HIF-1α in cerebral ischemia (Review)

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Abstract. Cerebral ischemic injury may lead to a series of serious brain diseases, death or different degrees of disability. Hypoxia-inducible factor-1α (HIF-1α) is an oxygen-sensitive transcription factor, which mediates the adaptive metabolic response to hypoxia and serves a key role in cerebral ischemia. HIF-1α is the main molecule that responds to hypoxia. HIF-1α serves an important role in the development of cerebral ischemia by participating in numerous processes, including metabolism, proliferation and angiogenesis. The present review focuses on the endogenous protective mechanism of cerebral ischemia and elaborates on the role of HIF-1α in cerebral ischemia. In addition, it focuses on cerebral ischemia interventions that act on the HIF-1α target, including biological factors, non-coding RNA, hypoxic-ischemic preconditioning and drugs, and expands upon the measures to strengthen the endogenous compensatory response to support HIF-1α as a therapeutic target, thus providing novel suggestions for the treatment of cerebral ischemia.

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1. Introduction

In 2017, stroke was recorded as the second leading cause of death in people >60 years old worldwide and the leading cause of permanent disability (1,2). The condition has become a huge global health problem (3). Ischemic stroke is the most common type of stroke and the third leading cause of disability worldwide (4,5). Ischemic stroke is a pathological state of insufficient blood supply in specific parts of the brain, particularly in the middle cerebral artery, due to sudden rupture of cerebral vessels or local ischemia caused by cerebral artery thrombosis or embolism, resulting in an insufficient supply of nutrients, oxygen and glucose, energy imbalance, and finally, neuronal cell death (6-9). The pathogenesis of ischemic stroke is complex, involving numerous mechanisms, including oxidative stress, neuroinflammation, excitatory neurotoxicity, ion imbalance, energy metabolism and apoptosis (10-12). At present, recombinant tissue plasminogen activator is the only drug approved by the Food and Drug Administration for the treatment of acute ischemic stroke (13). Therefore, the search for alternative treatment strategies for ischemic stroke has attracted increasing attention. Endogenous protection is an important mechanism of protection and recovery after cerebral ischemia. The hypoxia-inducible factor-1α (HIF-1α) signaling pathway serves an important role in endogenous protection. HIF-1α regulates angiogenesis, neuroprotection, neurogenesis, migration of neuronal stem cells to the ischemic area and proliferation to functional neurons by regulating the transcription of downstream target genes (14). Strengthening the endogenous compensatory response may become an interesting potential treatment strategy in stroke.

It is worth noting that the HIFs are a family of transcription factors involved in the hypoxia response and one of the key regulatory mechanisms of hypoxic stress at the cellular level (15,16). HIF-1 consists of an oxygen-regulated α subunit, HIF-1α, and a constitutively expressed β subunit, HIF-1β (17). Although mammals have a number of hypoxia adaptation mechanisms, including those that have a faster response time than the HIF-1α system, the unique degree of influence of the HIF system makes it a more important hypoxia response regulation mechanism (18). Under normoxic conditions, the proline and lysine residues on the oxygen-dependent degradation domain of HIF-1α are hydroxylated, and the modified HIF-1α interacts with the Von Hippel-Lindau E3 ubiquitin ligase complex via ubiquitin-proteasome pathway degradation (19). However, HIF-1α is stable under hypoxic conditions (20).

With the assistance of co-activators, such as cyclic adenosine monophosphate response element binding protein and acetyltransferase, HIF-1α forms a heterodimer with HIF-1β (21), and then HIF-1α is transferred to the nucleus and combines with the target gene hypoxia response element (HRE) to induce the expression of downstream genes (Fig. 1). HIF-1α regulates the transcription of >100 genes (22); its target genes encode molecules involved in vasomotor control, angiogenesis, erythropoiesis, cell proliferation and energy metabolism, and complex physiological and pathological processes, such as cell death and inflammation (23-26). During cerebral ischemia, HIF-1α is expressed in the chronic hypoxic area around the infarct area (27). Therefore, HIF-1α may become a novel and valuable therapeutic target.

2. Literature screening method

The literature was searched using PubMed and ScienceDirect databases. With use of 'pathogenesis of cerebral ischemia', 'HIF-1α' and 'cerebral ischemia and HIF-1α' as search terms, 2,199, 1,550 and 42 relevant articles were retrieved, respectively. The resulting articles were then screened according to the clarity and specificity of the research objectives, and the date of publication (from December 2000 to present). A total of 132 articles were selected for assessment in the current study.

3. Role of HIF-1α in cerebral ischemia

The role of HIF-1α in cerebral ischemia is related to hypoxia. During cerebral ischemia, the oxygen supply is insufficient and partial oxygen pressure in the tissue decreases, leading to the activation of HIF-1α (28). HIF-1α is mainly induced in the penumbra of the cerebral ischemic region and serves an important role in angiogenesis, glucose metabolism and cell survival following an ischemic stroke (29-31).

