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REVIEW PAPER

Hypoxia Inducible Factor-1: Its Potential Role In Cerebral Ischemia

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Abstract A divergence in the supply and consumption of oxygen in brain tissue initiates complex cycle of biochemical and molecular events resulting in neuronal death. To overcome such adverse situation, the tissue has to adopt some cellular mechanisms such as induction of various transcription factors, such as hypoxia inducible factor (HIF). It is a transcriptional regulator of oxygen homeostasis and key factor to generate the adaptive responses through upregulation of various target genes involved in the erythropoiesis, angiogenesis as well as glucose metabolism and transport. On the other hand, some studies do suggest that HIF also plays a detrimental role in ischemic reperfusion injury by inducing the pro apoptotic molecules, cytokines such as Nix, BNip3, and IL-20 which cause mitochondrial dysfunction leading to cell death. Hence, modulation of HIF-1 activity seems to provide an innovative therapeutic target to reduce the cellular damage, which arises from ischemic injury. Apart from traditional oxygen dependent HIF regulation, the focus has now shifted toward oxygen independent regulation in cell specific manner through reactive oxygen species involving hypoxia-associated factor, and heat shock protein 90, etc. Therefore, future development of such small molecule regulators for HIF-1 stability and signaling may prove useful to therapeutically target for enhancing recovery and repair in I/R injury.

Keywords HIF-1 · I/R injury · Neuroprotection · ROS · HSP90

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Abbreviations

17-AAG	17-Allylaminogeldanamycin
AD	Alzheimer disease
Ahr	Aryl hydrocarbon receptor
ARD1	Arrest defective protein
ARNT	Aryl hydrocarbon receptor nuclear transporter
CBP	cAMP-response element-binding protein
Cbz-LLL	*N*-carbobenzoxyl-L-leucinyl-L-leucinyl-L-norvalinal
DFO	Desferoxamine
DHB	Dihydroxybenzoic acid
DMOG	Dimethyloxallyl glycine
Epo	Erythropoietin
ePAS1	Endothelial PAS domain protein 1
FIH	Factor-inhibiting hypoxia
GSK	Glycogen synthase kinase
GLUT	Glucose transporter
HAF	Hypoxia-associated factor
HIF-1	Hypoxia inducible factor-1
HLH	Helix–loop–helix
HRD	Hypoxia-responsive domain
HO	Hemo oxygenase
HRE	Hypoxia-responsive element
HSP	Heat shock protein
iNOS	Inducible nitric oxide synthase
I/R injury	Ischemia reperfusion injury
LDH	Lactate dehydrogenase
MCAO	Middle cerebral artery occlusion
2ME	2-Methoxyestradiol
MMP	Matrix metalloproteinase
Ngb	Neuroglobin
NGF	Nerve growth factor
ODDD	Oxygen-dependent degradation domain
PHD	Prolyl 4-hydroxylase enzyme
pVHL	Von Hippel–Lindau tumor suppressor protein

PI3K	Phosphatidylinositol 3-kinase
ROS	Reactive oxygen species
PTEN	Phosphatase/tensin homolog deleted on chromosome 10
RACK	Receptor of activated protein kinase C
SUMO	Small ubiquitin-like modifiers
SENP	Sentrin-specific peptidase
VEGF	Vascular endothelial growth factor

Introduction

The brain comprises of only 2% of the total body weight, but utilizes nearly 20% of the cardiac output for proper function. It needs oxygen and glucose to execute all essential functions of human body. An average adult human consumes ~250 ml per minute of oxygen or about 360 l of oxygen per day. Hypoxia is usually defined as 2% ≤oxygen, and which may lead to severe hypoxia (anoxia), when oxygen concentration becomes ≤0.02%. Therefore, even a minor depletion in oblige amount of oxygen, may give birth to a number of pathological conditions such as cerebral stroke, spinal and traumatic brain injury. The adaptation to a sustained reduction in oxygen availability (hypoxia) necessitates alterations in gene expression, leading to reduced oxygen consumption and increased oxygen delivery, which would provide a means of counteracting the detrimental effects of hypoxia (Semenza 2004).

Stroke is a second leading neurocardiovascular disorder causing significant mortality and disability world over. In case of cerebral ischemia, the biochemical and molecular processes are the outcome of disturbances in cerebral blood flow. This occurs due to an embolism, thrombosis or systemic hypo-perfusion, which triggers excitotoxicity, acidotoxicity, and multiple cellular and molecular processes comprising ionic imbalance, peri-infarct depolarization, calcium overload, stress signaling, neuroinflammation, oxidative and nitrative stress, leading to cellular necrosis and apoptosis (Mehta et al. 2007; Nakka et al. 2008). Apart from these, some endogenous adaptive and regenerative mechanisms also get initiated to rescue damaged cells from these ischemic events. Regulation of many of these processes occurs at the transcriptional level, which involves the concerted activation of various transcription factors, including hypoxia inducible factor (HIF) which is an essential mediator of oxygen homeostasis. It mediates a large number of gene-cassettes, which promotes the neuronal adaptation for survival under hypoxic conditions. During hypoxic condition or in cerebral ischemia, HIF triggers some neuroprotective as well as few detrimental pathways. This review briefly deals with the general introduction of HIF, its structure and regulation besides its beneficial and detrimental role in case of cerebral ischemic reperfusion (I/R) injury.

HIF and Its Isoforms

The discovery about expression of the oxygen sensitive changes in erythropoietin (Epo) gene transcription in a particular human hepatoma-derived tissue culture lines (Goldberg et al. 1987), eventually led to the discovery of HIF (Wang and Semenza 1995). It belongs to PAS family (PER-ARNT-SIM) and composed of two protein subunits of basic helix–loop–helix (HLH) family: one is oxygen-regulated alpha (α) subunit and other is oxygen independent beta (β) subunit, which is also known as aryl hydrocarbon receptor nuclear transporter (ARNT) (Stolze et al. 2004). The transcription and translation of α subunit is constitutive but the stability of this protein subunit is dependent on oxygen concentration, whereas, the β subunit's transcription, mRNA and stability are oxygen independent (Wang and Semenza 1995; Kallio et al. 1998). The ARNT subunit gives transcriptional response to the xenobiotic compounds such as dioxin or benzo(a)pyrene by dimerising with aryl hydrocarbon receptors (Ahr) (Reyes et al. 1992; Reisz-Porszasz et al. 1994). In response to scarce oxygen concentration, dimerization of α and β subunits occur in the nucleus and this active hetrodimer binds to the *cis*-acting hypoxia-responsive element (HRE) in target genes with transcriptional co-activator p300/CBP (CREB-binding protein) and DNA polymerase II. After this, the transcription complex transcribes a number of genes responsible for angiogenesis, vascular tone, glycolysis, mitochondrial function, and cell survival (Ebert et al. 1995; Firth et al. 1995; Gleadle et al. 1995; Liu et al. 1995) as shown in Fig 1.

