Acta Physiologica Sinica, October 25, 2017, 69(5): 598–610
DOI: 10.13294/j.aps.2017.0054 http://www.actaps.com.cn

Review

Regulation of iron metabolism by hypoxia-inducible factors

XU Man-Man, WANG Jun, XIE Jun-Xia*

Department of Physiology, Shandong Provincial Key Laboratory of Pathogenesis and Prevention of Neurological Disorders, Shandong Provincial Collaborative Innovation Center for Neurodegenerative Disorders, Medical College of Qingdao University, Qingdao 266071, China

Abstract: Hypoxia-inducible factors (HIFs) are central mediators of cellular adaption to hypoxia. The heterodimeric HIF transcription factors consist of HIF-α and HIF-β, that form functional HIFs. Mammals contain HIF-1α, HIF-2α, and HIF-3α. HIFs play a key role in iron metabolism by regulating the expression of iron-related proteins, such as divalent metal transporter 1 (DMT1), ferroportin 1 (FPN1), duodenal cytochrome b (Dcytb), and transferrin receptor (TfR). Hepcidin and iron regulatory proteins (IRPs), the central mediators for systematic and intracellular iron homeostasis, are also regulated by HIFs. In this review, we summarized the regulatory effects of HIFs on iron-related proteins, thus providing insights into the control of HIFs as therapeutic strategies for some iron related disorders.

Key words: hypoxia-inducible factors; iron metabolism; divalent metal transporter 1; ferroportin 1

低氧诱导因子对铁代谢的调节

许曼曼，王 俊，谢俊霞*

青岛大学医学院生理学教研室，山东省神经相关疾病重点实验室，山东省神经退变疾病协同创新中心，青岛 266071

摘 要：低氧诱导因子(hypoxia-inducible factors, HIFs)是一类介导细胞内低氧反应的核转录复合体。HIF-α和HIF-β形成有功能的异质二聚体。哺乳动物中有HIF-1α、HIF-2α和HIF-3α。HIFs在铁代谢中发挥重要作用。受HIFs调节的铁代谢相关蛋白主要有二价金属转运蛋白1 (divalent metal transporter 1, DMT1)、铁转出蛋白(ferroportin 1, FPN1)、十二指肠铁细胞色素b (duodenal cytochrome b, Dcytb)和转铁蛋白受体(transferrin receptor, TfR)。铁调素(hepcidin)和铁调节蛋白(iron regulatory proteins, IRPs)是调节机体与细胞内铁代谢、维持铁稳态的重要因子，同样受到HIFs的调节。本文综述了HIFs对上述铁代谢相关蛋白的调节作用，以期为治疗铁代谢相关疾病提供可能的靶点。

关键词：低氧诱导因子；铁代谢；二价金属转运蛋白1；铁转出蛋白
中图分类号：Q4

1 Hypoxia-inducible factors (HIFs)

1.1 The structures and activations of HIFs

HIFs were initially discovered as a protein complex that binds to the erythropoietin (*EPO*) gene regulatory sequence induced by hypoxia[1]. They are the major transcription factors and key regulators of the genomic response to hypoxia. HIFs are highly conserved transcription factors that are ubiquitously expressed and are heterodimers whose expression are regulated by oxygen[2]. The heterodimeric HIF transcription factors, members of the basic helix-loop-helix Per-Arnt-Sim

Received 2017-04-25 Accepted 2017-07-11

Research from the corresponding author's laboratory was supported by grants from the National Natural Science Foundation of China (No. 31571054, 81430024), the National Basic Research Development Program of China (No. 2011CB504102), Excellent Innovative Team of Shandong Province and Taishan Scholars Construction Project, China.

*Corresponding author. Tel: +86-532-85955891; Fax: +86-532-83780136; E-mail: jxiaxie@public.qd.sd.cn

(bHLH-PAS) family[2, 3], comprise an oxygen-sensitive α subunit and a stable, constitutively expressed β subunit, also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) that forms a functional HIF[3]. Mammals contain HIF-1α[2, 4], HIF-2α[5], and HIF-3α[6]. HIF-α subunits are hydroxylated on two prolines by iron-dependent prolyl-hydroxylases (PHD) [7, 8] and then degraded through the ubiquitin-proteasome pathway via its interaction with the von-Hippel-Lindau (VHL) tumor suppressor protein during normal cellular oxygen tension[9]. Mammalian cells contain three PHDs, PHD1, PHD2 and PHD3, also known as EGL nine homolog (EGLN)1, EGLN2, and EGLN3[10]. PHDs are 2-oxoglutarate-dependent dioxygenases which require iron binding at an active site and oxygen as a co-substrate to active[11]. Under normoxic conditions, PHDs hydroxylate HIF α subunits at conserved prolines through the ubiquitin-proteasome pathway. Oxygen-dependent prolyl-hydroxylation is required for HIF to bind to VHL, and then to the E3 ubiquitin ligase complex, leading to rapid proteasomal degradation of HIF-α subunit[12–14]. Inactivation of VHL in normoxic cells results in HIF activation, demonstrating VHL is required for HIF degradation[15]. In addition, the HIF-3α2 splice variant (IPAS) hydroxylates HIF-1α via asparaginyl hydroxylase factor inhibiting HIF (FIH) on an asparagine residue of the C-terminal domain, which blocks the interaction with p300 transcriptional co- activator. This provides a second mechanism by which HIF-mediated transcription is inactivated[16–19]. HIFs are activated under hypoxia, and pseudo hypoxic, or other pathological conditions mediate adaptive responses of cells to these states. A decrease in cellular oxygen or iron availability inhibits PHD-dependent proline hydroxylation of HIF-α subunits, leading to the stabilization. Following stabilization, the regulator subunit accumulates and translocates into nucleus, partners with ARNT/HIF-β, recruits transcriptional cofactors such as p300/CBP[20], binds to hypoxic response elements (HREs) of target gene regulatory sequences, and mediates the robust induction of a battery of target genes to activate transcription of genes [5, 8, 14, 19].

