential value for tumour therapy [49]. Basic fibroblast growth factor (bFGF) activates HIF-1 via the PI-3K/Akt and MEK1/ERK pathways, and PI-3K/Akt and MEK1/ERK pathways synergistically and differently regulate the HIF-1 process, where the PI-3K/Akt pathway plays a more important role [50, 51]. Hypoxia-mediated enhancement of ERK1/2 and Akt activation requires a direct cell-cell interaction between mast cells and keloid fibroblasts, and the activation of ERK1/2 and Akt is involved in the accumulation of hypoxia-dependent HIF-1α protein and the expression of VEGF [52].

SENP1/HIF-1α signalling pathway

Sentrin-specific protease 1 (SENP1) is a member of the small ubiquitin-like modified protein (SUMO) specific protease family, and HIF-1α is the target protein modified by SUMO. Hypoxia can inhibit the activity of PHD and activate SENP1. The decreased activity of PHD increases the expression of HIF-1α. At the same time, the activated SENP1 de-SUMOises HIF-1α, and hence HIF-1α is stably expressed and activates downstream target genes [53]. Inhibiting the SENP1/HIF-1α pathway is important for controlling tumour growth. Hypoxia can promote the expression of HIF-1α and SENP1. Si-HIF-1α downregulates SENP1 expression and angiogenesis ability under hypoxia, Si-SENP1 down-regulates HIF-1α expression and angiogenesis ability under hypoxia. Under hypoxic conditions, SENP1 and HIF-1α form a positive feedback loop and are important for angiogenesis [54]. HIF-1α and SENP1 have a positive feedback loop in the regulation of osteosarcoma (OS) cell proliferation, invasion, and epithelial transformation under hypoxic conditions, suggesting that the SENP1/HIF-1α axis may become a new therapeutic drug for the treatment of osteosarcoma (OS) [55]. This positive feedback loop between SENP1 and HIF-1α is of great significance in the increase of tumour dryness in liver cancer and the occurrence of liver cancer under hypoxic conditions. Drugs that specifically target SENP1 may provide a potential new treatment for HCC [56].

HIF-1α/BNIP3/Bcline-1 signalling pathway

Bal-2/adenovirus E1B 19kD-related protein 3 (BCL-2/interacting protein 3, BNIP3) signalling pathway is important in the process of hypoxia-induced autophagy activation. Under hypoxic conditions, the expression level of HIF-1α increases, and it combines with the hypoxic response element of BNIP3 to promote the expression of BNIP3. BNIP3 belongs to the BH3-only subfamily in the Bal-2 protein family. It not only mediates non-caspases-dependent apoptosis, but also interacts with Bcline-1 to regulate the process of autophagy. When the expression of BNIP3 is increased, a large amount of free Beclin-1 is produced, and Beclin-1 mediates the localisation of other autophagy proteins in phagocytic vesicles, regulating the formation and maturation of autophagosomes. Strengthening the autophagy of tumour cells and inflammatory cells in medical treatment is a new method for targeted treatment of cancer and inflammation [57]. Ischaemia/reperfusion and hypoxia/reperfusion injury increase the expression level of HIF-1α and activate downstream BNIP3, thus triggering mitochondrial-dependent autophagy. Upregulating the expression of HIF-1, HIF-α, and BNIP3 may promote autophagy in H9C2 cells induced by ischaemia/reperfusion injury and hypoxia/reperfusion injury. In addition, downregulating the expression of HIF-1α or BNIP3 siRNA can reduce the autophagy ability of H9C2 cells under hypoxia/reperfusion injury. Therefore, HIF-1α synchronises the regulation of BNIP3 during the autophagy of H9C2 cells induced by hypoxia-ischaemia reperfusion injury [58]. Testosterone induces renal tubular epithelial cell death by activating the HIF-1α/BNIP3 pathway [59]. The protective effect of Panax notoginseng saponins on ischaemia-reperfusion injury is mainly through the HIF1α/BNIP3 pathway to promote mitochondrial autophagy in myocardial tissue [60]. Hypoxia-induced autophagy is involved in the invasion of salivary adenoid cystic carcinoma through HIF-α/BNIP3 signalling pathway [61]. Autophagy regulates hypoxia-induced osteoclastogenesis through HIF-1α/BNIP3 signalling pathway [62].