Both α and β subunits of HIF are divided into three subcategories on the basis of their genetic differences. The isoforms of HIF α subunit are: HIF-1α, HIF-2α, and HIF-3α and β isoform are ARNT1, ARNT2, and ARNT3 (Ikeda and Nomura 1997; Hogenesch et al. 2000). HIF-1α is the original HIF isoform discovered by affinity purification of oligonucleotides from Epo locus (Wang and Semenza 1995), whereas homology searches for interaction partners with HIF-1α led to the identification for HIF-2α and HIF-3α. They shares 48% of amino acid identity and 83% identity in their bHLH domain and ~70% homology in their PAS region. These findings are in conformity with in vitro functional studies as well (Ema et al. 1997; Hogenesch et al. 1997).

HIF-1α is having molecular weight of 120 kDa and found ubiquitously in most of the tissues under normoxic

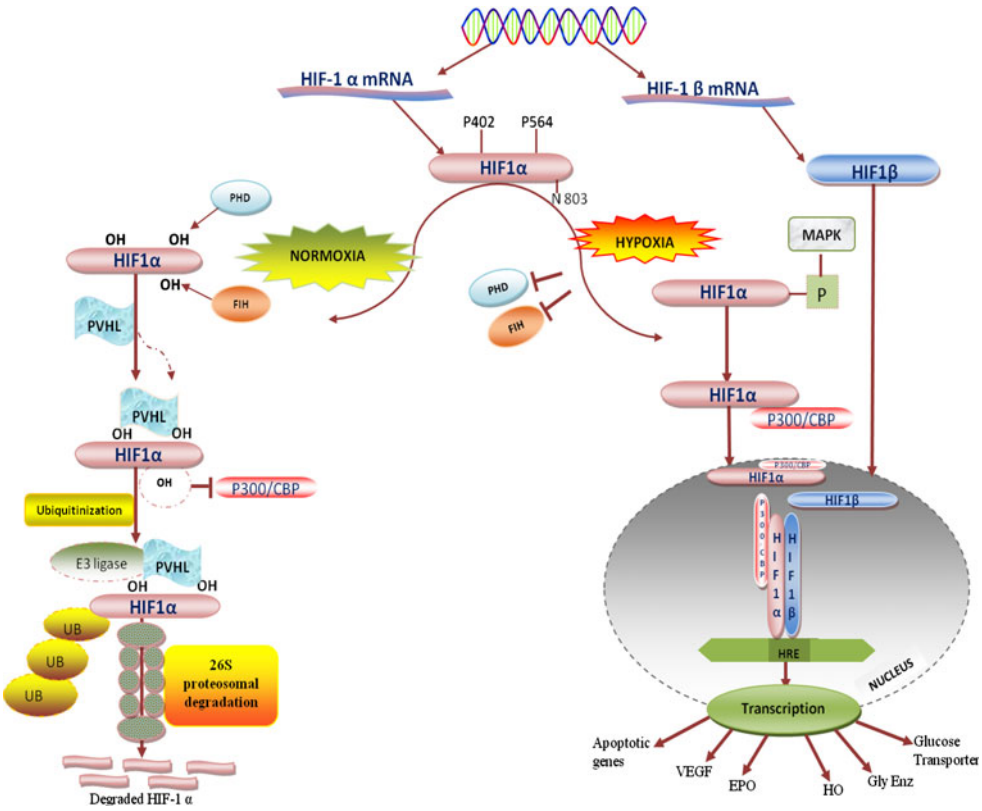

Fig. 1 A systematic representation of HIF regulation under normoxic and hypoxic conditions. Under normal condition HIF-1α subunit is hydroxylated by PHD and FIH, which makes it susceptible to the binding of pVHL leading to the recruitment of E3 ligase. The HIF-1α subunit is then subjected to ubiquitin dependent proteosomal degradation. Under hypoxia PHDs and FIH inhibit, and stabilize HIF-1, which moves into the nucleus and binds to HIF-1β and this heterodimer binds to HRE and transcribes a number of genes

condition, but degrades due to physiological insult. It plays an important role in embryonic development, as knockout mice of HIF-1α dies on day 11 during embryogenesis due to defective cardiac morphogenesis, vascularisation and neural tube closure (Kotch et al. 1999). Moreover, HIF-1 activity was induced under hypoxic conditions in all cell types tested, which suggests that HIF-1 plays a more general role in oxygen homeostasis.

HIF-2α also known as endothelial PAS domain protein 1 (ePAS1) or HIF-1α-like factor (HLF), was discovered in vascular endothelial cells and later on its presence was also reported in cells of different tissues such as kidney fibroblasts, hepatocytes, etc. (Tian et al. 1997; Rosenberger et al. 2002; Wiesener et al. 2003). The knockout studies and gene microarray techniques clearly indicates that although HIF-1α and 2α subunits have homology in their sequences but they execute diverse functions in embryonic development (Compernolle et al. 2002; Peng et al. 2000). Further HIF-2α also plays an important role in tissue homeostasis in developing and mature lung tissues. Therefore, it may not necessarily be just involved in hypoxia only, but also seems to have a distinct role in the management of hypoglycemia (Brusselmans et al. 2001).

The most distinct isoform, HIF-3α has 53 and 57% amino acid sequences similarity at N terminus with HIF-1α and 2α, respectively. The C-terminus of 3α contains 36 amino acids which shows 61% identity with the hypoxia-responsive domain 1 (HRD1) of HIF-1α. Due to the sequences similarity, it may compete with HIF-1α or 2α to make heterodimer with ARNT1 and may recognize the same DNA sequences (Gu et al. 1998). Inhibitory PAS domain protein, produced by the alternate splicing of HIF-3α transcript forms inactive heterodimer with HIF-1α (Makino et al. 2001, 2002). The functional properties of HIF-3α are still unclear, however, it is believed that HIF-3α may transcribe the Epo and glucose transporter-1 in response to hypoxia. It may also act as an offset to other HIF subunits as HIF-3α of human origin suppresses HIF-1 mediated target genes expression (Hara et al. 2001).

Regulation of HIF

The expression and activity of HIF-1α subunit is a determining factor for the biological activity of HIF-1 and is dependent on the availability of oxygen, where as the β subunit remains unaffected. HIF-1α subunit has a very short half life of about 5 min (Salceda and Caro 1997). The transcription and production of HIF-1α is not affected by oxygen concentration, but under normoxic condition, due to the degradation of HIF-1α by ubiquitin–proteasome pathway, its amount is undetectable. This is evidenced by using proteosomal pathway inhibitors, MG132 and *N*-carbobenzoxyl-L-leucinyl-L-leucinyl-L-norvalinal (Cbz-LLL;

Huang et al. 1998; Sutter et al. 2000). Moreover, hydroxylation, ubiquitination, acetylation, and phosphorylation reactions act as regulatory factors that affect the stability and transcriptional power of HIF-1α.

Oxygen-Dependent Regulation of HIF

Role of Prolyl Hydroxylase and Factor-Inhibiting Hypoxia

Prolyl 4-hydroxylase enzyme (PHD) is a member of 2-oxoglutarate-dependent dioxygenase family, which utilizes non-heme iron in the catalytic moiety. When sufficient oxygen is available, PHD hydroxylates the two proline residues pro 402 and pro 564 located in oxygen-dependent degradation domain (ODDD) of HIF-1α (Srinivas et al. 1999; Masson et al. 2001). These hydroxylated proline residues are susceptible for the binding to E3–pVHL complex, which further leads the ubiquitination of HIF-1α.

