REVIEW ARTICLE

Hypoxia-inducible Factor: Basic Biology and Involvement in Cardiovascular Pathology

Valeriya A. Soldatova, Alexei N. Demidenko, Vladislav O. Soldatov, Alexei V. Deikin, Olga Yu. Bushueva, Olesya A. Puchenkova

Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod - 308015, Russia

Abstract

The hypoxia-inducible factor (HIF) system plays a dominant role in the regulation of oxygen balance. There are three forms of HIF protein, whose function is being actively studied by the medical and biological environment. HIF-1 consists of α- and β-subunits. The α-subunit is destroyed under normoxia by oxygen-dependent enzymes such as prolyl hydroxylase domain and factor-inhibiting HIF-1. In hypoxic condition, a complex of HIF-1α and HIF-1β forms a transcription factor that controls the expression of several hundred genes. HIF-1 activity is primarily aimed at reducing mitochondrial respiration, activating glycolysis, and increasing the oxygen capacity of the blood and organs vascularization. Under hypoxic condition, HIF-1 reduces the activity of mitochondria, which prevents the generation of reactive oxygen species and protects the cells. On the transgenic animal models, as well as in the study of cardiac tissue biopsies in patients with myocardial infarction, the protective role of HIF in ischemic myocardial injuries was confirmed. During hypoxia of the brain, HIF plays an ambiguous role. There is evidence that astrocytic HIF-1 plays a negative role, and neuronal HIF-1 causes neuroprotection during hypoxia. The structure of HIF has a relatively low variability even in interspecific comparison. Molecular epidemiological studies conducted to date reveal a close relationship between the polymorphism of the HIF system genes with a wide range of cardiovascular, inflammatory, and oncological diseases. The study of the HIF system can contribute to the discovery of new targets and methods of pharmacological effects for the treatment of cardiovascular, oncological, rheumatological, and endocrinological pathology.

Key words: Cardiovascular pathology, hypoxia-inducible factor, hypoxia, oxygen

INTRODUCTION

Being one of the most common causes of cell alteration, hypoxia plays a key role in the pathogenesis of most diseases and critical conditions. This is associated with the presence of conservative O_2 balance regulation systems with pleiotropic, mutually overlapping effects. At the moment, the dominant role in the regulation of O_2 balance is assigned to the hypoxia-inducible factor (HIF) system, which includes three molecules: HIF-1, HIF-2, and HIF-3. HIF-1 and HIF-2 show to a certain extent similar activity.[1] HIF-3 has a structural similarity in some domains but acts as a negative regulator of two other family members.[2]

MOLECULAR BIOLOGY OF HIF-1

HIF-1 is a heterodimeric protein (122–132 Kd), consisting of α- (chromosome 14) and β- (chromosome 1) subunits. Both subunits have a helix-loop-helix motif and Per-Arnt-Sim (PAS) domains with DNA-binding ability.[3,4] An important feature of the α-subunit is instability and cytoplasmic localization. The β-subunit is permanent and localized in the nucleus. In addition to those mentioned, the a-subunit contains two more functionally significant domains: Oxygen-dependent degradation (ODD) domain and transactivation domain (TAD), which influence on its activity and existence duration. In the normoxia, the enzymes such as prolyl hydroxylase domain (PHD) and factor-inhibiting HIF-1 (FIH-1) were attached to join OH-groups

Address for correspondence:
Vladislav O. Soldatov, Department of Pharmacology and Clinical Pharmacology, Medical Institute, Belgorod State University, 85, Pobedy St., Belgorod - 308015, Russia.
E-mail: 1263220@bsu.edu.ru