MAPK/HIF-1α signalling pathway

Mitogen-activated protein kinase (MAPK) can promote cell proliferation and participate in HIF-1α activation [63]. Anti-apoptotic extracellular regulated kinase (ERK) is a member of the MAPK protein family.

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Hypoxia can induce ERK phosphorylation, which in turn activates oncogenes to produce cancer cells. Ras, a kind of upstream regulatory factor of ERK, can bind to the N-terminal domain of Raf, and activate it. Raf activates downstream MAPK/ERK kinase (MEK), which phosphorylates ERK and increases the expression level of HIF-1α [64]. Salceda confirmed by reporter gene analysis and EMSA experiments that in hypoxic Hep3B cells the ERK pathway inhibitor PD98059 can block the transcriptional activity of HIF-1 without affecting its DNA binding activity [65]. Richard showed that the HIF-1α subunit can be phosphorylated by activated ERK1 or ERK2, but the activity of ERK1 or ERK2 could not be detected. Other related experiments confirmed that the activation of the ERK pathway can directly participate in the activation of HIF-1 transcription activity [66]. Tanshinone IIA sodium sulfonate reduces the cigarette smoke-induced inflammation and oxidative stress by blocking the mitogen-activated protein kinase/HIF-1α signalling pathway [67].

Other related signalling pathways

In recent years, it has been found that proteins such as pVHL, heat shock protein 90 (Hsp90), and cyclooxygenase-2 (COX-2) can also form pathways that mediate hypoxic signalling with HIF-1α. The experiment found that the expression of pVHL in renal clear cancer cells resulted in decreased expression of HIF-1α and vascular endothelial growth factor (VEGF), which inhibited cancer cell proliferation, metastasis, and vascular regeneration [68].The mechanism is the hydroxylation of key proline residues of HIF-1α under normoxia, which results in HIF-1α being recognised by the pVHL/E3 ubiquitin ligase complex and degraded by polyubiquitination, thus affecting the metabolic activity of cells. During hypoxia, the proline residues of HIF-1α will not be recognised by pVHL, which makes HIF-1α stable in the hypoxic environment, and in turn activates downstream VEGF genes and induces blood vessels [69]. The Hsp90 signalling pathway is important for liver cancer, pancreatic cancer, and breast cancer. Under hypoxic conditions, Hsp90 binds to the bHLH-PAS domain of HIF-1α and activates the expression of HIF-1α, thereby regulating its downstream target genes and promoting cancer cell growth [70]. Under hypoxic conditions, HIF-1α is expressed in large amounts and binds to the hypoxic response element on the COX-2 promoter, thus promoting the expression of COX-2. This pathway has significance for tumour cell proliferation, blood vessel growth, and anti-apoptosis [71]. In retinoblastoma, the hypoxic microenvironment may enhance distant invasion and metastasis of tumour cells by up-regulating the HIF-1α/MMP9 axis. HIF-1α regulates glucocorticoid-induced osteoporosis through the PDK1/AKT/mTOR signalling pathway [72]. Circular ribonucleic acid PIP5K1A promotes the proliferation and metastasis of non-small cell lung cancer by regulating miR600/HIF1α [73]. ARHGAP4 mediates the Warburg effect of pancreatic cancer through mTOR and HIF-1α signalling pathway [74]. Necrostatin 1 (NEC-1) effectively protects renal ischaemia-reperfusion (I/R) injury by inhibiting necrotising ptosis, oxidative stress, and inflammatory response, and may function by mediating HIF-1α/miR-26a/TRPC6/PARP1 signalling pathway [75]. HIF-1α/phosphokinase 4/autophagy pathway has a protective effect on the vascular smooth muscle cell calcification induced by advanced glycation end products, i.e. AGEs promote autophagy through the HIF-1α/PDK4 signalling pathway, and autophagy helps to reduce AGE-induced calcification of vascular smooth muscle cells [76]. Through the miR-21/PDCD4 pathway, HIF-1a can work on the myocardial ischemia injury in rats [77]. The IL-6/stat3 pathway leads to metastasis and chemotherapy resistance of hepatocellular carcinoma after interventional therapy through the HIF-1α/SNAI1 axis [78]. MALAT1 affects hypoxia-induced vascular endothelial cell damage and autophagy by regulating the miR-19b-3p/HIF-1a axis [79]. Nuclear factor-kappaB hypoxia-induced ROS participates in the myoblast sagging during obstructive sleep apnoea through the NF-B/HIF-1 signalling pathway [80].