The catalytic mechanism of this enzyme can be divided into two phases: the first one is generation of hydroxyl species and the second is using these species for the formation of hydroxyproline. The one oxygen atom of dioxygen is incorporated into the 2-oxoglutarate which gives succinate and CO_2. Simultaneously ferryl ion is formed, which hydroxylates the proline residues in the substrate at the second half of the reaction by using second oxygen molecule (Hanauske-Abel and Günzler 1982). There are three different isozymes of PHD viz: PHD1, PHD2, PHD3, which are present in all mammals (Epstein et al. 2001) but in humans these PHDs are referred as HPH3, HPH2, and HPH1 (Bruick and McKnight 2001), whereas in case of *C. elegans*, these are referred as EGLN2, EGLN1, and EGLN3 (Taylor 2001). These three orthologs appear to be product of gene duplication as there is only one gene (EGL-9) in *C. elegans* (Wang and Semenza 1995) and (CG1114) in *D. melanogaster* (Lavista-Llanos et al. 2002). The PHD isozymes differ in their distribution, regulation and ability to hydroxylate HIF-1α due to the variation in amino acid sequence of the N-terminal region, whereas the C-terminal region shares homology in their sequences (Huang et al. 2002; Metzen et al. 2003).

PHD1, a 43.6 kDa protein is located in nucleus with high level of expression in the testis and low level of expression in liver, kidney, and heart. It hydroxylates both N and C-terminal of ODDD in HIF-1α. Regulation of PHD1 is not related to oxygen tension, despite it is induced by estrogen and helps in cell signaling (Seth et al. 2002), but growing evidence now also suggest that it is also regulated by oxygen as there is upregulation of PHD1 mRNA in HT22 cells as well as in brain of hypoxic mice (Khanna et al. 2006). Two isoforms of PHD1 having molecular weight 40 and 43 kDa are known to have same hydroxylation properties as that of PHD1.

PHD2 is located in cytoplasm having 46 kDa molecular weight and is present in high amount in heart with moderate amount in brain. It shows more specificity of hydroxylation toward HIF-1α than HIF-2α (Appelhoff et al. 2004). Only inhibition of PHD2 but not PHD1 or PHD3 by siRNA is sufficient to upregulate HIF-1α under normoxia in various cell lines (Berra et al. 2003). PHD2 expression is not only regulated by the change in oxygen concentration but also with all well-known inhibitors of PHDs.

PHD3 is a 27.3 kDa protein, distributed evenly in nucleus and cytoplasm (Metzen et al. 2003) and it shuttles between both the compartments. PHD3 shows higher specificity of hydroxylation toward HIF-2α rather than HIF-1α. Further PHD3 hydroxylates the CODD domain not the NODD domain (Appelhoff et al. 2004). Its expression is also regulated by hypoxia and by PHDs inhibition. The two alternative slice forms with molecular weights 24 and 17 kDa are also present. Major product is found in primary cancer tissue having PHD activity although minor product is not present (Cervera et al. 2006). A homolog of PHD3, SM-20, is a growth factor regulated gene present in muscle cells that can promote cell death in neurons (Wax et al. 1994; Freeman et al. 2003).

Regulation of HIF-1α is also governed by the factor-inhibiting hypoxia (FIH) present in the nucleus as well as in the cytoplasm. FIH, an asparginyl hydroxylase catalyzes the hydroxylation of a conserved aspargine residue Asn803 within the C-terminal TAD under normoxic condition. Further this complex associates with Von Hippel–Lindau protein bound at the N-terminal TAD to form a ternary complex that blocks CBP/p300 interaction. FIH is a Fe(II)-dependent enzyme that uses molecular oxygen to modify its substrate and serves as second oxygen sensor.

Under hypoxic condition, lack of oxygen arises due to which PHD and FIH are incapable to hydroxylate proline and aspargine residue, which further leads to the stabilization of HIF-1α. Now this dimerises with ARNT subunit in nucleus and transcribes a cassette of genes such as VEGF, Epo, glucose transporter (GLUT), and glycolytic enzymes, etc.

Polyubiquitination

The von Hippel–Lindau tumor suppressor protein (pVHL) is the substrate recognition component of the E3 ligase that ubiquitinates prolyl hydoxylated HIF-1α. Without functional pVHL, polyubiquitination and degradation of HIF-1α becomes compromised, which leads to HIF-1α accumulation and overexpression of HIF-1 target genes. The VHL gene encodes a 213-amino acid protein (pVHL),

which has two major functional domains: for the nucleation of the elongins BC/Cul 2/VHL complex α domain is required and for prolyl-hydroxylated HIF-1α recognition the β domain is required (Stebbins et al. 1999).

After the hydroxylation of proline residues, the hydroxylated HIF-1α is fitted specifically via Pro564 residue on the pocket like structure present on the surface of pVHL protein by excluding water molecule, which further ligase with E3 complex (Hon et al. 2002; Min et al. 2002). This E3 complex associates along with proteins like elongin C, elongin B, cullin-2, and Rbx1 to form a VCB-Cul2 E3 ligase complex, which is responsible for polyubiquitination of HIF-1α (Ivan and Kaelin 2001). Under normal condition, HIF-1α and target genes are constitutively expressed in renal carcinoma cells lacking functional VHL (Cockman et al. 2000). The co-immunoprecipitation studies of HIF-1α and pVHL in presence of proteosome inhibitors show a physical interaction between them. The complex of pVHL–E3 ligase is localized in cytoplasm and shuttles in between cytoplasm and nucleus.

Acetylation

Regulation of HIF-1α is done by the acetylation of side chain of lysine 532 residue present on the ODDD. This reaction is catalyzed by a protein known as arrest defective protein (ARD1).The acetylation step induces interaction between HIF-1α and pVHL and stimulates ubiquitination of HIF-1α protein.

The lysine residues for acetylation are conserved in HIF-1α and 2α but not in 3α. The binding of ARD1 with HIF-1α seems to be strong in normaxia as compared to hypoxic condition, suggesting that the activity of acetyltransferases is influenced by oxygen. Inhibition of ARD1 expression by using transfection with an antisense ARD1 expression vector significantly increased protein stability of HIF-1α by using yeast two hybrid system. This indicates that ARD1 is a negative regulator of HIF-1α expression (Jeong et al. 2002).

SUMOylation

Another oxygen-dependent regulatory process for HIF-1α expression is SUMOylation. Small ubiquitin-like modifiers (SUMO) is a reversible post-translational protein modifier, involved in various cellular processes such as nuclear transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle.

Hypoxia induces SUMO, which regulates HIF-1 expression by SUMOylation. The degradation of HIF-1α takes place by hydroxyproline independent pVHL-E3 ligase complex binding. This process is prevented by SUMO1/sentrin-specific peptidase 1 (SENP1), a nuclear SUMO protease that deconjugates SUMOylated HIF-1α to inhibit its degradation. SENP1−/− embryos exhibit severe fetal anemia owing to deficient Epo production and die mid-gestation, this provides strong evidence for a physiological role of SENP1 in regulating HIF-1α (Cheng et al. 2007). However, there is growing evidence in contrast to above, that an increase in HIF-1α SUMOylation increases the stabilization of HIF-1α (Carbia-Nagashima et al. 2007).