1.2 The functions of HIFs

HIF-1α is ubiquitously expressed and transcriptionally upregulates a large number of genes, including those encoding transferrin (Tf), vascular endothelial growth factor (VEGF), glucose transporters, glycolytic pathway enzymes, insulin-like growth factor-2, endothlin-1, and inducible nitric oxide synthetase [21, 22]. However, HIF-2α and HIF-3α expression is largely tissue restricted[6, 23, 24]. HIF-2α is expressed on specific cell types, including renal interstitial fibroblast-like cells, endothelial cells and glia, plays an important role in enterocyte iron uptake and is the primary regulator of EPO production[5, 18, 25] . HIF-1α and HIF-2α have been extensively demonstrated to regulate glucose metabolism, angiogenesis, cell survival, proliferation, migration, and HIF signaling way also plays an important role in inflammatory responses[26–28] and regulating the immune environment[29, 30].

Although HIF-1 and HIF-2 share a high degree of sequence identity, and are regulated in a similar fashion, bind to the same HRE and share many overlapping genes and function, there exists specially distinct function[31]. In cancer progression, HIF-1α plays the dominant role in the response to acute hypoxia, whereas HIF-2α drives the response to chronic hypoxia[32]. Importantly, depletion of one HIFα-subunit provokes a compensatory increase of the other one, such as some studies[33] reported that knockdown of HIF-1α increased the expression HIF-2α via the reciprocal regulatory mechanism, and this switch from HIF-1α to HIF-2α dependent ways provides a clue to suppress hypoxia-inducible pathways. Yu *et al*.[32] analyzed results of published reports about HIFs inhibitors for cancer therapy, and reported that it may be essential to target HIF-1 and HIF-2 together, rather than one factor, so as to suppress hypoxia-inducible pathways. Shah *et al*.[34] used the generation of knockout mice and tissue-specific conditional mice, and reported that HIF-2α, but not HIF-1α, promotes iron absorption in intestine.

HIF-3 is the newest member of HIF family. A few studies indicate that HIF-3α have multiple variants due to different promoters, transcription initiation sites and alternative splicing[32]. Some HIF-3α variants are dominant-negative regulators of HIF-1/2α, while others inhibit HIF-1/2α actions by competing for common β-subunits[35]. Further studies on HIF-3α will provide new insights[31].

Inhibition of PHD activates HIF target genes and genetic disruption of PHDs increases expression of HIF and reactivates hepatic expression of EPO in mice. That constitutive activation of HIF can lead to inflammation and cancer [10, 11, 21, 36].

1.3 Regulation of HIFs

HIFs protein levels are tightly regulated by several mechanisms. HIF-1α and HIF-2α are degraded through

the ubiquitin-proteasome pathway via PHDs interaction with VHL, which is mediated by E3 ubiquitin ligase[30]. PHDs are regulated not only by oxygen availability, but also by several metabolites[37]. The decreased O_2 tension or inflamed tissue promotes activation of HIFs. Under hypoxia conditions, HIFs are stabilized, dimerize with ARNT, and translocate to the nucleus to regulate transcription. In addition to O_2-dependent regulation, inflammation intimately links to direct HIF regulation[38, 39]. In macrophages, bacterial lipopolysaccharide (LPS) activates NF-κB, directly increases HIF-1α mRNA and the expression of HIF-1α by increasing intracellular ferritin. And that, several cytokines and intermediate metabolites such as succinate can lead to HIF activation[40–43]. However, The cytokines such as IL-4 and IL-13 selectively increase HIF-2α expression[44]. Additionally, it is demonstrated that cytokine-induced reactive oxygen species (ROS) and specially mitochondrial ROS directly activate HIF, and its activation is modulated by mitochondrial membrane potential increased by mitochondrial ROS[43–45].

Iron-driven pathways specially regulate HIF-2α in intestine. However, chelation of iron activates both HIF-1α and HIF-2α in cell lines, while iron-deficient diets selectively induce HIF-2α expression in mice intestine[46–48]. Moreover, it has been demonstrated that iron regulatory protein 1 (IRP1) interaction is a physiological regulator of HIF-2α protein expression[49, 50].

Besides oxygen-dependent regulation of HIF-1α, there are other mechanisms. Hypoxia-associated factor (HAF) could regulate HIF-1α protein stability, its action is selective for HIF-1α and it is a novel HIF-1α isoform specific E3 ligase[51]. Extracellular heat shock protein 90 (Hsp90), Hsp70 and carboxyl terminus of Hsp70-interacting protein (CHIP) selectively regulate ubiquitination and degradation of HIF-1α, but not HIF-2α[51, 52]. In addition, phosphorylation is another mechanism of HIF-α stabilization. ERK-MAPK phosphorylated HIF-1α, leading to HIF-1α activation[53]. HIF-1α and HIF-2α undergo phosphorylation by P42/44 and p38 kinase *in vivo*[53–55].