Received: 10-12-2018
Revised: 23-12-2018
Accepted: 28-12-2018

to the proline residue (Pro-402 or Pro-564) of ODD domain and the asparagine residue (Asn-803) of TAD domain. These modifications make HIF-1α available for ubiquitination with further 26-S-proteasomal degradation[5,6] and block binding to natural coactivators such as CREB-binding protein (CBP) and p300.[7-9] Both of these enzymes belong to the group of Fe–, a-ketoglutarate-dependent dioxygenase and are used as substrate O_2 [Figure 1]. Thus, their activity is directly related to the concentration of oxygen and decreases with hypoxic condition.[10]

In the case of an increase in the concentration of HIF-1a, the HIF-1a/CBP/p300 complex moves to the nucleus, where after joining with HIF-1b, it forms a transcription factor interacting with DNA. The method of immunoprecipitation of chromatin and the use of biochips have shown that in response to an increase in HIF-1 when exposed to hypoxia, it's shows several hundred to 1000 genes are expressed.[11,12]

PHYSIOLOGICAL EFFECTS OF HIF-1

Semenza[13] distinguished two categories of HIF-dependent genes. The first group includes genes that mediate the increase in oxygen delivery. The second group includes genes that reduce its consumption. The first category activates/modulates O_2 transport systems. The products of these genes are molecules such as erythropoietin (EPO), vascular endothelial growth factor, transferrin, transferrin receptor, and endothelial NO-synthase (NOS). The second category acts by changing the ratio between mitochondrial respiration and glycolysis. The first measure is to block the entry of pyruvate into the tricarboxylic acid cycle (TCA) and to activate a glycolysis enzymes. For example, activation of pyruvate dehydrogenase kinase leads to inactivation of pyruvate dehydrogenase, an enzyme necessary to convert pyruvate to acetyl-CoA, which is used as the first link in the TCA.[14] HIF-1 also inhibits the activity of the TCA enzymes, since acetyl coenzyme A can be obtained by the cell not only from pyruvate.

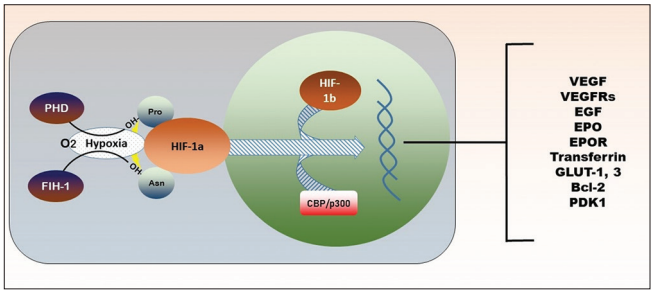

Figure 1: Molecular physiology of hypoxia-inducible factor (HIF)-1. Hypoxia decreases the activity of prolyl hydroxylase domain and FIH-1 activity and prevents the joining of OH- to HIF-1a. HIF-1a without hydroxyl groups can penetrate the nucleus, form a complex HIF-1a/HIF-1b/CREB-binding protein/p300, and interact with target genes. VEGF: Vascular endothelial growth factor, VEGFRs: Vascular endothelial growth factor receptors, EGF: Epidermal growth factor, EPO: Erythropoietin, EPO receptor, PDK1: Pyruvate dehydrogenase kinase isozyme 1

The next measure is more radical and is aimed at the induction of mitochondrial autophagy using bNIP3, Beclin-1, and Atg5.[13,15] Finally, HIF-1 through increased expression of glucose transporters GLUT-1, 2, and 3 enhances the flow of glucose into the cell.[16,17] We can also select another group of genes - proapoptotic factors (bNIP3, Noxa, Nix, and RTP801), the expression of which prevails in the early response to critical ischemia.[18]

In addition, the effects of HIF-mediated hypoxic adaptation strategies can be divided into (1) intracellular, due to reorganization of the mechanisms of energetic homeostasis of the cell and control of intracellular organelles or apoptosis; (2) local, associated with the secretion of regulatory molecules into the extracellular space; and (3) systemic aimed at increasing the overall resistance of the organism to hypoxia and mediated by the release of regulatory molecules into the blood [Figure 1].