The formation of neovascularization promotes the nerve recovery of ischemic injury after cerebral ischemia (32). Angiogenesis is one of the most important modes of neovascularization, which depends on endothelial progenitor cells (EPCs) (32). HIF-1α is a transcription factor that regulates angiogenesis. It has been widely accepted that HIF-1α serves a role in regulating angiogenesis by regulating endothelial cells (ECs). For example, in the acute phase of ischemia, HIF-1α serves an important role in homing and germination of bone marrow-derived EPCs (bmEPC) in Sprague Dawley rat brain tissues. This effect is related to maintaining proper astrocyte responses in the ischemic brain. From a molecular perspective, the signals of the chemokine (C-X-C motif) ligand 12 (CXCL12)/chemokine C-X-C-motif receptor 4 (CXCR4) axis, high mobility group protein B1 (HMGB1) and vascular endothelial growth factor A (VEGF-A)/vascular endothelial growth factor receptor 2 (Flk1)-neuropilin-1 (Nrp1)/delta-like ligand-4 (Dll4) axis between astrocytes and bmEPCs are the basis for HIF-1α to regulate the homing and sprouting of bmEPCs (33). After a stroke, bmEPCs are mobilized from the bone marrow to the peripheral blood and recruited to the ischemic brain (34), and CXCL12 and HMGB1 expression is upregulated. CXCL12/CXCR4 and HMGB1 are important for the homing of EPCs (35,36). EPCs then attach to damaged blood vessels and repair them or migrate outward to start angiogenesis (37). Bud angiogenesis is another important mode of angiogenesis guided by apical cells (38,39). Apical cells are usually mobile and located at the top of vascular buds (38,39). Morphologically, apical cells are rich in filamentous pseudopodia, which contributes to the formation of vascular tubes (40). ECs serve as a bank for apical cells. The differentiation of ECs into apical cells is controlled by the VEGF-A/Flk1/Dll4 signaling pathway (38,39). Specifically, when Flk1-positive ECs are stimulated by VEGF-A, the levels of Dll4 are increased in these cells, and thus, these cells begin to differentiate into tip cells (38,39). In addition, Nrp1 is a co-receptor of Flk1 and is necessary for apical cell formation (41,42). The role of astrocytes in angiogenesis is that activated astrocytes recruit bmEPCs by secreting HMGB1 and CXCL12 (35). These two substances are ligands of advanced glycosylation end product-specific receptor and recombinant CXCR4, which are expressed on the cell membrane of EPCs (35,43). It has been revealed that inhibition of HIF-1α can reduce the expression levels of CXCR4 in bmEPCs, as well as reducing the expression levels of VEGF-A, VEGF-A/Flk-1, NRP1 and Dll4 in bmEPCs. These findings suggest that HIF-α may participate in the homing of bmEPCs via CXCL12/CXCR4 and HMGB1, and promotes the germination of bmEPCs via VEGF-A/Flk1-Nrp1/Dll4 (33). Additionally, knockdown of HIF-1α *in vivo* reduces the number of reactive astrocytes in the ischemic brain (33,44). Furthermore, a previous study (31) found that the number of reactive astrocytes increased in the brain of ischemic mice with insufficient prolyl hydroxylases (PHDs), and insufficient PHDs led to the stabilization of HIF-1α. This indicated that astrocytes serve a key role in the homing and germination of bmEPCs, and HIF-1α serves a direct role in the homing and germination of bmEPCs via regulation of the pathway between astrocytes and bmEPCs. Conversely, affecting the number of astrocytes has an indirect effect on the homing and germination of bmEPCs. In addition, HIF-1α induces bone marrow dendritic cell (BMDC) homing to ECs and regulates angiogenesis (45). In the molecular mechanism of BMDC transport, pituitary adenylate cyclase-activating peptide 38 (PACAP38) increases the expression levels of adhesion/migration-related proteins cellular prion protein (PrPc), α6-integrin, B1 integrin, focal adhesion kinase and CXCR4 (46-52), enhances the activities of MMP9 and MMP2 in BMDCs, and promotes the homing and migration of BMDCs. The PACAP38-pituitary adenylate cyclase-activating polypeptide type I receptor isoform 1 (PAC1) signal is an important part of the homing mechanism. During ischemia and hypoxia, HIF-1α upregulates PACAP38 by binding to the HRE on the PACAP38 promoter. The PACAP38 receptor PAC1 is widely expressed on BMDCs. PACAP38 binds to the receptor and promotes the homing of BMDCs to the ischemic area. In addition, PACAP38 upregulates the expression levels of α6-integrin and PrPc on the surface of BMDCs. This may stimulate the bone marrow mesenchymal cells to move to the blood vessels and increase their binding to laminin. Laminin is concentrated on the surface of the blood vessel (53,54). The interaction can enable BMDCs to integrate into the vascularized parenchymal area of the ischemic brain to promote tissue repair (Fig. 2) (53,54).
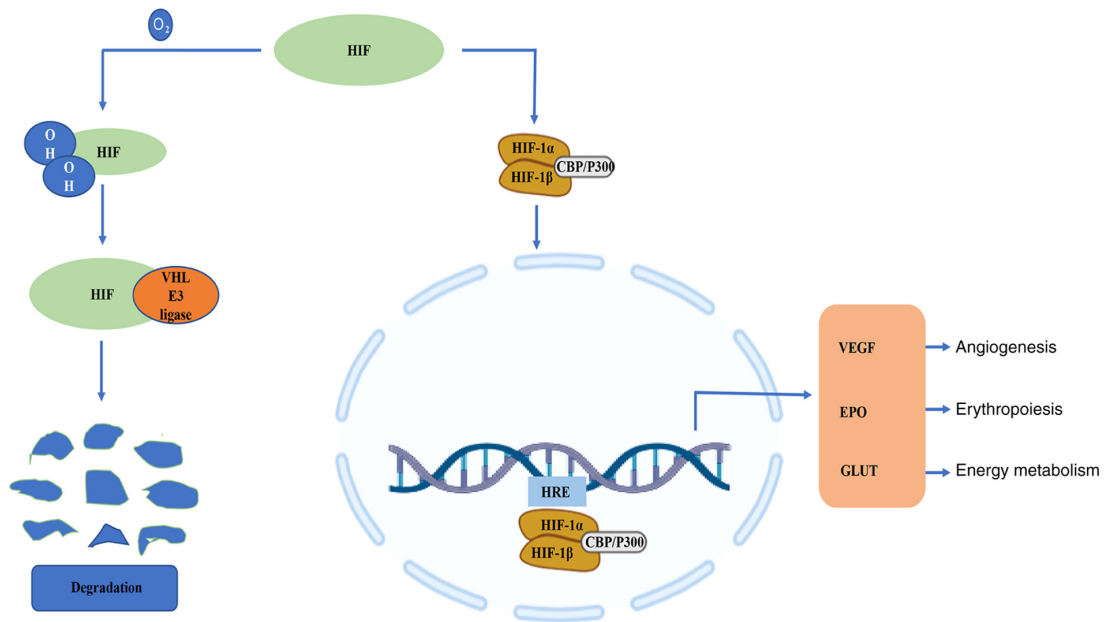
Figure 1. Under normoxic conditions, the proline and lysine residues on the oxygen-dependent degradation domain of HIF-1α are hydroxylated, and modified HIF-1α interacts with the Von Hippel-Landau E3 ubiquitin ligase complex via ubiquitin-proteasome pathway degradation. However, HIF-1α is stable under hypoxic conditions. With the help of co-activators, such as CBP and p300, HIF-1α forms a heterodimer with HIF-1β, and then HIF-1α is transferred to the nucleus and combines with the target gene HRE to induce downstream gene expression. HIF, hypoxia-inducible factor; HRE, hypoxia response element; CBP, cyclic adenosine monophosphate response element binding protein; p300, acetyltransferase; VEGF, vascular endothelial growth factor; EPO, erythropoietin; GLUT, glucose transporter type.
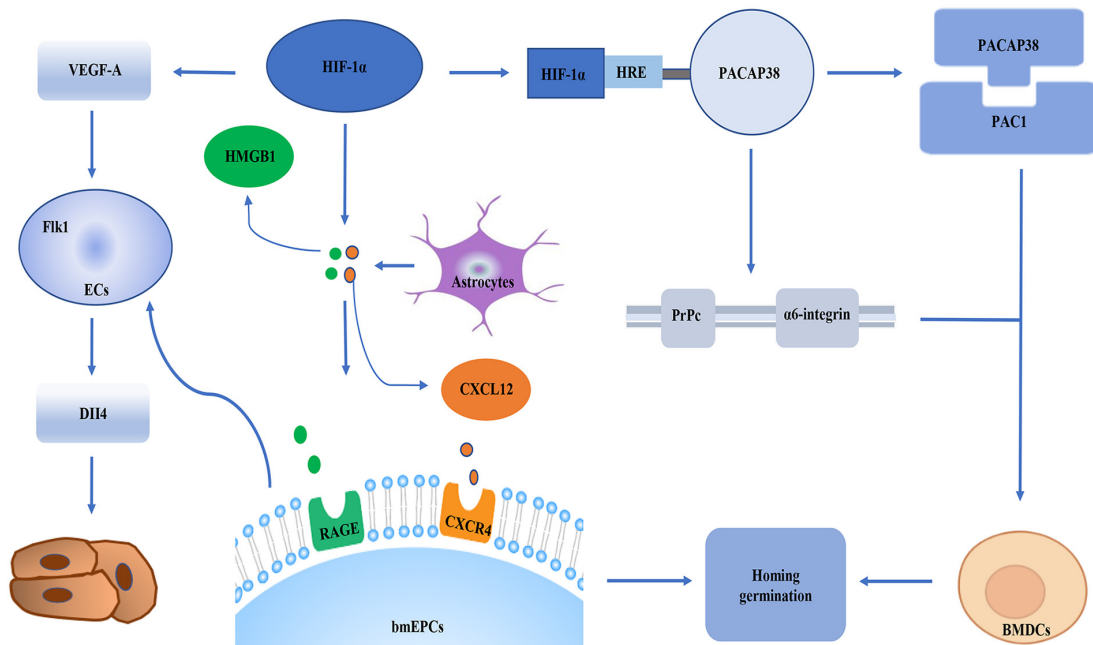