2 Iron metabolism

Iron, an essential micronutrient in all living organisms, is required for oxygen delivery and is a cofactor in several enzymatic and redox reactions[56]. Seventy to eighty percent of iron is used for red blood cell synthesis as an essential prosthetic group in hemoglobin. One to two milligrams of iron are lost on a daily basis, and intestinal iron absorption is the only known mechanism to recover the daily loss[57]. It is essential for many biological processes. Iron metabolism, oxygen homeostasis and erythropoiesis are consequently strongly interconnected. Iron levels are controlled by a multi-tissue homeostatic process and need to be tightly regulated[56]. Iron insufficiency induces a hypoferric anemia in mammals, coupled to hypoxia in tissues, whereas excess iron is toxic, and causes generation of free radicals by the Fenton reaction[16, 56, 57].

Divalent metal transporter-1 (DMT1), also known as Nramp2, SLC11A2, and DCT1, is a member of the natural resistance associated macrophage protein family[58]. It locates to cellular membranes and endosomal membranes, where it is a key player in non-transferrin-bound iron (NTBI) uptake[59]. DMT1 has 12 transmembrane domains, membrane targeting motifs, one consensus transport motif, and two asparagine-linked glycosylation signals in an extra cytoplasmic loop. Both the N- and C-termini of the protein are located in the cytoplasm[60, 61]. There are four isoforms, one with the 3' untranslated region (UTR) iron responsive element (IRE) (+IRE), one without an IRE (−IRE), which are two alternative transcriptions. And the others are two additional transcripts in the 5'-regulatory regions, which are exon 1A and exon 1B[59, 62]. DMT1+IRE are localized to the cell membrane to transfer Fe^{2+} across the endosomal membrane into cytoplasm[63–67]. This complexity reflects the fine balance required in iron homeostasis, as this metal is indispensable in many cell functions but highly toxic when appearing in excess[61, 68].

Ferroportin 1 (FPN1) is a transmembrane domain protein present within intracellular compartments as well as on the basolateral surface of epithelia and on the surface of non-polarized cells[69–71]. It is the only characterized, mammalian, basolateral iron exporter[72]. It transports Fe^{2+}, which must be oxidized to Fe^{3+} by one of several ferroxidases that also serve to stabilize FPN1, before iron can bind to Tf[73–75]. FPN1 is remarkable insofar as it is not only the effector of cellular iron export, but also the receptor of hepcidin, its primary regulator[76, 77].

DMT1 and FPN1 are expressed in cells that are critical in maintaining iron homeostasis, mainly macrophages, hepatocytes and enterocytes[78–85]. However, on central nervous system (CNS), DMT1 both with and without IRE are expressed in neurons, astrocytes, microglia, but not in oligodendrocytes[61, 86–89].

Hepcidin (encoded by *HAMP*) is a 25-amino acid peptide, which plays a critical role in the regulation of iron homeostasis[82] and is produced predominately by the liver in response to iron stores and serum iron availability[80, 90]. It was initially discovered as an anti-microbial peptide that was mainly synthesized in the liver, could be detected in the urine, and was induced by inflammation[82, 90, 91]. Shortly after its discovery, the link between hepcidin and iron homeostasis was uncovered and high systemic levels of iron increased the expression of hepcidin[77]. It acts as a negative regulator of iron release from cells, binding with FPN1 (the only known mammalian iron exporter) to promote its rapid internalization and degradation[76, 92]. The function of hepcidin correlates with its expression, which is regulated by systemic levels of iron. Low systemic levels of iron reduce hepcidin expression and high systemic levels of iron increase its expression[93, 94]. Overall, hepcidin production simultaneously leads to decreased intestinal iron absorption and sequestration of iron in macrophages, limiting its availability for erythropoiesis[57]. In addition, diminished hepcidin expression permits more non-heme iron to be released from internal liver and macrophage stores and increases iron transfer through intestinal epithelial cells, effectively controlling the bioavailable iron supply[81].

Iron regulatory proteins (IRPs), including IRP1 and IRP2, regulate cellular iron homeostasis. Proteins involved in iron uptake, storage, utilization, and export must be regulated in a coordinated fashion[35, 95–97]. It is an intricate process and their integrated regulation is influenced by iron at both transcriptional and post-transcriptional levels[98]. More than 25 years ago, the presence of RNA motif called IRE was localized in the 3' or 5' UTR of several mRNAs involved in iron metabolism and homeostasis[56]. IRP1 and IRP2 bind to IRE in a manner dependent on cellular iron levels[99]. Binding of IRPs to IREs can block translation or increase mRNA stability and affect levels of several proteins involved in iron homeostasis[98]. Intracellular levels of iron regulate expression and activity of IRPs. Decreased cellular levels of iron activate IRPs. At low intracellular iron concentration, IRPs bind to the IRE of FPN1 mRNA in its 5'-UTR, and repress translation. Alternatively, transcripts with an IRE in the 3'-UTR of DMT1 are stabilized by IRPs binding. The converse regulation occurs in cells with high iron levels, which decreases IRPs function. High levels of cellular iron lead to assembly of iron-sulfur clusters (4Fe-4S) in the IRE binding pocket of IRP1, which inhibits its interaction with IREs. IRP2 is degraded under high concentrations of cellular iron. Loss of IRP1 and IRP2 activity results in translation of mRNAs that contain IREs in the 5'-UTR and decreases stability of mRNAs with IRE in the 3'UTR[100]. Both IRP1 and IRP2 are also regulated by nitric oxide, but in opposite ways; IRP1 is also an oxygen sensor, binding to IREs in response to high oxygen tensions. IRP2 seems to be the predominant regulator of iron homeostasis in mammalian cells at physiological oxygen tensions[96, 98]. Even though the regulation of either protein is not entirely dependent on the other, it's possible that similar pathways. The ability of IRPs to stabilize the transcript and increase protein levels of DMT1 could be coordinated with hepcidin-mediated repression of FPN1[35, 69, 72, 93, 94, 101].