HIF AND OXIDATIVE STRESS

Of the above effects of HIF, the most important and fundamental is the reduction of mitochondrial respiration by inhibiting the operation of the electron transport chain and the induction of mitochondrial autophagy. In the process of sequential movement of electrons along the components of the respiratory chain to oxygen (which as a result is reduced to water), an electrochemical gradient is created, which is used to synthesize adenosine-5'-triphosphate (ATP). However, some of the electrons combine with O_2 prematurely. It upsets the electron/O_2 balance and leads to the formation of reactive oxygen species (ROS).[19]

Interesting data were obtained by culturing mouse fibroblasts knocked out by HIF-1 with 0.5–1% pO2. When compared with normal mouse fibroblasts cultured with 20% pO_2, it turned out that, in the first, the level of ATP was even higher than in the second, but their death occurred much earlier due to the accumulation of ROS.[14]

If we draw an analogy, then we can compare the mitochondria with a nuclear power plant, glycolysis, with a thermal power plant, and oxygen, with a cooling system. A nuclear power plant is much more productive, but in conditions of a cooling deficit, its work becomes unsafe. The function of the HIF is to change the energy source to a more primitive but safe (glycolysis) one in time. Thus, the primary adaptive significance of HIF-1-dependent transition from redox to glycolytic metabolism under hypoxic conditions is determined by the need to maintain redox homeostasis, rather than oxygen levels. There is reason to believe that the regulation of mitochondrial activity can occur through the direct effect of HIF-1a on mitochondria without the participation of nuclear DNA.[19] HIF-2 has a similar antioxidant potential.[20]

Despite this, the antioxidant properties of HIF-1 can be replaced by prooxidant with changing conditions of hypoxia. It has been demonstrated that, in some tissues, chronic ischemia-reperfusion leads to the generation of ROS through the activation of HIF-1.[21]

CARDIOVASCULAR PATHOLOGY

Mice with HIF-1a -/- genetic knockout die in the prenatal period with multiple cardiac, vascular, and erythropoiesis development disorders.[22] Depending on the genetic background, mice lacking HIF-2α: Died on day 12 of embryogenesis with vascular defects[21] or bradycardia due to insufficient catecholamine production.[19]

Depending on the organ, degree, and duration of ischemia, as well as the speed of its onset, HIF has various effects: From protector to damaging. This ambiguity is determined; among other things, by the fact that, in parallel with the activation of HIF, a large number of molecular cascades are triggered in response to ischemia and the total effect is determined by their joint contribution.

Heart stroke

HIF-1 plays an important role in the pathogenesis of myocardial infarction. A study of cardiac tissue biopsies in patients with various forms of coronary artery disease showed that HIF-1a levels increased during the 1st h of infarction, with high levels of elevation associated with a more favorable prognosis.[23]

Knockout of one HIF allele is associated with severe maladaptation to hypoxia and ischemia.[24,25] Transgenic mice with overexpression of HIF-1a with occlusion of the coronary arteries show a smaller infarction area and a higher degree of vascularization than rodents with normal gene expression.[26] Moreover, it seems that HIF-1a plays a key role in the phenomenon of ischemic preconditioning, which is to increase resistance to prolonged hypoxia after first conducting one or more short ischemia-reperfusion cycles. During intermittent ischemia/reperfusion, there is a significant increase in the concentration of HIF-1a, as well as other isoforms of the HIF-1a subunit, and this event is critical and determines the effectiveness of cardioprotection. It is shown that the degree of increase in the activity of the main effector of preconditioning, the purinergic system, depends on the induction of HIF-1. In particular, HIF-1α is involved in the coordinated induction of ecto-5'-nucleotidase, adenosine A2B receptor, and adenosine kinase enzyme.[27-30]