Figure 2. HIF-1α may participate in the homing of bmEPCs via CXCL12/CXCR4 and HMGB1, and promotes the germination of bmEPCs via VEGF-A/Flk1/Dll4. HIF-1α upregulates PACAP38 by binding to the HRE on the PACAP38 promoter during ischemia and hypoxia. PACAP38 binds to the receptor PAC1 and promotes the homing of BMDCs to the ischemic region. In addition, PACAP38 upregulates the expression levels of α6-integrin and PrPc on the surface of BMDCs. ECs, endothelial cells; CXCL12, chemokine (C-X-C motif) ligand 12; CXCR4, chemokine C-X-C-motif receptor 4; HMGB1, high mobility group protein B1; RAGE, ligands of advanced glycosylation end product-specific receptor; VEGF-A, vascular endothelial growth factor A; Flk1, vascular endothelial growth factor receptor 2; Dll4, delta-like ligand-4; BMDCs, bone marrow dendritic cells; HIF-1α, hypoxia-inducible factor 1α; HRE, hypoxia response element; PACAP38, pituitary adenylate cyclase-activating peptide 38; PAC1, PACAP type 1 receptor; PrPc, cellular prion protein; bmEPCs, bone marrow-derived EPCs.

Previous research has revealed that HIF-1α serves a dual role in promoting survival or death of nerve cells during cerebral ischemia. First, endogenous neurogenesis is enhanced during cerebral ischemia and hypoxia (55), which may be related to the activation of endogenous neural stem cells (NSCs) during cerebral ischemia (56,57). Previous studies

have demonstrated that both global and focal cerebral ischemia can increase the proliferation and neural differentiation of NSCs located in the subgranular area of the dentate gyrus, the anterior subventricular area and the posterior peripheral area of the ventricle adjacent to the hippocampus (58,59). HIF-1α, by increasing activation of the Wnt/β-catenin signaling pathway, stimulates NSC proliferation (60). The Wnt signaling pathway regulates the embryonic NSC pattern, cell fate determination and cell proliferation (61). Wnt signaling may regulate hippocampal neurogenesis in adult rats (62). In fact, Wnt3α mutant mice exhibit hippocampal hypoplasia due to a lack of proliferation (62). Wnt family members are expressed in hippocampal astrocytes, while hippocampal stem/progenitor cells express Wnt protein receptors and signal components (63). It has been reported that HIF-1α signaling is inhibited under oxygen-deprived conditions, which may reduce β-catenin nuclear translocation and cyclin D1 expression, delaying NSC proliferation (60). In addition, HIF-1α has different effects on the occurrence of apoptosis in different periods of cerebral ischemia (64,65). A previous study has demonstrated that HIF-1α may have a neuroprotective effect in the early stage of an ischemic stroke (66). HIF-1α is highly expressed in rat brain tissue in the early stage of ischemic stroke and may markedly reduce infarct cell apoptosis. This effect may be related to the inhibition of acyl CoA synthase long chain family member 4 (ACSL4) by HIF-1α (67). ACSL4 is an important metabolic isoenzyme of polyunsaturated fatty acids. ACSL4 promotes neuronal death by enhancing lipid peroxidation (a marker of iron drop disease). In addition, ACSL4 may promote the microglia mediated inflammatory response. HIF-1α inhibits ACSL4 expression, thereby reducing lipid peroxidation and inflammation, and exhibiting a neuroprotective effect on cerebral ischemia (67). On the contrary, a previous study conducted by Panchision (68) demonstrated that HIF-1α expression could promote neuronal apoptosis after long-term severe ischemia and hypoxia. HIF-1α may regulate the inflammatory response through the NLR family containing pyrin domain protein 3 (NLRP3) inflammasome complex, thereby promoting apoptosis and pyrophagocytic cell death after stroke (69). NLRP3 inflammatory bodies are the main mediators of the inflammatory response during ischemic stroke (70,71). Upregulation of NLRP3 inflammatory bodies activates pre-caspase-1 by cleavage (72), thus promoting the maturation of IL-1B and IL-18. HIF-1α regulates the NLRP3 inflammatory focal pathway, resulting in brain cell death (69). In addition, in the transient focal cerebral ischemia model, on the one hand, HIF-1α upregulates erythropoietin (EPO) expression, thereby inhibiting the expression of activated caspase-3 in neurons and inhibiting neuronal apoptosis to improve the recovery of nerve function (73). On the other hand, HIF-1α exerts a neuroprotective effect on transient focal cerebral ischemia by upregulating VEGF and downregulating caspase-9 (74). A previous study also found that the anti-apoptotic effect of HIF-1α gene therapy effectively reduced the neurological deficit score and brain edema at 24 and 72 h after reperfusion, and inhibited the pathological damage and apoptosis of nerve cells in a rat middle cerebral artery occlusion model (75). Additionally, a previous study reported that HIF-1α may improve brain damage after ischemia/reperfusion (I/R) via BCL2/adenovirus E1B interacting protein 3 (BNIP3)

and Bcl-2 family proteins containing BH3 domain-dependent enhancement of autophagy cell survival (76). Taken together, these observations suggest that HIF-1α may induce cell death in severe and long-term ischemia, but that activation in mild ischemic stress could promote cell survival (Fig. 3). This may be related to different mechanisms being involved in the regulation of the response to ischemic stroke by HIF-1α. The role of HIF-1α in neuroprotection requires further study.