IRP1 has three functional HREs in its 5'-regulatory region, which is regulated by the HIF-1/HRE system under hypoxia. There are HREs in the promoters of DMT1 and IREs in the 3'-UTR mRNA of DMT1. Therefore, DMT1 is transcriptionally regulated by HIF/HRE system and post-transcriptionally regulated by IRP/IRE system[49, 102]. Cheng *et al.*[103] founded that IRP1 suppressed hypoxia-induced DMT1 expression, restricted intracellular iron levels, including the labile iron pool and total cellular iron content, and reduced lactate dehydrogenase (LDH) release.

In intestine, ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) by the duodenal cytochrome b (Dcytb) (iron reductase) in luminal membrane cells of intestine firstly. Then Fe^{2+} enters cells through DMT1. After the iron enters the cells, it can be stored in ferritin or be transferred out of the cells by FPN1 in basolateral membrane[104, 105].

3 HIFs and iron metabolism

HIFs play a key role in iron metabolism by regulating the expression of iron-related proteins, such as DMT1, FPN1, Dcytb, and transferrin receptor (TfR)[106, 107]. Hepcidin and IRPs, the central mediators for systematic and intracellular iron homeostasis, are also regulated by HIFs (Fig. 1).

3.1 HIFs, DMT1 and FPN1

HIF-2α is the key transcription factor that promotes intestine iron absorption by regulating the expression of Dcytb, DMT1 and FPN1by binding to HREs on their