Another phenomenon - distant preconditioning (DPC) has been studied for its association with HIF-1. The phenomenon is that pre-ischemia of one organ reduces damage to another organ during its ischemia. Knockout or pharmacological inhibition of PHD-2 appeared to enhance the effect of DPC. The in-depth study showed that the second substrate PHD-2 plays the leading role in this process, not HIF-1 (the co-substrate required for the hydroxylation of HIF-a-ketoglutarate. A-ketoglutarate accumulates, enters the liver, is metabolized to kinureric acid [kynurenic acid], and finally determines the effects of DPC).[31]

Brain stroke

HIF-1 is widely expressed in the brain.[32] HIF has been shown to affect brain development and memory consolidation processes.[33,34]

Both systemic hypoxia and brain hypoxia increase the concentration of HIF-1a,[35,36] and its expression is maximal in the penumbra zone.[37] Concerning the participation of HIF-1 in various forms of cerebral ischemia, a large amount of data have been accumulated, indicating both its positive and negative role.

Many studies demonstrate the involvement of HIF-1 in neuroprotection in ischemia-reperfusion.[38] By studying ischemic preconditioning on genetically modified, it was found that HIF-1-dependent adaptive pathways are only relevant in the early response to hypoxia since the HIF-1 knockdown did not affect the volumes of ischemic damage in the delayed ischemic preconditioning model.[18,39]

Studies using various models of focal and global cerebral ischemia have shown that the accumulation of HIF-1α protein correlates with the expression of target genes that encode the proteins involved in various adaptive responses. As mentioned, the HIF-responsive protein EPO has neuroprotective properties in acute brain hypoxia. Introduction of soluble EPO receptors to rats eliminates the neuroprotective effects of ischemic preconditioning.[40]

However, some experiments have demonstrated that HIF-1 is involved in the induction of proapoptotic factors [bNIP3 and p53] and an increase in the infarction zone.[39,41-45] Apparently, the difference in effects is explained by the degree and model of ischemia.

Local knockout of HIF-1 in astrocytes, while maintaining normal expression in neurons, significantly reduces hypoxic brain damage, which indicates the pathological effect of astrocytic HIF-1 on the viability of neurons. In contrast, the loss of HIF-1 in neurons slightly reduced the ability of neurons to resist hypoxia. These results are associated with an increased activity of inducible NOS (iNOS) in astrocytes.[39,46]

Lower limb ischemia

Not surprisingly, results comparable to those described were obtained in studies on ischemia models of other localization.

For example, ligation of the femoral artery in HIF-1a-defective mice leads to more serious damage compared to the wild type, and local genetic modification by intramuscular injection of adenovirus encoding an O_2-resistant form of HIF-1a (AdCA5) led to improved blood circulation and prevention lower limb necrosis in models of peripheral artery disease and diabetes in mice.[1]

GENETICS OF HIF

Currently, 34 SNPs are found in the HIF-1a gene. More serious mutations with a significant effect on the activity and expression of the protein were not registered, probably due to the fundamental importance of the molecule for the development of the organism. In addition, the HIF structure has relatively low variability, even when interspecific comparison. Phylogenetic analysis demonstrated the highly conservative nature of the oxygen-dependent and transactivating HIF domains.[47]

The assumption that the same variations in the HIF gene structure may entail beneficial or harmful properties arising from the ambiguity of participation in various pathological processes and is confirmed by the data of molecular genetic studies.

HIF-1a

At present, a significant association of the HIF-1a gene polymorphism with a wide range of diseases and conditions is shown from the increased stamina to inflammatory connective tissue diseases. This agrees well with the above information about the biology of the work of this factor. More detailed and complete information is given in the review of Gladek *et al*.[48]

HIF-2a

Genetic studies of HIF-2a endothelial PAS domain protein 1 show that this gene is propped up by hypoxic selection in highland residents. Several mutations are associated with phenotypes such as mountain sickness, hemoglobin concentration, high-altitude pulmonary hypertension, and high-altitude polycythemia. Mutations that increase the function of the gene are common in Tibet, and this applies to both the human population and many animals. It is believed that the human population carries these mutations due to the introgression of the Denisovan genes.[49-53]