4. HIF-1α protects the brain via the regulation of endogenous substances

During cerebral ischemia, endogenous factors, such as neurotransmitters, amino acids and inorganic salts, serve a protective role against cerebral ischemia by regulating HIF-1α to influence the angiogenesis and neuroprotection of ischemic brain tissue (77-80). For example, choline may increase the levels of α7 nicotinic acetylcholine receptor, induce the expression of HIF-1α and VEGF, and promote the formation of cerebral arteries and cerebral cortex capillaries, thereby effectively reducing cerebral ischemic damage in permanent middle cerebral artery occlusion (MCAO) rats (77). Peroxynitrite promotes neurogenesis by activating HIF-1α and enhancing the Wnt/β-catenin signaling pathway (78). Arginine reduces the inflammatory response mediated by HIF-1α and protects against the death of ischemic neurons after I/R injury in rats (79). Glycine inhibits HIF-1α by inhibiting the upregulation of NF-κB/p65 after I/R injury, thereby inhibiting pro-inflammatory activity (80).

MicroRNAs (miRNAs/miRs) are a type of small endogenous non-coding single-stranded RNA that regulate protein expression by inducing mRNA degradation or interfering with translation. miRNAs have been found to be involved in the pathogenesis of stroke. To date, numerous miRNAs have been determined to be involved in the molecular process of the ischemic cascade (81,82). Several miRNAs, including miR-376b-5p, miR-433, miR-335, miRNA-210 and miR-155-5p, have also been demonstrated to regulate HIF-1α during cerebral ischemia. Notably, among these miRNAs, miRNA-210 is positively associated with HIF-1n expression. When miRNA-210 expression is upregulated, the gene and protein expression levels of HIF-1α and VEGF are increased, and their expression trends are consistent (83). Recently, it has been revealed that elevated levels of miRNA-210 increase neuronal cell apoptosis by activating the HIF-1α-VEGF signaling pathway. By contrast, downregulation of miRNA-210 expression markedly inhibits the gene and protein expression of HIF-1α and VEGF (83). The expression levels of miR-376b-5p, miR-433, miR-335 and miR-155-5p are negatively associated with the expression levels of HIF-1α (84-87). miR-376b-5p inhibits angiogenesis after cerebral ischemia via the HIF-1α-mediated VEGFA-Notch1 signaling pathway (84). Overexpression or downregulation of miR-433 alters the mRNA and protein levels of HIF-1α and its downstream genes, VEGF, glucose transporter 1 (GLUT1) and angiopoietin 2 (Angpt2) (85). In addition, miR-335, as a direct regulator of HIF-1α, serves different roles in different periods of cerebral ischemia, which may be related to the different effects of HIF-1α in different periods of cerebral ischemia. In the early stage of cerebral ischemia, miR-335 mimic may reduce the area of cerebral infarction, while the levels
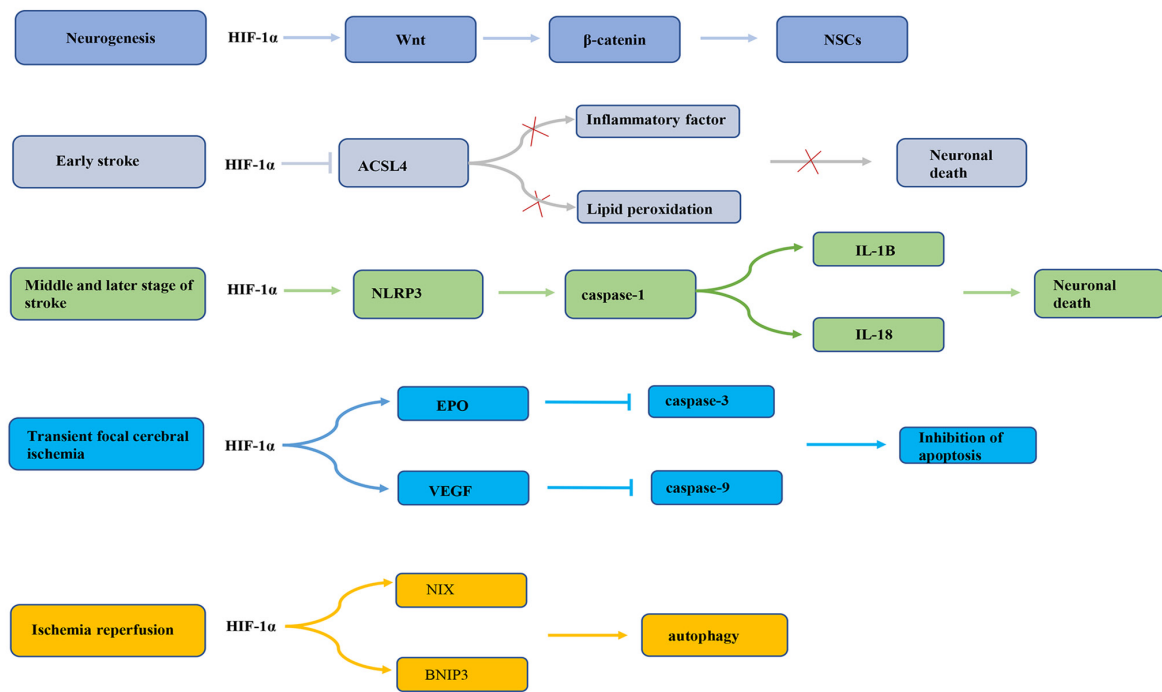
Figure 3. HIF-1α expression in ischemic stroke is controlled by different mechanisms. NSCs, neural stem cells; HIF-1α, hypoxia-inducible factor-1α; ACSL4, acyl CoA synthase long chain family member 4; NLRP3, NLR family containing pyrin domain protein 3; IL-18, interleukin 18; IL-1B, interleukin 1B; EPO, erythropoietin; VEGF, vascular endothelial growth factor; NIX, Bcl-2 family proteins containing BH3 domain; BNIP3, BCL2/adenovirus E1B interacting protein 3.