HIFs inhibitors

Inhibitors affecting the synthesis of HIF-A Mrna or HIF-A protein

The synthetic antisense oligodeoxynucleotide EZN-2968 contains 16 nucleotide residues complementary to hHIF-1αmRNA, which downregulate the expression of hHIF-1α subunit in a dose-dependent manner, and it has complete inhibitory activity at a concentration of 5 nmol/L. EZN-2968 and HIF-2α mRNA have three base pair mismatches, so the inhibitory effect on HIF-2α subunit is weak. The results of the tumour biopsy in the phase I clinical trial of EZN-2968 showed that EZN-2968 reduced the mRNA levels of HIF-1α subunit and target genes [81]. MicroRNAs (miRNAs) can regulate the synthesis of HIF-α through interaction with HIF-α mRNA [82, 83]. For example, through the principle of base pairing, miR-145 and miR-558 would respectively combine with the three non-coding regions and five non-coding regions of HIF-2α mRNA to inhibit HIF-2α's transcription and translation. Hutt et al. [84] discovered histone deacetylases inhibitors (HDACis) in HCC cells. Vorinostat can reduce the protein level of HIF-1α subunit by inhibiting HDAC9 with an eIF3G (eukaryotic translation initiation factor)-dependent translation mechanism. Vorinostat and another HDACis

romidepsin have been approved by the US Food and Drug Administration (FDA) for the treatment of skin T cell lymphoma. Topotecan (TPT), a semi-synthetic analogue of camptothecin, is an inhibitor of topoisomerase I (Top I), which can inhibit the production of the HIF-1α subunit at the translation level. Camptothecin drugs can be used for clinical treatment of small cell lung cancer and ovarian cancer [85]. The oestrogen metabolite 2-methoxyestradiol (2ME2) can inhibit tumour growth and angiogenesis by inhibiting the translation synthesis of HIF-1α and HIF-2α subunits and the nuclear translocation process [86]. In addition, Shukla et al. [87] found that HIF-1α mediates the resistance of pancreatic cancer cells to gemcitabine by upregulating the expression of cytidine triphosphate synthase (CTPS1) and transketolase (TKT). After digoxin inhibits the translation process of the HIF-1α subunit, pancreatic cancer cells become more sensitive to gemcitabine.

Inhibitors affecting the stability or dimerisation of HIF-α subunits

Geldanamycin (GDM) and its synthetic derivative 17-allylamino-17-demethoxygeldanamycin (17-AAG) can inhibit heat shock protein 90 (HSP90) activity prevents the HIF-α subgene from folding and positioning properly, and thus degrades in a pVHL-independent manner. EC154, another small molecule HSP90 inhibitor, has a stronger ability to inhibit HSP90 activity than 17-AAG [88]. The PAS domains in HIF-αs and HIF-1β subunits are involved in the assembly of HIFs heterodimers. Therefore, small molecules targeting the PAS domain can affect the dimerisation of HIF-αs and HIF-1β. The disinfectant acriflavine (acriflavine) destroys the stability of the HIFs heterodimer by binding to the interface between the HIF-αs subunit PAS-B domain, and it destabilises the heterodimers of HIFs [89]. Cyclic peptide inhibitors (cyclic-CLLFVY) selectively act on the PAS-B domain of HIF-1α subunit, thus disrupting the dimerisation process of HIF-1, without affecting the dimerisation process of HIF-2 [90]. Compound PT2385 selectively acts on the PAS-B domain of HIF-2α subunit but has no effect on HIF-1 [91]. The bicyclic compound OX3 can bind to the hydrophobic pocket of the PAS-B domain of the HIF-2α subunit, which affects the conformational stability of HIF-2 and the HREE sequence binding activity, but it has little effect on HIF-1 [92].