HIF-3a

From the point of view of human genetics, the HIF-3a molecule remains poorly understood. The human HIF-3α gene undergoes extensive alternative splicing, which leads to a large structural diversity.[54] At present, strict associations have been proven only for a few phenotypes, including lung cancer for rs3810302[55] and obesity for rs3826795.[56] In addition, epigenetic modifications, in particular, the methylation level of the HIF-3a gene in peripheral blood and adipose tissue is associated with body mass index and the risk of developing type 2 diabetes.[57]

CONCLUSION

HIF, as one of the fundamental intracellular systems, deserves close attention from biologists, doctors, and pharmacologists. The information currently available on the biology of the HIF system and its involvement in the pathogenesis of various diseases is to some extent fragmentary and insufficient. A more detailed definition of the role of this system will provide insights into new pharmacological targets for the treatment of atherosclerosis, diabetes mellitus, cancer, obesity, coronary heart disease, retinopathy, etc.

REFERENCES

1. Semenza GL. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. Annu Rev Pathol 2014;9:47-71.
2. Loboda A, Jozkowicz A, Dulak J. HIF-1 versus HIF-2-is one more important than the other? Vascul Pharmacol 2012;56:245-51.
3. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995;92:5510-4.
4. Jiang BH, Semenza GL, Bauer C, Marti HH. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. Am J Physiol 1996;271:C1172-80.
5. Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. Science 2001;294:1337-40.
6. Kamura T, Sato S, Iwai K, Czyzyk-Krzeska M, Conaway RC, Conaway JW, *et al.* Activation of HIF1alpha ubiquitination by a reconstituted von hippel-lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci U S A 2000;97:10430-5.
7. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK, *et al.* FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 2002;16:1466-71.
8. Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science 2002;295:858-61.
9. Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, *et al.* An essential role for p300/CBP in the cellular response to hypoxia. Proc Natl Acad Sci U S A 1996;93:12969-73.