of HIF-1α protein are lower. This results in the decreased expression of downstream target genes of HIF-1α, including Angpt2, BNIP3, MMP9, plasminogen activator inhibitor-1 and VEGF-A. By contrast, in the middle and late stages of cerebral ischemia, the use of anti-miR-335 is beneficial. HIF-1α protein is upregulated and then its downstream gene expression levels increase (86). miR-335 regulates HIF-1α expression and also affects neurovascular permeability, cell death and the blood-brain barrier, resulting in a reduction in infarct volume (86). Furthermore, a recent study has demonstrated that miR-155-5p targets HIF-1α in NSCs (87). Inhibition of miR-155-5p may promote the viability of NSCs and inhibit cell apoptosis induced by oesophago-gastro-duodenoscopy (OGD). Additionally, after transplantation, NSCs inhibit miR-155-5p, and this also enhances the inhibition of inflammation and oxidative stress, which enhances the protection against cerebral infarction. Long non-coding RNAs (lncRNAs) are a relatively newly discovered class of non-coding RNA, ranging in length from ~200 nucleotides to several kilobases (88). lncRNAs are dynamically expressed in tissues, based on differentiation stages and cell type-specific patterns, and participate in numerous normal cellular processes. lncRNAs can compete with specific mRNAs for the same miRNA pool. The result is that the binding of miRNAs to target mRNAs is inhibited or reduced, and the function of miRNA post-transcriptional silencing is impaired (89,90). HIF-1α-AS2 is an antisense lncRNA derived from the natural antisense transcript of HIF-1α (91). A previous study has revealed that the expression levels of HIF-1α-AS2 are upregulated in hypoxic human umbilical vein ECs (HUVECs) (92). Upregulation of HIF-1α-AS2 leads to downregulation of miR-153-3p, which can reduce the post-transcriptional silencing of HIF-1α.

MCAO reduces the level of miR-153-3p RNA in the infarct area and increases the protein levels of HIF-1α, VEGF-A and Notch1. This function of HIF-1α-AS2 promotes the activation of the HIF-1α/VEGFA/Notch1 cascade, thereby promoting the vitality, migration and tube formation of HUVECs (92).

5. Role of HIF-1α in cerebral ischemic preconditioning (IPC)

Preconditioning has a certain protective effect on cerebral ischemia. To date, several preconditioning methods have been used to induce ischemic tolerance (IT). Studies have revealed that during pretreatment, HIF-1α is activated to serve a neuroprotective role (93,94). Hypoxic preconditioning is a phenomenon in which mild hypoxia may induce a strong state of IT to resist the subsequent damage caused by severe hypoxia, which exists in a number of organs, especially the brain (95-98). Hypoxic preconditioning improves the survival rate of rats with cerebral ischemia, reduces neurological deficits, increases the object recognition and social recognition memory of the rat, and inhibits the inflammatory response caused by cerebral ischemia. These effects are regulated by HIF-1α (99). During hypoxia preconditioning, IT levels increase, which is related to the activation of HIF-1α and the expression of its target genes GLUT1, EPO, VEGFα, Bcl-2 and inducible nitric oxide synthase. Also during hypoxic preconditioning, olfactory mucosal mesenchymal stem cells activate HIF-1α in vitro to inhibit the pyrolysis and apoptosis of microglia after cerebral I/R injury (100). In addition to hypoxic preconditioning, IPC is also used as a means of cerebral ischemia protection. IPC is defined as a transient sublethal ischemic injury, which may mobilize protective mechanisms