Oxygen Independent Regulation of HIF

Besides oxygen, HIF-1α is also regulated by an oxygen independent manner as well. Certain key factors like growth factors, cytokines and phosphorylation, do affect the HIF-1α synthesis as well as its transcriptional activity and these have been discussed in the succeeding lines.

Phosphorylation

Direct phosphorylation or mitogen-activated protein kinase (MAPK) plays an important role in regulation of HIF-1α. Phosphorylation does not affect its stability or binding affinity to DNA, and surprisingly it increases the transcriptional activity of HIF (Richard et al. 1999). The phosphorylation sites on HIF-1α and 2α is threonine at positions 796 and 844, respectively. Whereas phosphorylated HIF-1β subunit preferentially binds to the phosphorylated HIF-1α (Suzuki et al. 2001).

Hypoxia-Associated Factor

Hypoxia-associated factor (HAF) is also known as squamous cell carcinoma antigen recognized by T-cell (SART1), is expressed in proliferating cells as a nuclear protein (Shichijo et al. 1998). It is a complex essential for the assembly of mature splicesomes, having the ability to bind the promoter of the Epo, which, contributes to its hypoxia inducible characteristics. It is the mediator of new mechanism of HIF-1α degradation which is independent of pVHL and oxygen.

HAF shows increased level of HIF-1α which is independent of pVHL or oxygen as demonstrated by the knockout studies, whereas there is no change found in HIF-2α level. This suggests that HAF is specific for HIF-1α only. The HAF SiRNA also increases the endogenous level of HIF-1α in normoxic condition. HAF mediates oxygen independent HIF-1α degradation pathway that is complementary to oxygen dependent pathways mediated by pVHL, thus providing an additional level of control that allow the HIF regulation under diverse condition. HAF has a dual nature as on one side it inhibits the HIF-1α signaling

by degradation and on other side it promotes the down regulation of HIF-1α key gene. HAF also serves as a potential target for SUMOylation (Vertegaal et al. 2004).

HSP90 versus RACK1

The oxygen pVHL independent regulation of HIF-1α is also mediated by the molecular chaperon heat shock protein 90 (HSP90) and receptor of activated protein kinase (RACK1). The binding of HSP90 to HIF-1α provides proper folding and stability under normoxic condition. It binds to the bHLH-PAS domain of HIF-1α and this has been confirmed by the co-immunoprecipitation, using HSP90 antibodies (Minet et al. 1999). Under hypoxic condition, the dimer of HSP90 and HIF-1α dissociate and HIF-1α translocate toward the nucleus. Disruption of HSP90 function by the HSP90 inhibitor geldanamycin promotes HIF-1α degradation via a novel, oxygen-independent E3 ubiquitin ligase which diminishes transcriptional activity of HIF-1α (Isaacs et al. 2002).

The RACK1 competes with HSP90 for binding with HIF-1α. It dimerises with HIF-1α and recruits elongin C and other component of the E3 ligase complex for ubiquitination and degradation. RACK1 interact with residues 531–826 of HIF-1α and its strongest interaction is with the amino-terminal half of HIF-1α and which is sufficient to promote HIF-1α degradation (Liu et al. 2007). Loss of function of RACK1 by RNA interference increases HIF-1α protein level and promotes HIF-1 downstream target genes expression. The calcineurin, a calcium and calmodulin-dependent regulatory factor for RACK1 pathway interestingly mediates the dephosphorylation of RACK1 and inhibits dimerization of HIF-1α-RACK1 leading to stabilization of HIF-1α (Liu et al. 2007).

Further signaling pathways like phosphatidylinositol 3-kinase (PI3K), AKT protein kinase B, mTOR regulate both HIF-1α transcription as well as translation in a tissue-specific manner. Under hypoxia, growth factors, cytokines, and other signaling molecules stimulate HIF-1α synthesis via activation of the PI3K or MAPK pathways. These pathways can be activated by signaling via receptor tyrosine kinases, non-receptor tyrosine kinases or G-protein-coupled receptors. PI3K activates the downstream serine/threonine kinases AKT and mammalian target of rapamycin (mTOR). This mTOR phosphorylates the translation initiation factors, which increases the rate at which a subset of mRNAs (including HIF-1α mRNA) are translated into protein. It is well established that AKT is responsible for the neuronal survival, neurogenesis, angiogenesis and synaptic transmission. Since the wortmannin, an inhibitor of PI3K/AKT pathway inhibits the transcription and translation of HIF1 and VEGF in oxygen–glucose deprived cell culture (Zhang et al. 2009). Phosphatase/tensin homolog deleted on chromosome 10 (PTEN), a tumor suppressor gene also inhibits the AKT signaling, which leads to the inhibition of HIF-1 and its related gene, whereas constitutively active AKT stimulates this process (Zundel et al. 2000). Several growth factors, most notably insulin-like growth factor-2 (IGF2) and transforming growth factor-α (TGF-α), are also HIF-1 target genes (Feldser 1999,Krishnamachary 2003). Binding of these factors to their associated receptors, the insulin-like growth factor 1 receptor (IGF1R) and epidermal growth factor receptor (EGFR), respectively, activates signal-transduction pathways that lead to HIF-1α expression and cell proliferation. Glycogen synthase kinase 3 (GSK3), consists of two isoforms (alpha and beta), is phosphorylated and inactivated by AKT. GSK3beta overexpression results in prolyl-hydroxylation and pVHL-independent HIF-1α ubiquitylation and proteosomal degradation via GSK3beta-mediated HIF-1α phosphorylation (Flugel et al. 2007). Besides the classical pattern of regulation of HIF that is oxygen dependent, the oxygen independent regulation do provides a new avenue to discover more precisely physiological significance of HIF.

Role of HIF in Cerebral Ischemia

Following cerebral ischemia, HIF helps neuronal cells to cope up with such adverse situation and generates some adaptive responses at transcriptional level. During hypoxia, HIF triggers an increase in expression of several genes involved in angiogenesis, glycolysis, glucose transport, vascular tone and mitochondrial function, which collectively initiate cell survival mechanisms under such adverse conditions. Apart from it, HIF is also involved in the activation of several genes such as p53, BNip involved in apoptosis.

Recently, there have been extensive efforts to explore the beneficial role of HIF in several neurological diseases such as cerebral stroke and Alzheimer disease (AD). In response to inadequate supply of oxygen, HIF-1 accumulation is altered. The very first time HIF-1 mRNA expression was observed in the brain of rats and mice subjected to hypoxia for 30 to 60 min. There was an increase in mRNA encoding HIF-1α in case of permanent focal cerebral ischemia (Wiener et al. 1996). A 15–17-fold increase was observed in HIF-1α expression after 7.5 h of ischemia which remained consistent up to 19–24 h in the penumbral region (Bergeron et al. 1999). Further 1.8–2.5-fold increase in both HIF-1α and HIF-1β proteins, 20 h post-reperfusion was also observed (Sharp et al. 2001).