10. Adams JM, Difazio LT, Rolandelli RH, Luján JJ, Haskó G, Csóka B, *et al.* HIF-1: A key mediator in hypoxia. Acta Physiol Hung 2009;96:19-28.
11. Schödel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR, *et al.* High-resolution genome-wide mapping of HIF-binding sites by chIP-seq. Blood 2011;117:e207-17.
12. Xia X, Lemieux ME, Li W, Carroll JS, Brown M, Liu XS, *et al.* Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis. Proc Natl Acad Sci U S A 2009;106:4260-5.
13. Semenza GL. Regulation of oxygen homeostasis by hypoxia-inducible factor 1. Physiology (Bethesda) 2009;24:97-106.
14. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006;3:177-85.
15. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, *et al.* Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 2008;283:10892-903.
16. Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. J Biol Chem 2001;276:9519-25.
17. Gleadle JM, Ratcliffe PJ. Induction of hypoxia-inducible factor-1, erythropoietin, vascular endothelial growth factor, and glucose transporter-1 by hypoxia: Evidence against a regulatory role for src kinase. Blood 1997;89:503-9.
18. Baranova O, Miranda LF, Pichiule P, Dragatsis I, Johnson RS, Chavez JC, *et al.* Neuron-specific inactivation of the hypoxia inducible factor 1 alpha increases brain injury in a mouse model of transient focal cerebral ischemia. J Neurosci 2007;27:6320-32.
19. Semenza GL. Hypoxia-inducible factor 1: Regulator of mitochondrial metabolism and mediator of ischemic preconditioning. Biochim Biophys Acta 2011;1813:1263-8.
20. Scortegagna M, Ding K, Oktay Y, Gaur A, Thurmond F, Yan LJ, *et al.* Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in epas1-/-mice. Nat Genet 2003;35:331-40.
21. Peng YJ, Yuan G, Ramakrishnan D, Sharma SD, Bosch-Marce M, Kumar GK, *et al.* Heterozygous HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. J Physiol 2006;577:705-16.
22. Compernolle V, Brusselmans K, Franco D, Moorman A, Dewerchin M, Collen D, *et al.* Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor-1alpha. Cardiovasc Res 2003;60:569-79.
23. Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, Thistlethwaite PA, *et al.* Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N Engl J Med 2000;342:626-33.
24. Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, *et al.* Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. J Clin Invest 1999;103:691-6.
25. Semenza GL. Regulation of physiological responses to continuous and intermittent hypoxia by hypoxia-inducible factor 1. Exp Physiol 2006;91:803-6.
26. Kido M, Du L, Sullivan CC, Li X, Deutsch R, Jamieson SW, *et al.* Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. J Am Coll Cardiol 2005;46:2116-24.
27. Synnestvedt K, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, *et al.* Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. J Clin Invest 2002;110:993-1002.
28. Kong T, Westerman KA, Faigle M, Eltzschig HK, Colgan SP. HIF-dependent induction of adenosine A2B receptor in hypoxia. FASEB J 2006;20:2242-50.
29. Morote-Garcia JC, Rosenberger P, Kuhlicke J, Eltzschig HK. HIF-1-dependent repression of adenosine kinase attenuates hypoxia-induced vascular leak. Blood 2008;111:5571-80.
30. Eltzschig HK, Faigle M, Knapp S, Karhausen J, Ibla J, Rosenberger P, *et al.* Endothelial catabolism of extracellular adenosine during hypoxia: The role of surface adenosine deaminase and CD26. Blood 2006;108:1602-10.
31. Olenchock BA, Moslehi J, Baik AH, Davidson SM, Williams J, Gibson WJ, *et al.* EGLN1 inhibition and rerouting of α-ketoglutarate suffice for remote ischemic protection. Cell 2016;164:884-95.
32. Wiener CM, Booth G, Semenza GL. *In vivo* expression of mRNAs encoding hypoxia-inducible factor 1. Biochem Biophys Res Commun 1996;225:485-8.
33. Tomita S, Ueno M, Sakamoto M, Kitahama Y, Ueki M, Maekawa N, *et al.* Defective brain development in mice lacking the hif-1alpha gene in neural cells. Mol Cell Biol 2003;23:6739-49.
34. Adamcio B, Sperling S, Hagemeyer N, Walkinshaw G, Ehrenreich H. Hypoxia inducible factor stabilization leads to lasting improvement of hippocampal memory in healthy mice. Behav Brain Res 2010;208:80-4.
35. Bernaudin M, Nedelec AS, Divoux D, MacKenzie ET, Petit E, Schumann-Bard P, *et al.* Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain. J Cereb Blood Flow Metab 2002;22:393-403.
36. Stroka DM, Burkhardt T, Desbaillets I, Wenger RH, Neil DA, Bauer C, *et al.* HIF-1 is expressed in normoxic