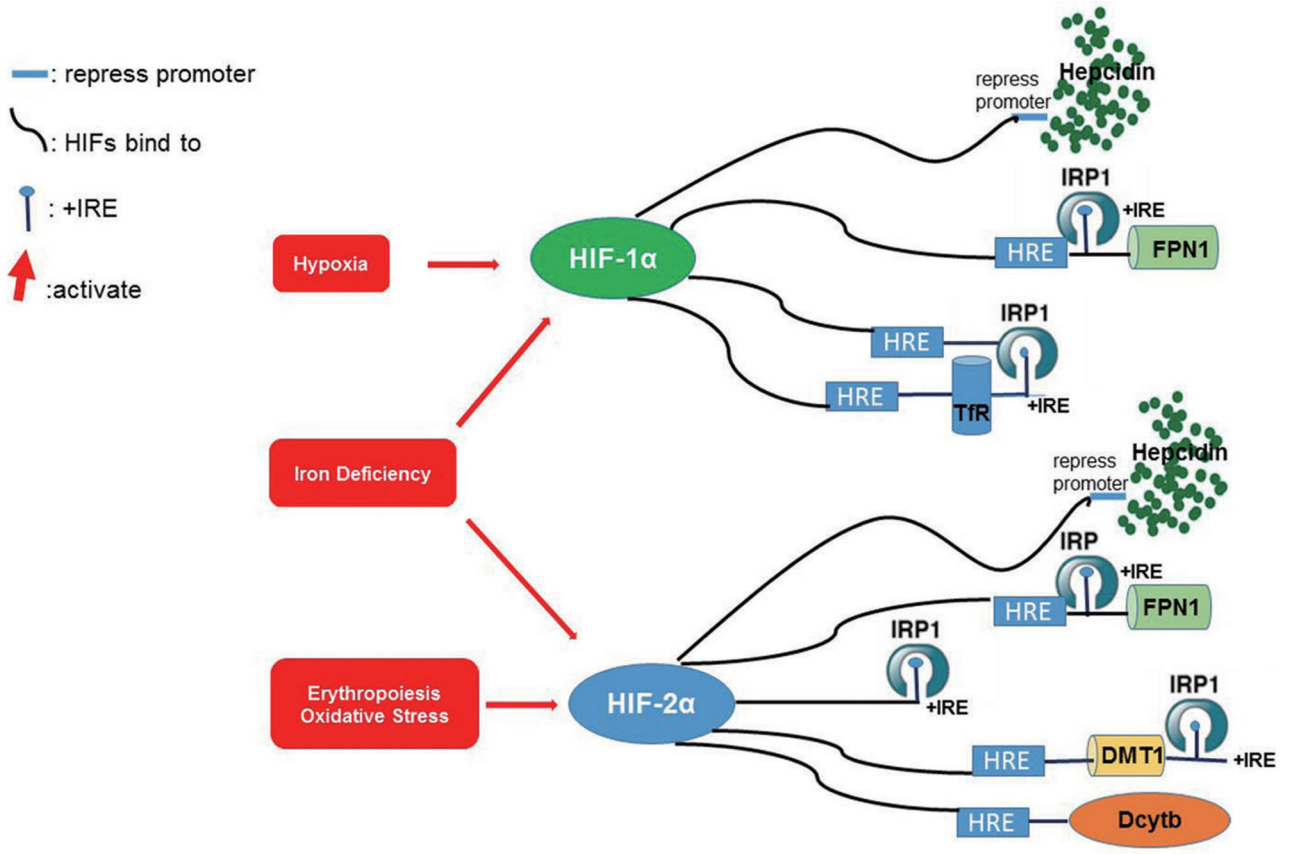

Fig. 1. Graphical summary about HIFs regulating iron-related proteins. HIFs regulate the expression of DMT1, FPN1, Dcytb, TfR and IRP1 by binding to HREs in their mRNAs. HIFs regulate the expression of hepcidin by binding to its repress promoter. IRP1 regulates translation of HIF-2α mRNA via its 5'-UTR IRE. DMT1: divalent metal transporter 1; FPN1: ferroportin 1; Dcytb: duodenal cytochrome b; TfR: transferrin receptor; IRP1: iron regulatory protein 1; HRE: hypoxia response element; UTR: untranslated region; IRE: iron responsive element.

respective promoters[9, 31, 34, 36, 57, 78, 108].

The DMT1 promoter maintains HIF-2α specificity on a 200-bp proximal promoter region, which contains a HRE and adjacent binding sites for CAAT enhancer binding proteins[34]. Mastrogiannaki *et al*.[109] in their study suggested that the direct transcriptional regulation of DMT1 by HIF-2α indicated that HIF-2α regulation of DMT1 isoforms that contain IREs in their transcripts operates upstream of the IRP/IRE system. In their study, they used conditional knockout mice of HIF-1α and HIF-2α in the intestine (vil-Cre^{+}/HIF-1α$^{fl/fl}$ mice and vil-Cre^{+}/HIF-2α$^{fl/fl}$ mice) to analyze the expression of DMT1+IRE, FPN1 and of Dcytb mRNA, observed that the expression of DMT1 and FPN1 significantly decreased, as well as the decrease of Dcytb mRNA in the vil-Cre^{+}/HIF-2α$^{fl/fl}$ mice. However, they didn't observe the same effects in the vil-Cre^{+}/HIF-1α$^{fl/fl}$ mice, suggesting that HIF-2α specifically regulates the expression of DMT1 and FPN1. Then they further found that HIF-2α bound and directly trans-activated the DMT1-1A promoter. They next examined the vil-Cre^{+}/HIF-2α$^{fl/fl}$ mice after 2-month iron-deficient diet, and observed lower mRNA levels of DMT1 upregulation in vil-Cre^{+}/HIF-2α$^{fl/fl}$ mice compared with wild type (WT) mice. This study extended and confirmed the conclusion of Shah *et al*.[46] that HIFs regulate the expression of DMT1 and FPN1 in intestine.

During iron deficiency, the iron-dependent PHDs have decreased activity, leading to HIF-2α activation[34]. HIF-2α was required for increases in FPN1 mRNA in response to acute changes in iron levels. Taylor *et al*.[78] reported that low-iron induced expression of FPN1 was completely lost in HIF-2α$^{F/F}$ mice and FPN1 mRNA was increased under long-term deprivation of iron in a HIF-2α-dependent manner. In their study, they first used microarray analysis and showed that FPN1 mRNA

was significantly induced in a HIF-2α-dependent manner following 2-weeks of a low-iron diet treatment. Then to confirm this result, they used qPCR to detect total FPN, FPN1A, and FPN1B isoforms, and no increases were found in the HIF-2α$^{\Delta IE}$ mice (mice that lacked HIF-2α specifically in the intestine), however, both increased in the WT mice. And they used Vhl$^{\Delta IE}$/HIF-2α$^{\Delta IE}$ mice to investigate the FPN protein expression and FPN1 mRNA. In addition, they used qPCR ChIP analysis and found that HIF-2α is able to bind to the FPN1B regulatory region *in vivo* similarly to what is observed on the DMT1 promoter region. Above all, they demonstrated that acute, adaptive induction of FPN1 occurs via increased transcription of FPN1 and HIF-2α-dependent mechanism regulates that long-term responses to systemic iron[110]. This biphasic regulation provides an efficient system to maintain a rapid and sustained response to increase systemic iron requirements[111].

The expression of DMT1 and Dcytb were up-regulated via overexpression of HIF-2α under acute or long-term iron deprivation. In HIF-2α intestine knockout mice, the expression of DMT1 and Dcytb are completely lost [46, 109], and the adaptive increase in DMT1 and Dcytb following increased erythropoiesis is also lost. Above all, these findings demonstrated that HIF-2α, but not HIF-1α, directly regulates FPN1, DMT1 and Dcytb [46, 78, 109].

Transferrin receptor 1 (TfR1) and DMT1 are regulated by HIF-1α in response to hypoxia in extra-neural organs and cells. Yang *et al*.[112] demonstrated that hypoxia preconditioning (HP), which could produce significant protective effects on neuron in experimental cells, animals and humans[113–118], activated HIF-1α to increase the expressions of TfR, DMT1 and FPN1, and thus increased transferrin-bound iron (Tf-Fe) and non-transferrin-bound iron (NTBI) uptake and iron release in astrocytes[112]. They proposed that HP led to a progressive increase in cellular iron content and increased iron transport speed in astrocytes. However, our latest unpublished studies showed that HIF-2α, but not HIF-1α, plays a key role in regulating the expression of DMT1 and FPN1 in astrocytes under pathology. The effect of HIF-2α on iron metabolism of astrocytes is similar with small intestine, therefore, astrocytes may be "iron pump" and enhanced iron traffic in astrocytes may be the source of iron accumulated in dopaminergic neurons of Parkinson's disease (PD), which is a common neurodegenerative disorder characterized in its late phase by the sustained loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), selective deposition of iron in the substantia nigra (SN) has been observed, while no iron content increases in other brain regions.