to improve neuronal damage following fatal ischemia (101). A previous study has revealed that IPC protects CA1 pyramidal neurons from non-IPC lethal ischemia by increasing HIF-1α expression in CA1 pyramidal neurons, thereby enhancing the expression of VEGF and the activation of NF-κB (102). The increase of HIF-1α may be related to the continuous increase of P2X7 receptors in astrocytes. Activation of P2X7 receptors leads to the increase of HIF-1α. This hypoxia-independent, but P2X7 receptor-dependent mechanism could induce persistent expression of HIF-1α in astrocytes, thereby effectively inducing IT and neuroprotection against ischemia (103). Studies have also demonstrated that remote IPC (RIPC) could improve the response of peripheral immune cells by regulating the upregulation of HIF-1α (104,105). This effect may be the protective effect of cerebral ischemia mediated by RIPC activation of the HIF-1α/AMP-activated protein kinase (AMPK)/70-kilodalton heat shock protein (HSP70) signaling pathway. Highly conserved HSPs, as molecular chaperones of abnormally folded proteins in cellular stress, are induced through the HIF-1α pathway during hypoxia, and the existing evidence also demonstrates that, in newborns, HSPs transform into the mature conformation, and HSP70 and HSP90 serve an important role in the post-translational process (106). The AMPK-histone deacetylase 5 signaling pathway promotes HIF-1α through the deacetylation of HSP70 in the cytoplasm (107,108). The nuclear accumulation of HIF-1α and the activation of HIF-1α function indicate that RIPC mediates ischemic protection through the interaction between AMPK, HIF-1α and HSP70 (109). In addition, exercise preconditioning is a special neuronal IPC, which may induce brain tolerance to ischemia, enhance neuroprotection and resist a series of brain damages caused by ischemia (110,111). Previous studies have demonstrated that exercise pretreatment 3 weeks before stroke improves the structural integrity of the brain microvascular structure in rats (112-114). This effect may be related to HIF-1α. The pre-IPC exercise induces cerebral IT mediated by neurons and astrocytes by increasing the expression levels of HIF-1α (115,116). In addition, HIF-1α triggers the expression of endothelin 1, increases the expression of B-type natriuretic peptide and has a neuroprotective effect (117).

6. Role of HIF-1α in the protective effects of natural compounds against cerebral ischemia

Platelet drugs and thrombolytic drugs are the only ischemic stroke drugs supported by strong clinical evidence (118). However, the application of these two treatment methods is often limited by the potential risk of cerebral hemorrhage and a narrow treatment time window. In general, after decades of practice and investigation, the number of effective interventions for ischemic stroke is limited. Therefore, it is necessary to study further feasible and effective treatment options. A number of studies (119-122) have demonstrated that certain natural compounds, chemical drugs and traditional Chinese medicine compounds may alleviate cerebral ischemic injury through HIF-1α. A previous study found that certain compounds and traditional Chinese medicines serve a role in angiogenesis and vascular protection through the HIF-1α/VEGF signaling pathway, thereby protecting against cerebral ischemic injury (123). *In vivo* and *in vitro* studies have demonstrated

that catalpol directly activates the HIF-1α/VEGF signaling pathway in the brain and primary cerebral microvascular ECs of rats with cerebral ischemia, protects the vascular structure and promotes blood vessel generation (120). Astragaloside IV activates the HIF-1α/VEGF/Notch signaling pathway through miRNA-210 to promote angiogenesis (121). Fluoxetine induces a cascade of events leading to the upregulation of the expression of HIF-1α-Netrin/VEGF protein, promotes angiogenesis after ischemic stroke and improves long-term functional recovery after ischemic stroke (122,123). Racemic dl-3-n-butylphthalide treatment could also promote functional recovery after focal transient cerebral ischemia, and this recovery and dl-NBP may upregulate the expression of HIF-1α-VEGF and Notch-Dll4, and then affect the integrity of white matter, the number of capillaries and the expression of tight junction protein occludin (124). In addition, certain compounds serve a neuroprotective role by regulating HIF-1α. Berberine pretreatment enhances the accumulation of HIF-1α by activating PI3K/Akt and induces the production of sphingosine-1-phosphate (S1P) by promoting HIF-1α-mediated sphingosine kinase 2 (Sphk2) transcription and activation of Sphk2. S1P protects neuronal cells against hypoxia and ischemia by activating high-affinity G protein-coupled receptors, and serves an important protective role in tissue I/R injury (125). Methylene blue protects hippocampal-derived neuronal cells from OGD-reoxygenation damage by increasing the content of HIF-1α protein and activating the EPO signaling pathway (126). Salidroside induces the production of HIF-1α subunit and EPO via the PI3K/Akt signaling pathway, and exerts anti-inflammatory effects on cerebral ischemia and reperfusion (127). Certain drugs inhibit HIF-1α to exert neuroprotective effects, while others upregulate HIF-1α to produce the same effect. Curcumin exerts a neuroprotective effect by inhibiting the interaction between HIF-1α and autophagy in cerebral I/R injury (128). A recent study has reported that nateglinide stabilizes HIF-1 cerebral I/R injury by inhibiting STAT-3 phosphorylation and stops the expression of HIF-1α-dependent inflammation and mediators of apoptosis, namely phorbol-12-myristate-13-acetate-induced protein 1 and NF-κB (129). Different drugs have a neuroprotective effect through the opposite regulation of HIF-1α, which may be due to the different mechanisms of HIF-1α in the neuroprotective effect of cerebral ischemia. Different drugs affect different pathways by activating or inhibiting HIF-1α, and serve a role in cerebral ischemia. *Angelica sinensis* has a neuroprotective effect on astrocyte-mediated infarct expansion through HIF-1α-mediated angiogenesis, as well as HIF-1α-mediated anti-apoptotic effects. *Angelica sinensis* activates p38/MAPK/HIF-1α/VEGF-A/cAMP-response element binding protein/von Willebrand factor signaling to mediate angiogenesis (130). In addition, the anti-apoptotic effect of *Angelica sinensis* has been attributed to the activation of the HIF-1α/VEGF-A/p-Bad signaling pathway mediated by p38/MAPK (130). This activation could lead to Bad inactivation, maintain the integrity of the outer mitochondrial membrane and prevent cytochrome caspase-3-mediated apoptosis in the cortical ischemic penumbra, thereby exerting an anti-apoptotic effect (130). In addition, a previous study demonstrated that ligustilide can inhibit the upregulation of HIF-1α, VEGF and aquaporin 4 in an OGD-induced blood-brain barrier model and reduce the permeability of the