tissue and displays an organ-specific regulation under systemic hypoxia. FASEB J 2001;15:2445-53.
37. Bergeron M, Yu AY, Solway KE, Semenza GL, Sharp FR. Induction of hypoxia-inducible factor-1 (HIF-1) and its target genes following focal ischaemia in rat brain. Eur J Neurosci 1999;11:4159-70.
38. Shi H. Hypoxia inducible factor 1 as a therapeutic target in ischemic stroke. Curr Med Chem 2009;16:4593-600.
39. Vangeison G, Rempe DA. The janus-faced effects of hypoxia on astrocyte function. Neuroscientist 2009;15:579-88.
40. Prass K, Scharff A, Ruscher K, Löwl D, Muselmann C, Victorov I, *et al.* Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. Stroke 2003;34:1981-6.
41. Althaus J, Bernaudin M, Petit E, Toutain J, Touzani O, Rami A, *et al.* Expression of the gene encoding the pro-apoptotic BNIP3 protein and stimulation of hypoxia-inducible factor-1alpha (HIF-1alpha) protein following focal cerebral ischemia in rats. Neurochem Int 2006;48:687-95.
42. Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, *et al.* Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998;394:485-90.
43. Halterman MW, Federoff HJ. HIF-1alpha and p53 promote hypoxia-induced delayed neuronal death in models of CNS ischemia. Exp Neurol 1999;159:65-72.
44. Halterman MW, Miller CC, Federoff HJ. Hypoxia-inducible factor-1alpha mediates hypoxia-induced delayed neuronal death that involves p53. J Neurosci 1999;19:6818-24.
45. Chen C, Hu Q, Yan J, Lei J, Qin L, Shi X, *et al.* Multiple effects of 2ME2 and D609 on the cortical expression of HIF-1alpha and apoptotic genes in a middle cerebral artery occlusion-induced focal ischemia rat model. J Neurochem 2007;102:1831-41.
46. Vangeison G, Carr D, Federoff HJ, Rempe DA. The good, the bad, and the cell type-specific roles of hypoxia inducible factor-1 alpha in neurons and astrocytes. J Neurosci 2008;28:1988-93.
47. Poyya J, Joshi CG, Kumar DJ, Nagendra HG. Sequence analysis and phylogenetic studies of hypoxia-inducible factor-1α. Cancer Inform 2017;16:1176935117712242.
48. Gladek I, Ferdin J, Horvat S, Calin GA, Kunej T. HIF1A gene polymorphisms and human diseases: Graphical review of 97 association studies. Genes Chromosomes Cancer 2017;56:439-52.
49. Buroker NE, Ning XH, Zhou ZN, Li K, Cen WJ, Wu XF, *et al.* EPAS1 and EGLN1 associations with high altitude sickness in han and Tibetan Chinese at the Qinghai-Tibetan plateau. Blood Cells Mol Dis 2012;49:67-73.
50. Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, Knight J, *et al.* Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. Proc Natl Acad Sci U S A 2010;107:11459-64.
51. Newman JH, Holt TN, Cogan JD, Womack B, Phillips JA 3rd, Li C, *et al.* Increased prevalence of EPAS1 variant in cattle with high-altitude pulmonary hypertension. Nat Commun 2015;6:6863.
52. Miao B, Wang Z, Li Y. Genomic analysis reveals hypoxia adaptation in the Tibetan mastiff by introgression of the gray wolf from the Tibetan plateau. Mol Biol Evol 2017;34:734-43.
53. Xu J, Yang YZ, Tang F, Ga Q, Tana W, Ge RL, *et al.* EPAS1 gene polymorphisms are associated with high altitude polycythemia in Tibetans at the Qinghai-Tibetan plateau. Wilderness Environ Med 2015;26:288-94.
54. Pasanen A, Heikkilä M, Rautavuoma K, Hirsilä M, Kivirikko KI, Myllyharju J, *et al.* Hypoxia-inducible factor (HIF)-3alpha is subject to extensive alternative splicing in human tissues and cancer cells and is regulated by HIF-1 but not HIF-2. Int J Biochem Cell Biol 2010;42:1189-200.
55. Putra A, Hiyama K, Tanimoto K. The role of HIF3A polymorphism in lung cancer patients. J Thorac Oncol 2017;12:1142-3.
56. Pfeiffer S, Krüger J, Maierhofer A, Böttcher Y, Klöting N, El Hajj N, *et al.* Hypoxia-inducible factor 3A gene expression and methylation in adipose tissue is related to adipose tissue dysfunction. Sci Rep 2016;6:27969.
57. Main AM, Gillberg L, Jacobsen AL, Nilsson E, Gjesing AP, Hansen T, *et al.* DNA methylation and gene expression of HIF3A: Cross-tissue validation and associations with BMI and insulin resistance. Clin Epigenetics 2016;8:89.

Source of Support: Nil. **Conflict of Interest:** None declared.