There is growing evidence to suggest that HIF-1α accumulation starts as early as 1 h of recovery and persist

up to 7 days after transient global ischemia, induced by cardiac arrest and resuscitation (Chavez and LaManna 2002). HIF-1α protein accumulates significantly in the rat brain during continuous hypobaric hypoxia up to 14 days and which returns back to basal level on 21st day (Chavez et al. 2000). In case of focal cerebral ischemia, the induction of HIF-1α and transcriptional activation of its target genes occurs in the ischemic penumbra after permanent focal cerebral ischemia (Bergeron et al. 1999; Halterman et al. 1999). There is an increase in HIF-1α level, which initiates supply of blood, oxygen, and nutrients to penumbra region in mice injected with HIF-1α DNA lacking an ODDD (Vincent et al. 2000; Trentin et al. 2006). Recently, Baranova et al. (2007) performed middle cerebral artery occlusion (MCAO) in knockout mice and demonstrated a biphasic activation of HIF in the neurons. Its expression being maximum at 6 h, which declined at the end of day one and again it increased from day two and remained elevated till 8 days of recovery. Moreover brain-specific knockout of HIF-1α exhibits neuroprotection against hypoxic ischemic damage (Helton et al. 2005).

A recent study by Zhou et al. (2008) suggests that a natural metabolite of estrogen 2-methoxyestradiol (2ME) inhibits the induction of HIF-1α, which decreases the neuronal cell survival in case of global ischemia in rat. On the other hand, there are several studies in which preconditioning and some inhibitors of PHDs show an increase in HIF-1α expression which exhibits beneficial as well as some detrimental effect too. This can be explained by a recent observation that HIF-1α shows biphasic expression pattern, the early phase expression facilitated apoptosis, but late-phase expression increased cell survival (Yeh et al. 2011).

Protective Face of HIF

In response to lower oxygen concentration, HIF-1α accumulates and triggers the expression of several genes which initiate angiogenesis, erythropoiesis, vascular tone maintenance, mitochondrial function, cell survival following ischemic/hypoxic injury. There is growing evidence which shows that activation of HIF-1α offer protection in case of cerebral ischemia. Preconditioning or inhibition of PHDs is well known to trigger the accumulation of HIF-1α and exhibits protection against cerebral stroke damage (Giusti and Plazas 2011). Preconditioning of hypobaric hypoxia provides profound protection against focal cerebral ischemia in mice (Bernaudin et al. 2002). The pre-treatment with $CoCl_2$ and desferoxamine (DFO) the HIF-1α inducer exhibit protection against cerebral ischemia by 75 and 56%, respectively (Sharp et al. 2001, 2004). The preconditioning with $CoCl_2$ increases the accumulation of HIF-1α which exerts its prosurvival role via upregulation of a sodium–calcium exchanger-1 (NCX1) (Valsecchi et al., 2011). DFO and dihydroxybenzoic acid (DHB) prevent neuronal damage against permanent cerebral ischemia by inhibiting PHD, which elevates HIF level (Siddiq et al. 2005; Li et al. 2008). Recent studies also suggest that dimethyloxallyl glycine (DMOG), a PHD inhibitor provides protection against permanent and transient focal cerebral ischemia by the induction of HIF (Nagel et al. 2011). Therefore, more precise molecular studies on mechanisms of HIF-1α in cerebral ischemia on preconditioning will shed new light on the role and modulation of HIF-1.

HIF-Regulated Tools for Neuroprotection

The HIF-1α down regulated genes responsible for protection in cerebral ischemia/hypoxic injury has been discussed in the following lines:

Erythropoietin

Semenza and Wang (1992) reported that Epo was the first target gene for HIF-1 and it is still one of the best-characterized gene activated by reduced oxygen level (Wenger 2002). There is an increase in in vivo expression of Epo receptors, indicating the increased sensitivity of neuronal cells toward Epo during ischemic condition (Bernaudin et al. 1999; Sadamoto et al. 1998; Chin et al. 2000). Inhibition of hypoxia-induced apoptosis and ischemic tolerance in astrocytes by Epo may be mediated by HIF-1α as evidenced by various in vitro and in vivo ischemic preconditioning models (Ruscher et al. 2002; Prass et al. 2003). Marti and Risau (1999) suggested that Epo might leads to the formation of new blood vessels, which may further increase the blood flow as well as oxygen concentration that neutralizes the damaging effect of hypoxia on neuronal cells. Moreover, Epo also protects hippocampal neurones from delayed cell death in a global cerebral ischemia model in gerbils (Sakanaka et al. 1998). Further, the programmed cell death is also inhibited by increased Epo expression in the ischemic penumbra following MCAO in rats as well (Siren et al. 2001). An increase in Epo mRNA level in the penumbra region by treatment with DFO induces tolerance against focal cerebral ischemia. This increase is also associated with the activation of HIF-1 DNA binding (Bernaudin et al. 2000; Prass et al. 2003).

Vascular Endothelial Growth Factor

The endothelial cell growth, differentiation, and most important its survival is specifically regulated by vascular endothelial growth factor (VEGF) (Risau 1997).

The human and rodent VEGF genes contain in HRE at 5¢-flanking region (Liu et al. 1995) and their activation are triggered by the HIF-1α binding (Forsythe et al. 1996). During cerebral ischemia, the damaged tissue tries to increase oxygen delivery by the induction of angiogenesis via VEGF. This hypothesis is supported by the fact that there is an increase in the number of microvessels in the infarct area of brain at various survival times in stroke patients as compared with normal hemisphere (Krupinski et al. 1994).The VEGF and its receptors are upregulated in the brain by HIF-1α and 2α during 6 to 24-h post-ischemia. There is upregulation of HIF-1α and down regulation of VEGF gene by preconditioning the normobaric hypoxia, which induces tolerance against focal permanent cerebral ischemia (Bernaudin et al. 1999). It is not only the expression of VEGF but also the other survival factors like angiogenic factor such as angiopoietin-2 and insulin growth factor (IGF-2) and their receptors induced by hypoxia are regulated by HIF-1α as shown by HIF-1α gene therapy. The role of angiopoietin induced by HIF-1α in case of cerebral ischemia is yet to be established. Matrix metalloproteinase 2 (MMP2), cathepsin D (CATHD), and keratin (KRT), are the selected targets of the HIF-1α transcription complex involved in the angiogenesis to facilitate blood supply in the penumbra. It is believed that increased HIF-1α levels lead to proportional increase in these proteins (Dery et al. 2004; Lakhani et al. 2003).

Vascular Tone

During cerebral ischemia, the disturbance in vascular tone is one of the major consequences, which results in neuronal cell death. The inducible nitric oxide synthase (iNOS) is one such gene which is regulated by HIF-1 and helps the cells to cope up with these adverse conditions. In case of permanent cerebral ischemia, inactivation of iNOS expression is mediated via HIF-1α in the ischemic core as well as in the surrounding areas of core region, which helps the neuronal cells to regain their viability (Matrone et al. 2004). The involvement of HIF-1α in either promotion or protection against cell death depends on its context, which is very similar to that of iNOS, as iNOS knockout mice have reduced brain injury following focal cerebral ischemia. But in another study HIF-1α increases the expression of nNOS and iNOS which show significant protection in anemic rats (McLaren et al. 2007). A recent study also suggests that there is an upregulation of eNOS by the treatment with a PHD inhibitor DMOG, which affords neuroprotection against permanent and transient focal cerebral ischemia (Nagel et al. 2011).