Inhibitors affecting the binding of HIFs to DNA

Hypoxia-inducible factors mainly play the role of transcriptional activation by binding to HRE sequences in target genes. In vitro studies on human glioma cell line U251 using ChIP assay have confirmed that echinomycin can specifically inhibit HIF-1 and HRE sequences in the VEGF promoter region (5'- TACGTG-3') binding,

hence inhibiting hypoxia-induced VEGF expression [93]. However, the clinical trials of echinomycin are not effective. In addition, HIF-1 inhibitors targeting HRE sequences also include polyamide compounds, doxorubicin and daunorubicin [94].

Inhibitors affecting the formation of HIFs transcription complexes

The chetomin from the fungus Chaetomium chrysogenum can act on the zinc binding site in the p300 CH1 domain to efflux Zn2 + and change the conformation of the CH1 domain, thereby destroying p300 and HIF-1α interaction [95]. Reece et al. [96] confirmed that chaetocin can reduce the expression of secreted VEGF, lactate dehydrogenase A (LDHA) and enolase 1 (ENO1) in a dose-dependent manner, which would result in the growth of rat prostate cancer xenograft cells significantly inhibited in the end. The antitumour activity of bortezomib is through enhancement of the binding of aspartate hydroxylase FIH and HIF-1α, and destruction of the enrichment effect of HIF-1α on p300 [97]. In addition, the anti-platelet agglutinating agent YC-1 and thiazolidinone compounds also inhibit the transcription activation activity of HIFs to target genes by disrupting the interaction between HIF-αs and p300. The compound CJ-3k designed and synthesised according to the structure of YC-1 can also effectively inhibit the activity of HIF-1α [98].

Degradation mechanism of HIFs

The above indicate that HIFs are closely related to the occurrence and development of human diseases. In particular, HIFs regulate angiogenesis, tumour cell differentiation, tumour cell metabolism reprogramming, tumour angiogenesis, glucose metabolism, and cell apoptosis and autophagy. But there are many specific questions that remain unanswered. For example, how do HIF-1α and COX-2 work together on tumour cells? Is there some connection between the simultaneous expression of STAT3 and HIF-1α? What is the interaction mechanism between PKM2 and HIF-1α in the process of tumourigenesis and development? HIF-1α, HIF-2α, and HIF-3α are similar in protein structure, regulation of stability, and regulation of transcriptional activation, but the three show complexity in the functional relationship in the occurrence and development of different types of tumours. For example, Jiang et al. [99] found that HIF-1α and HIF-2α have similar effects on the survival, apoptosis, and cell cycle of cervical cancer cell line CaSki. When only inhibiting the expression of HIF-1α or HIF-2α, the cell cycle of CaSki can be blocked in the G1 phase. As another example, in vitro studies on the bladder cancer T24 cell line show that under long-term

hypoxia, HAF expression levels increase and play the role of E3 ubiquitin ligase, while activating the NF- B pathway and degrading HIF-1α via the polyubiquitinated proteasome pathway in an oxygen-independent manner. At this time, the expression of HIF-2α increased compensatorily, which accelerated the deterioration of T24 cells and facilitated the maintenance of T24 stem cell markers [100]. This indicates that there is a compensatory mechanism between HIF-1α and HIF-2α. In addition, overexpression of the HIF-1α subunit can slow the growth of pVHL-deficient renal cell carcinoma (RCC) xenograft cells, while overexpression of HIF-2α subunit can promote the growth of RCC transplanted cells. This indicates that in some tumours HIF-1α and HIF-2α play opposite roles [101]. Hydroxylase inhibitors may promote the growth of existing tumours by promoting angiogenesis. However, there is little evidence for its role in promoting tumours in animal models. On the contrary, there is a lot of evidence that they can effectively increase serum EPO levels, which may lead to unnecessary erythropoiesis in non-anaemic patients. Therefore, it is very attractive to develop a targeted drug delivery system for hydroxylase inhibitors to avoid the side effects of erythropoietin under non-anaemic conditions [102]. At the same time, the following question should be considered: The tumour will secrete relevant protein factors to promote angiogenesis in the tumour, so will the regulation of HIFs to promote angiogenesis induce a tumour?

Conclusion

The research potential for HIF is still very large, and we believe that further comprehensive research on HIF will be of vital significance for curing many diseases.

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