Du *et al*.[118] also investigated and found that HP increased the expression of HIF-1α as well as TfR1, DMT1 and FPN1, thus increased Tf-Fe, NTBI uptake and iron release in neurons. A progressive increase in cellular iron content as well as increased iron transport rate in neurons were observed. This work consists with findings of Yang *et al*.[112] in astrocyte, suggesting that increased iron transport rate and cellular iron in astrocytes or neurons might be one of the mechanism in neuroprotection or increased hypoxia tolerance in brain[112, 118].

3.2 HIFs and TfR

TfR, which is a membrane protein important for cellular iron uptake, is regulated transcriptionally through the binding of HIF-1 to HREs under hypoxic conditions[107, 119, 120]. There are HREs in the promoters of TfR1, as well as IREs in 3'-UTR mRNA of TfR1, and IRP1 has three functional HREs in its 5'-regulatory region[107, 119, 120]. Therefore, TfR1 is transcriptionally regulated by the HIF-1/HRE system and post-transcriptionally regulated by the IRP1/IRE system. Moreover, IRP1 is also regulated by the HIF-1/HRE system under hypoxia [100]. It has been proposed that hypoxia can regulate iron uptake, storage, and utilization via the HIF-1/HRE regulatory system[120]. Bianchi *et al*. [119] reported that HIF-1 is involved in transcriptional activation of the TfR in response to iron deprivation. The transcription rate of the TfR gene in isolated nuclei was up-regulated by treatment of Hep3B human hepatoma cells with the iron chelator desferrioxamine (DFO). They firstly observed DFO-dependent activation of a luciferase reporter gene in transfected Hep3B cells was mediated by a fragment of the human TfR promoter containing a putative HRE sequence. Secondly, they found that mutation of this sequence prevented stimulation of luciferase activity. Next, they identified that, by competition experiments and supershift assays, binding to this sequence of HIF-1α was induced by DFO. Furthermore, in mouse hepatoma cells unable to assemble functional HIF-1, inducibility of TfR transcription by DFO was lost, and TfR mRNA up-regulation was reduced. Above all, they demonstrated that HIF-1 controlled TfR gene expression in conditions of

iron depletion. Thus, they provide the insights into the mechanisms of transcriptional regulation which concurs with the well-characterized post-transcriptional control of TfR expression to expand the extent of response to iron deficiency[119].

Previous studies demonstrated that DFO treatment led to deficiency in iron-reducing capacity, resulting in inhibition of PHD activity, induction of HIF-1α stabilization, and downstream HO-1, TfR, and Tf [107, 119, 120], and decreased mitochondrial ROS production in primary neurons[45, 107, 119, 120]. Weinreb *et al*.[121] and Guo *et al*.[122] demonstrated that the iron chelator M30 upregulated HIF-1α in various brain regions of adult mice, leading to differentially induced levels of TfR, tyrosine hydroxylase (TH), and EPO[123]. Taken together, iron chelators activated the transcriptional HIF-1α, upregulated TfR genes, and decreased iron-reducing capacity to provide neuroprotection. Iron chelators plays the protective role in neurodegenerative pathologies through the HIF-1 signaling system[122].

3.3 HIFs, IRPs and hepcidin

Iron acts directly as a cofactor for HIF-PHD enzymes, and iron-driven pathways are the best-known pathways regulating HIF-2α in the intestine. PHD are iron-dependent enzymes, and iron depletion can stabilize HIF and activate HIF-1α and HIF-2α[2, 57, 96]. However, iron-deficient diets selectively induce HIF-2α expression in mice intestines[46]. In addition, IRPs regulate translation of HIF-2α mRNA via its 5'-UTR IRE [47, 57, 124]. IRP1 and IRP2 bind with high affinity to IREs on HIF-1α and HIF-2α to control iron-dependent regulation of the transcription. However, a high activity of the IRP1, which is the major regulator of the HIF-2α translation, leads to the inhibition of HIF-2α, but not HIF-1α[47]. Recent study[125] demonstrated that IRP1 interaction is a physiological regulator of HIF-2α protein expression. *Irp1*- (but not *Irp2*-) knockout mice led to selective HIF-2α activation and increased expression of HIF-2α target genes. Three research groups[48, 126, 127] found that IPR1 but not IRP2 knockouts increased HIF-2α mRNA in IRP1 global knockout mice. Furthermore, Shah and Xie[57] showed low levels of iron in the intestine led to activation of HIF-2α and upregulations of DMT1, FPN1 and Dcytb, which are HIF-2α target genes. Another research groups[93, 128] demonstrated that following iron deficiency, the expression of hepcidin decreased in liver and the expressions of DMT1, Dcytb and FPN1 increased in intestine.

On the other hand, IRP1 has three functional HREs in its 5'-regulatory regions, and Luo Q *et al*.[129] demonstrated that the HRE of IRP1 5'-regulation regions could combine with HIF-1 *in vitro*. HIF-1α regulates IRP1 by directly binding to its HREs of 5'-regulatory regions.