Hemo oxygenase (HO) is an antioxidant enzyme and its activation is regulated by HIF-1α, even its modest expression shows neuroprotection in case of cerebral ischemia by its two by-products, one being carbon monoxide, which acts as anti-apoptotic agent and second one is bilirubin a potent antioxidant (Vulapalli et al. 2002; Akamatsu et al. 2004). The protective effect of HO mainly depends upon the severity of ischemic insult. A recent study by suggests that upregulation of HO1 by HIF-1α promotes cell survival and angiogenesis and helps in collateral vessel development in a tissue-specific manner (Loor and Schumacker 2008).

Glycolytic Enzymes

Since ischemic disturbance in cerebral blood flow results in high oxygen and glucose starvation. Glucose is the only source of ATP generation by the neuronal cells in brain and needs to be supplied to meet its required amount. Hence, to overcome this unfavorable situation the neuronal cells have to fulfill the glucose demand by increasing the activity of glycolytic enzymes and GLUTs, which enhance the concentration of glucose and thereby ameliorate neuroprotection. Glucose transport and glycolytic flow as a result of HIF-1α activation by hypoxia has been linked to cell survival (Lawrence et al. 1996). It has been shown that there is an HIF-1α associated increase in GLUT1 expression, which starts from 7.5 h and last up to 24 h after permanent MCAO (Bergeron et al. 1999). The study also provided evidence that there is an increased expression of HIF-1α dependent glycolytic enzymes in cingulate/retrosplenial cortex following permanent MCAO. There is another study, which also showed the upregulation of enolase gene by the inhibition of PHD responsible for HIF-1α degradation also offered neuroprotection in permanent MCAO (Siddiq et al. 2005). The increase in level of lactate dehydrogenase (LDH), enolase1 and other glycolytic enzymes by treatment with DFO, an inhibitor of PHD confirmed protection against oxidative stress induced apoptosis (Zaman et al.1999).

Necrosis and apoptosis are the main pathways of cell death in cerebral ischemia. HIF-1α may show its protection by the inhibition of cytochrome c release, caspase activation or PARP cleavage (Li et al. 2004; Piret et al. 2004; Sasabe et al. 2005). Chong et al. (2002) suggesting that inhibition of cytochrome c release, caspase activity and activation of AKT1 play an important role in preventing the DNA fragmentation and cell injury. In some cases, the HIF-1α restricted the apoptosis by the suppression of p53 genes too but this is totally dependent upon the severity or duration of hypoxia/ischemia (Li et al. 2004).

Brain-derived neurotrophic factor (BDNF) shows neuroprotection in case of cerebral focal and global ischemia (Schabitz et al. 1997; Larsson et al. 1999). Recent study also show that HRE regulates the expression of adenovirus-mediated BDNF, constructed by adenoviral vector with

five copies of HRE found in VEGF gene responsible for the regulation of BDNF in hypoxia (Shi et al. 2009). These findings suggest that HIF-1 acts as sensor to express protective genes in case of cerebral I/R injury. PHD inhibition may also be beneficial for the treatment of cardiovascular diseases by inhibiting renin-angiotensin system via AT_1R down regulation (Matsuura et al. 2011).

In addition, hypoxia up-regulated mitochondrial movement regulator (HUMMR), a HIF-regulated gene which plays an important role in mitochondrial transport in neuron and astrocytes. It regulates the movement of mitochondria in axons and in absence of it the mitochondria are reduced in axon and motile mitochondria moves toward retrograde direction. So it may play a critical role to overcome injury caused by mitochondrial dysfunction (Li et al. 2009). Neuroglobin (Ngb), an oxygen-binding heme protein also plays an important role in survival of neurons during ischemia/hypoxia injury. Studies have proved that hypoxic preconditioning or DFO or $CoCl_2$ treatment enhance the expression of HIF-1α and Ngb, which afforded protection against focal cerebral ischemia in mice (Bergeron et al. 1999; Siddiq et al. 2005; Sun et al. 2001).

Detrimental Face of HIF

Although on one side HIF do play a significant role in neuroprotective mechanisms but other side it also behaves as apoptotic inducer in cerebral stroke. This is substantiated by the studies of Jiang et al. (1996) as HIF-1α in primary culture from neonatal mouse with the help of dominant negative form of HIF, which, inhibited the HIF-1 activity, leading to reduced neuronal damage in response to glucose and oxygen deprivation. This is further supported by the observation that HIF-1α is responsible for induction of apoptosis on deprivation of glucose and oxygen (Carmeliet et al. 1998). Among number of genes, the p53 and p21 involved in cell cycle control are also regulated by HIF-1α. The p53 protein is concerned in regulating apoptosis, induced by hypoxia through regulation of apoptosis related genes such as PERP, NOXA, PUMA, and Bax (Schuler and Green 2001). Inhibition of NOXA by antisense nucleotide reduces cell death induced by hypoxia in ischemia associated with decrease in HIF-1 expression. It means that NOXA activation depends on HIF-1 in hypoxia and is involved in hypoxic cell death (Kim et al. 2004). The apoptosis induced in cortical neurons associates with upregulation of HIF-1α and prevents degradation of p53 protein suggesting the detrimental role of HIF-1 (Banasiak and Haddad 1998). The upregulation of HIF-1 is also associated with the increase in LDH (Bergeron et al. 1999) and this increase correlates with increased production of lactate, which is responsible for negative consequences after cerebral ischemia.

Cerebral ischemia initiates the inflammatory process cells, which contributes to the pathogenesis of the disease. The iNOS plays an important role in inflammation during cerebral ischemia and is one of the target genes of HIF-1, responsible for its proapoptotic property (Iadecola et al. 1996; Jung et al. 2000). Recent studies by Chang et al. (2009) demonstrated the puerarin, an isoflavonoid offered neuroprotection by significant decrease in HIF-1α levels and consequently affecting iNOS expression following ischemic insult. Inflammatory cytokines such as IL-20, IL1β are also regulated by HIF-1. The very evidence that IL-20 is involved in the pathogenesis of ischemic stroke with the increased expression of HIF and inhibition of HIF-1 by 2ME provided neuroprotection by decreasing the IL-20 expression (Chen and Chang 2009). Inhibition of HIF-1α by D609 tricyclodecan-9-ylxanthogenate triggers the down regulation of VEGF, cleavage of caspase 3 as well as BNIP3 which are the causative agent for cell death in case of cerebral ischemia. The D609 inhibits the HIF-1α via inhibiting the biosynthesis of phosphatidic acid (Temes et al. 2004). BNIP and BNIP3, members of Bcl2 family proteins are activated in hypoxic condition. Bruick (2003) suggested that BNIP3 contains HRE in their promoter site which confirms that it is a direct target for HIF-1 and its activation leads to ischemic and myocardial cell death by membrane depolarization (Kubasiak et al. 2002; Regula et al. 2002) and MPTP opening (Mellor and Harris 2007).