Table I. Regulation of drugs affecting HIF-1α.

Material	Impact on HIF-1α	Related pathway	Function	(Refs.)
Catalpol	Enhancement	HIF-1α/VEGF	Protects vascular structure, promotes angiogenesis	(120)
Astragaloside IV	Enhancement	HIF-1α/VEGF/Notch	Promotes angiogenesis	(121)
Fluoxetine	Enhancement	HIF-1α/Netrin/VEGF	Promotes angiogenesis	(122,123)
Dl-NBP	Enhancement	HIF-1α/VEGF, HIF-1α/Notch-Dll4	Increases the number of microvessels and tight junction protein occludin	(124)
Berberine	Enhancement	HIF-1α/Sphk2/S1p	Protects neurons against hypoxia and ischemia	(125)
Methylene blue	Enhancement	HIF-1α/EPO	Protects hippocampal derived neurons	(126)
Salidroside	Enhancement	HIF-1α/PI3K/AKT, HIF-1α/EPO	Anti-inflammatory effect	(127)
Curcumin	Inhibition	To be studied	Anti-apoptosis, neuroprotection	(128)
Nateglinide	Inhibition	HIF-1α/NF-κB, TNF-β	Anti-inflammatory, neuroprotective	(129)
Angelica sinensis	Inhibition	HIF-1α/VEGF-A/CREB/vWF, HIF-1α/VEGF-A/P-Bad	Angiogenesis, anti-apoptosis, neuroprotection	(130)
Ligustilide	Inhibition	HIF/VEGF/AQP-4	Reduces the permeability of blood-brain barrier	(131)
Bu Yang Huan Wu Decoction	Inhibition	HIF/VEGF	Maintains the integrity of blood-brain barrier	(132)

Dl-NBP, dl-3-n-butylphthalide; HIF-1α, hypoxia-inducible factor-1α; VEGF, vascular endothelial growth factor; Sphk2, sphingosine kinase 2; S1p, sphingosine 1-phosphate; EPO, erythropoietin; PI3K, hosphatidylinositol 3-kinase; AKT, protein kinase B; NF-κB, nuclear factor κB; TNF-β, tumor necrosis factor β; MAPK, mitogen-activated protein kinase; VEGF-A, vascular endothelial growth factor-A; CREB, cAMP-response element binding protein; vWF, von Willebrand factor; AQP-4, aquaporin 4.

OGD-induced blood-brain barrier in an *in vitro* model (131). Bu Yang Huan Wu decoction has a protective effect on the brain I/R injury in MCAO rats by inhibiting the activation of the HIF-1α/VEGF signaling pathway in the brain and stabilizing the β epithelial Na⁺ channel ion channel, suggesting that Bu Yang Huan Wu decoction may be used to treat acute brain injury during stroke (Table I) (132).

7. Concluding remarks and future perspectives

An increasing number of studies have demonstrated that HIF-1α serves a key role in cerebral ischemia. The present review describes the means by which HIF-1α is activated during cerebral ischemia and how it serves a protective role in cerebral ischemic tissues in terms of angiogenesis and neuroprotection. In terms of neuroprotection, since HIF-1α expression in ischemic stroke may be controlled by different mechanisms, HIF-1α has a dual effect. In addition, when cerebral ischemia occurs, endogenous regulatory factors directly or indirectly regulate HIF-1α, which may be the key mechanism of endogenous protection during cerebral ischemia. Recent research has also revealed that preconditioning has a positive therapeutic effect on cerebral ischemia and may become a novel clinical treatment for cerebral ischemia. Natural medicines and traditional Chinese medicines could be used to treat cerebral ischemia by regulating HIF-1α. HIF-1α is expected to become a novel target for the treatment of cerebral ischemic diseases, and identifying the effect of natural products on HIF-1α is also a future research direction.

Whether the signals and pathways initiated by HIF-1α in hypoxia (or hypoxic diseases) serve the same role in cerebral ischemia needs to be further confirmed. As HIF-1α regulates multiple downstream target genes, and the related pathways and mechanisms are complex, the current literature on the mechanism of HIF-1α in cerebral ischemia is not comprehensive and in-depth. Therefore, it is necessary to further study the role and mechanism of HIF-1α in the pathophysiology of cerebral ischemia.

Availability of data and materials

Not applicable.

Authors' contributions

HH conceived and designed the review. QL retrieved the relevant literature and wrote the manuscript. PD reviewed and

edited the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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