HIFs regulate hepcidin transcription by directly binding to and repressing its promoter[19]. Some studies have suggested direct regulation of hepcidin by HIF-1α. In normoxic or iron-sufficient conditions, HIF-1α associates with VHL protein that promotes HIF-α subunit degradation. In hypoxia or iron deficiency, the hydroxylases are inactive, and the regulatory subunit accumulated, translocated into the nucleus, associated with HIF-1β and bound to promoter elements to modulate gene transcription[33]. The link between HIF and hepcidin demonstrated that HIF-1α bound to HREs of Hamp 1 promoter and decreased its transcription[19]. In *in vivo* studies, Peyssonnaux *et al*.[56] found that conditional disruption of liver VHL mice (alb-*cre*/vHL$^{flox/flox}$), which is the E3 ligase responsible for degradation of HIF-1α and HIF-2α, under iron deficient conditions showed a significant decrease in hepcidin level, an increase in HIF-1α level, and an increase in FPN1, suggesting the effect of the stabilization of HIF transcription factors on metabolism through hepcidin regulation. Fleming[77] also showed that stabilization of HIF in hepacytes increases FPN1 expression. Thus, a HIF-dependent FPN1 mRNA upregulation or/and a post-translational stabilization of FPN1 and deletion of vHL could prevent an ubiquitin-mediated degradation of FPN1. These works confirmed that the specific role of IRP1 and not IRP2 in regulation of HIF-2α expression, but not other HIFs[98].

It also has been demonstrated that hypoxia can increase iron uptake by cells as well as the expression of IRP1, IRP2 and DMT1. Hypoxia inhibited degradation of HIF-1α, which downregulated IRP1 expression. However, Luo *et al*.[130] recently found that hypoxia inhibited degradation of HIF-1α, leading to a decrease first and then increase, rather than a stable decrease, in IRP1 expression. Moreover, they used HIF-1α and/or HIF-2α siRNA in HepG2 cells under hypoxia for 4, 8, 12, and 24 h, further demonstrated that under hypoxia, expression of IRP1 is regulated by not only HIF-1 but also phosphorylation of the transcription factor cyclic AMP response element-binding protein (pCREB), which is crucial for stimulus-transcription coupling.

They suggested that HIF-1 plays its role in the early phase, while pCREB plays its role in the late phase of hypoxia[130].

4 Conclusion

HIFs are essential in regulating iron metabolism by combining with HRE. Hypoxia activated HIF-1α, and erythropoiesis, oxidative stress activated HIF-2α. HIF-1α and HIF-2α were activated by iron deficiency. HIF-2α-specific inhibitors have been characterized and may be useful for treatments in iron overload diseases, and several PHD inhibitors that activate HIF-2α are under preclinical or clinical investigation for potential therapeutic use in the treatment of iron-related diseases[32]. It is also important to learn more about new generation of HIF modulators, to determine if they might be effective therapeutics for iron-related disorders.

REFERENCES

1 Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A 1993; 90(9): 4304–4308.

2 Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Natl Acad Sci U S A 1997; 94(9): 4273–4278.

3 Kaelin WG, Jr., Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 2008; 30(4): 393–402.

4 Flamme I, Frohlich T, von Reutern M, Kappel A, Damert A, Risau W. HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels. Mech Dev 1997; 63(1): 51–60.

5 Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997; 11(1): 72–82.

6 Gu YZ, Moran SM, Hogenesch JB, Wartman L, Bradfield CA. Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. Gene Expr 1998; 7(3): 205–213.

7 Makino Y, Cao R, Svensson K, Bertilsson G, Asman M, Tanaka H, Cao Y, Berkenstam A, Poellinger L. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. Nature 2001; 414(6863): 550–554.

8 Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. Genes Dev 2002; 16(12): 1466–1471.

9 Shah YM. The role of hypoxia in intestinal inflammation. Mol Cell Pediatr 2016; 3(1): 1.

10 Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ, Gleadle JM. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem 2004; 279(37): 38458–38465.

11 Myllyharju J. Prolyl 4-hydroxylases, master regulators of the hypoxia response. Acta Physiol (Oxf) 2013; 208(2): 148–165.

12 Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998; 394(6692): 485–490.

13 Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000; 157(2): 411–421.

14 Locatelli F, Fishbane S, Block GA, Macdougall IC. Targeting hypoxia-inducible factors for the treatment of anemia in chronic kidney disease patients. Am J Nephrol 2017; 45(3): 187–199.

15 Poellinger L, Johnson RS. HIF-1 and hypoxic response: the plot thickens. Curr Opin Genet Dev 2004; 14(1): 81–85.

16 Peyssonnaux C, Zinkernagel AS, Schuepbach RA, Rankin E, Vaulont S, Haase VH, Nizet V, Johnson RS. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). J Clin Invest 2007; 117(7): 1926–1932.

17 Hubbi ME, Semenza GL. Regulation of cell proliferation by hypoxia-inducible factors. Am J Physiol Cell Physiol 2015; 309(12): C775–C782.

18 Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Rev 2013; 27(1): 41–53.

19 Liu Q, Davidoff O, Niss K, Haase VH. Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. J Clin Invest 2012; 122(12): 4635–4644.

20 Yamashita K, Discher DJ, Hu J, Bishopric NH, Webster KA. Molecular regulation of the endothelin-1 gene by hypoxia. Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, AND p300/CBP. J Biol Chem 2001; 276(16): 12645–12653.

21 Fandrey J. Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression. Am J Physiol Regul

Integr Comp Physiol 2004; 286(6): R977–R988.