After I/R injury, brain edema is a critical problem which increase the mortality. Higashida et al. (2011b) provided evidence that HIF-1α is also involved in edema formation after stoke by increasing the intracellular glycerol concentration, associated with the increase expression of aquaporins (AQP-4 and AQP-9). Inhibition of HIF-1 by 2ME suggests that HIF-1α serves as an upstream regulator of cerebral glycerol concentrations and brain edema via a molecular pathway involving AQP-4 and AQP-9. Pharmacological blockade of this pathway in stroke patients may provide novel therapeutic strategies. Further, these workers also supported that inhibition of HIF-1α reduced brain edema formation and BBB disruption via a molecular pathway cascade involving AQP-4 and MMP-9 in traumatic brain injury (Higashida et al. 2011a).

It is not possible to postulate the reason for the positive and negative face of HIF in pathophysiology of cerebral ischemia. Several studies do suggest about the dual face HIF-1α, which is totally dependent on the severity or duration of the ischemic or hypoxic insult. A 30-min transient focal ischemia (Baranova et al. 2007) versus 75-min bilateral common carotid artery occlusion Helton et al. (2005) support dual character of HIF. Whereas some recent observations suggest that HIF-1α exhibits cell-specific dual nature as HIF-1α expression in neuron and glial cells had different outcomes after an ischemic brain injury.

The loss of HIF-1α function in neurons reduced neuronal viability after hypoxia, whereas the selective loss of HIF-1α function in astrocytes protected neurons from hypoxia-induced neuronal death (Vangeison et al. 2008). Study by Yan et al. (2011) also support that HIF-1 function differently in different cells depending on the functions of the proteins coded by its downstream genes in the specific type of cells. Therefore, HIF-1 induction, accumulation under ischemic condition in different neuronal cell type may be responsible for its dual character and the factors affecting its regulation might open a new therapeutic window for cerebral stroke. During I/R injury, reactive oxygen species (ROS) and heat shock proteins affect the HIF-1 regulation and might provide new approaches for the effective treatment of cerebral stroke.

HIF and Oxidative Stress

In previous section it has been highlighted that HIF-1 has a dual face, hence regulation of HIF-1 level is most important factor for the future of the hypoxic cells. Regulation of HIF-1 level occurs both in oxygen dependent and independent manner. An increased generation of ROS is one of the main causative agents in I/R injury. Mitochondria and NADPH oxidase are the two main sources for the generation of ROS. In physiological condition, ROS acts as a signaling molecule during cell growth and differentiation. But under several pathophysiological states ROS may be serving as putative signaling molecule, which stimulates various cell death pathways.

Mitochondria a main source of ROS production and its dysfunction leads to neuronal death in case of cerebral ischemia. It is well established that mitochondrial complexes I, II, III are responsible for ROS production, and this may play an important role in the stabilization of HIF-1 in hypoxic injury. Various inhibitors of mitochondrial electron transport chain such as rotenone and stigmatellin inhibit HIF-1 expression (Agani et al. 2000; Chandel et al. 2000). The free-radical scavengers *N*-*t*-butyl-α-phenylnitrone, edaravone also inhibit the protective action of hypoxic exposure by the inhibition of ROS (Rauca et al. 2000; Zhang et al. 2011). ROS induced by hypoxic preconditioning increases the HIF-1α and its downstream gene Epo expression by lowering its threshold of activation and hence shows protection in neuronal cells. Superoxide dismutase (SOD1), an antioxidant enzyme added in neuronal culture due to low level of ROS exhibited low level of HIF-1 expression (Liu et al. 2005). The exogenous H_2O_2 induces the HIF-1 expression in normoxic cells by increasing the ROS and hypoxic induction of HIF can be blocked by ROS scavengers (Guzy et al. 2005; Mansfield et al. 2005). Sanjuan-Pla et al. (2005) suggested that mitoubiquinone, a mitochondria- targeted antioxidant, destabilized HIF-1α protein and ablated ROS generation induced by hypoxia and hence terminated the inhibition of HIF-1 transcriptional activity. In addition, the upregulation of HIF-1 and its downstream gene such as VEGF, Epo, and glycolytic enzymes exhibited reduced level by treatment with antioxidant pyrrolidine dithiocarbamate or ebselen (Chandel et al. 1998).

Recent studies have suggested that both mitochondria and ROS enhance the protective role of HIF. Low level of exogenous H_2O_2 protects the primary cortical neurons against ischemia by increasing HIF-1 expression (Chang et al. 2008). The preconditioning induced by short period of glucose–oxygen deprivation and H_2O_2 seems to offer neuroprotection (Furuichi et al. 2005). There is growing evidence which suggests that the increase of mitochondrial ROS may prevent the hydroxylation of HIF-1, which provides upregulation of HIF and its downstream genes in hypoxic condition (Guzy et al. 2005; Mansfield et al. 2005).

There is another school of thought that an increase in ROS destabilizes the HIF-1 expression in ischemic neurons. The ROS scavenger *N*-acetyl cysteine upregulates HIF-1 expression but an oxidative stress inducer L-buthionine sulfoximine downregulates the HIF expression in hypoxic neuronal cells. Interestingly, such inverse relationship between HIF-1α and H_2O_2 level was also observed by Guo et al. (2008). Further, Xanthine or xanthine oxidase also downregulates the HIF-1α level by generating superoxide radicals in explants of human pial arteries (Wellman et al. 2004).

Recent studies have suggested that HIF-1 inhibits the ROS generation in hypoxic condition mediated by upregulation of PDK1, a target for HIF-1 (Kim et al. 2004). The 2,3-dimethoxy-1,4-naphthoquinone, a superoxide generator offers destabilization of HIF-1α protein by increasing PHD activity under hypoxic condition and this effect can be reversed by ROS scavengers (Callapina et al. 2005). PHD inhibitors DHB, DMOG inhibit the cell death by increasing HIF-1 expression in nerve growth factor (NGF) deprived cell culture owing to the inhibition of ROS (Lomb et al. 2009). A probable mechanism of HIF regulation by ROS has been proposed by us in Fig. 2.

The possible mechanism by which ROS destabilizes the HIF-1 is not yet well characterized. It may be that ROS acts as oxygen which activates the PHD, responsible for HIF-1 α degradation (Haddad 2002; Liu et al. 2004). Another possibility could be that HIF-1α is oxidized by an increase in ROS levels and the oxidized protein is most likely degraded by ubiquitin–proteasome system. There is another proteosome degradation system, 20S, which may be upregulated by the ROS. The oxidized protein may be degraded by 20S proteosome pathway under oxidative stress (Shringarpure et al. 2003; Ding et al. 2006).

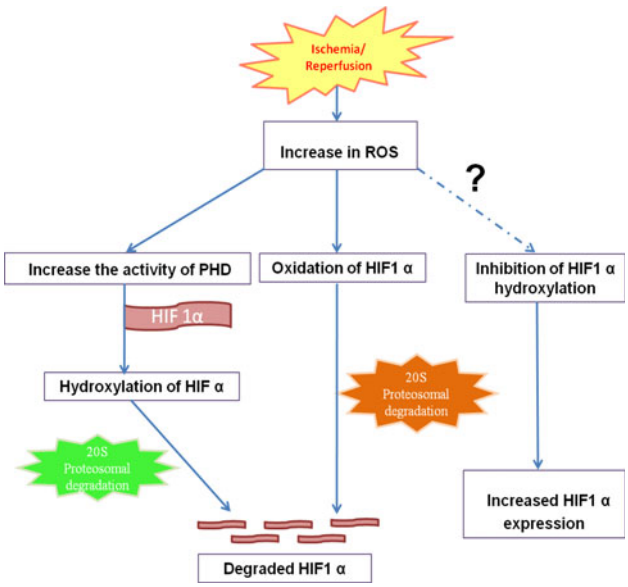

Fig. 2 A proposed mechanism for regulation of HIF by ROS during cerebral ischemia

Sometimes HIF-1 itself regulates its degradation by feedback mechanism via acetylation (Demidenko et al. 2005).

All these conflicting results raise unresolved questions that how ROS modulate the degradation or upregulation of HIF under hypoxic condition in cerebral ischemia and the mechanisms by which ROS level affects the HIF-1 stability. There are several reports which show the effect of mitochondrial ROS on HIF-1, whereas NADPH oxidase is another well-known ROS generator in cerebral ischemic reperfusion injury. How does NADPH oxidase modulate the HIF-1 and PHD activity is yet to be established and further how does ROS generated by NOX modulates HIF-1? The unsolved mystery, which is commonly asked that how severity of ischemic insult and reperfusion period are the deciding factor for ROS generation, which may ultimately regulates the dual behavior of HIF, still awaits outcome of future researches.

HIF and Heat Shock Proteins

Molecular chaperones are responsible for the folding and activation of various transcription factors and protein kinase. HSP90, an ATP-dependent chaperon, is regulated by many transcriptional factors such as a basic helix loop helix protein, bHLH Per-ARNT-Sim (PAS) factors Sim and Ahr (Wang and Semenza 1995). It is one of the most common proteins found in the eukaryotic cytoplasm. HSP90 prevents the aggregation of unfolded protein due to the oxidative stress, heat shock or ischemia. HSP90 binds to its substrate protein and leads the confirmational changes in both, which are due to ATP hydrolysis by intrinsic factor ATPase (Pratt and Toft 2003). HIF-1 belongs to HLH protein and it binds to the HSP90 with its PAS domain.

Under normal condition, HSP90 binds to HIF-1 and prevents misfolding or aggregation but under hypoxic condition, this association is broken down and HIF-1 translocate to the nucleus and forms active HIF (Suzuki et al. 2001; Katschinski et al. 2002). Several studies showed that geldanamycin and 17-allylaminogeldanamycin (17-AAG) inhibitors of HSP90 promote the degradation of HIF-1α in renal carcinoma cells, which lack VHL (Isaacs et al. 2002; Mabjeesh et al. 2002).

HIF-1α requires the unmask specific domain (NLS) of C-terminal for translocation toward the nucleus. In normoxic condition, this domain is covered and HIF-1 is found in cytosol. HSP90 when binds to the HIF-1, also covers the specific NLS domain, therefore HIF-1-HSP90 dimers does not translocate to the nucleus. This view is also supported by the co-immunoprecipitation studies (Minet et al. 1999). Under hypoxic condition, this dimer is broken down, and due to unmasking of RKRK motif in the C-terminal domain of HIF-1, it translocate to the nucleus (Kallio et al. 1998) but under hypoxic condition, HIF-1 is activated and this association breaks up. Factors which are involved in the HIF-1 activation and dissociation of HIF-1-HSP90 are still elusive. An HSP90 inhibitor radicicol and its derivative, KF58333 inhibits the expression of hypoxia-induced VEGF. It also shows that the inhibition of HSP90 binding does not reduce the expression of HIF-1 or its translocation toward the nucleus, but it reduces the binding affinity of HIF-1/ARNT dimer to the HRE region on DNA, which suggests that HSP90 binding results in conformational changes, and helps HIF-1/ARNT dimer binding to the HRE (Hur et al. 2002). Trichostatin A reduces the HSP90-HIF interaction by increasing acetylation of HIF-1α which is responsible for the proteosomal degradation of HIF-1α in oxygen independent manner (Kong et al. 2006). There is growing evidence, which suggests that HSP70 expression is also evoked in response to hypoxia (Kim et al. 2001; Lee et al. 2001). These HSP proteins are regulated by several protein kinases such as PI3K. Because an active PI3K/Akt is required to prevent the oxygen independent degradation of HIF with the help of HSP70 and/HSP90 in RCC4 cells (Zhou et al. 2004).

Further elucidation that breaks down HSP90-HIF-1 complex in hypoxic condition and regulation of HIF-1by HSP90 may provide a new avenue, which may offer new approaches for treatment of cerebral ischemia and other neurodegenerative disorders.

Summary

HIF-1, an essential transcriptional factor for oxygen homeostasis shows an adaptive response in order to cope

up with the pathological condition arising due to hypoxia. It upregulates various genes which show protective responses against cerebrovascular diseases and neurodegenerative disorders. Recent studies do suggest that HIF could serve as a novel therapeutic target for cerebral ischemia. The stroke is the second leading cause of death world over, and there are continuous efforts to ascertain the novel classes of therapeutic molecules to treat cerebral stroke in humans. During ischemic condition major damage occurs due to the free-radicals generation, leading to oxidative stress and neuronal death. Therefore, early efforts were directed to discover new therapeutic molecules having potential anti-oxidant effect. The attempt was not clinically very fruitful as the disease is multifactorial and combating single pathway may not yield very encouraging results. Now, the adaptive endogenous pathways may open a new path to reveal the underlying mechanisms related to the ischemic injury. HIF is a transcription factor responsible for the upregulation of various genes responsible for cell survival in ischemic condition. Therefore, extensive research on role and regulation of HIF-1 in ischemic injury may provide some new insights for the development of better therapeutic approaches for the treatment of cerebral stroke.

HIF plays both a beneficial as well as detrimental role in hypoxia. The severity of ischemia/hypoxia may also play a deciding factor for its dual face. HIF-1 induces expressions of a wide range of genes and functions of these genes may depend on the specific cell types as HIF-1 may function differently in different cells. Therefore, the future studies need to focus on specific cell types and cellular targets to better understand the role of HIF-1 in stroke as well as other pathological conditions. Moreover, specific PHD inhibitors appear as a potential target and efforts are on to develop new therapeutic moiety for ischemic condition. ROS are key factors in regulating HIF-1 in ischemic brain. The discovery of potential antioxidant molecules which prevent the neuronal death by regulating this pathway will also help to develop a new approach for the treatment of human stroke. Regulation of HIF-1 by HSP90 opens up new avenues in understanding its crucial role in cerebral ischemia. Thus the field of HIF-1 appears to be a novel therapeutic target for the management of stroke and other neurodegenerative diseases in humans.

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