22 Koivunen P, Hirsila M, Gunzler V, Kivirikko KI, Myllyharju J. Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases. J Biol Chem 2004; 279(11): 9899–9904.

23 Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Horstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, Ratcliffe PJ, Bachmann S, Maxwell PH, Eckardt KU. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. FASEB J 2003; 17(2): 271–273.

24 Wiener CM, Booth G, Semenza GL. *In vivo* expression of mRNAs encoding hypoxia-inducible factor 1. Biochem Biophys Res Commun 1996; 225(2): 485–488.

25 Warnecke C, Zaborowska Z, Kurreck J, Erdmann VA, Frei U, Wiesener M, Eckardt KU. Differentiating the functional role of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha (EPAS-1) by the use of RNA interference: erythropoietin is a HIF-2alpha target gene in Hep3B and Kelly cells. FASEB J 2004; 18(12): 1462–1464.

26 Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, Shima H, Johnson RS, Hirao A, Suematsu M, Suda T. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell 2010; 7(3): 391–402.

27 Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP. Pharmacologic stabilization of HIF-1alpha increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation. Blood 2013; 121(5): 759–769.

28 Xue X, Ramakrishnan S, Anderson E, Taylor M, Zimmermann EM, Spence JR, Huang S, Greenson JK, Shah YM. Endothelial PAS domain protein 1 activates the inflammatory response in the intestinal epithelium to promote colitis in mice. Gastroenterology 2013; 145(4): 831–841.

29 Kikuchi H, Pino MS, Zeng M, Shirasawa S, Chung DC. Oncogenic KRAS and BRAF differentially regulate hypoxia-inducible factor-1alpha and -2alpha in colon cancer. Cancer Res 2009; 69(21): 8499–8506.

30 Taniguchi CM, Finger EC, Krieg AJ, Wu C, Diep AN, LaGory EL, Wei K, McGinnis LM, Yuan J, Kuo CJ, Giaccia AJ. Cross-talk between hypoxia and insulin signaling through Phd3 regulates hepatic glucose and lipid metabolism and ameliorates diabetes. Nat Med 2013; 19(10): 1325–1330.

31 Duan C. Hypoxia-inducible factor 3 biology: complexities and emerging themes. Am J Physiol Cell Physiol 2016; 310(4): C260–C269.

32 Yu T, Tang B, Sun X. Development of inhibitors targeting hypoxia-inducible factor 1 and 2 for cancer therapy. Yonsei Med J 2017; 58(3): 489–496.

33 Suzuki N, Gradin K, Poellinger L, Yamamoto M. Regulation of hypoxia-inducible gene expression after HIF activation. Exp Cell Res 2017; 63(1): 75–85.

34 Ramakrishnan SK, Shah YM. Role of intestinal HIF-2alpha in health and disease. Annu Rev Physiol 2016; 78: 301–325.

35 Meyron-Holtz EG, Ghosh MC, Rouault TA. Mammalian tissue oxygen levels modulate iron-regulatory protein activities *in vivo*. Science 2004; 306(5704): 2087–2090.

36 Triner D, Shah YM. Hypoxia-inducible factors: a central link between inflammation and cancer. J Clin Invest 2016; 126(10): 3689–3698.

37 Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, Weir TL, Ehrentraut SF, Pickel C, Kuhn KA, Lanis JM, Nguyen V, Taylor CT, Colgan SP. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe 2015; 17(5): 662–671.

38 Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. Cancer Res 2003; 63(8): 1764–1768.

39 Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 2005; 7(1): 77–85.

40 Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG, Karin M. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 2008; 453(7196): 807–811.

41 Siegert I, Schodel J, Nairz M, Schatz V, Dettmer K, Dick C, Kalucka J, Franke K, Ehrenschwender M, Schley G, Beneke A, Sutter J, Moll M, Hellerbrand C, Wielockx B, Katschinski DM, Lang R, Galy B, Hentze MW, Koivunen P, Oefner PJ, Bogdan C, Weiss G, Willam C, Jantsch J. Ferritin-mediated iron sequestration stabilizes hypoxia-inducible factor-1alpha upon lps activation in the presence of ample oxygen. Cell Rep 2015; 13(10): 2048–2055.

42 Haddad JJ, Harb HL. Cytokines and the regulation of hypoxia-inducible factor (HIF)-1alpha. Int Immunopharmacol 2005; 5(3): 461–483.

43 Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H,

Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature 2013; 496(7444): 238–242.

44 Takeda N, O'Dea EL, Doedens A, Kim JW, Weidemann A, Stockmann C, Asagiri M, Simon MC, Hoffmann A, Johnson RS. Differential activation and antagonistic function of HIF-{alpha} isoforms in macrophages are essential for NO homeostasis. Genes Dev 2010; 24(5): 491–501.

45 Haddad JJ, Land SC. A non-hypoxic, ROS-sensitive pathway mediates TNF-alpha-dependent regulation of HIF-1alpha. FEBS Lett 2001; 505(2): 269–274.

46 Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab 2009; 9(2): 152–164.

47 Sanchez M, Galy B, Muckenthaler MU, Hentze MW. Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. Nat Struct Mol Biol 2007; 14(5): 420–426.

48 Zimmer M, Ebert BL, Neil C, Brenner K, Papaioannou I, Melas A, Tolliday N, Lamb J, Pantopoulos K, Golub T, Iliopoulos O. Small-molecule inhibitors of HIF-2α translation link its 5'UTR iron-responsive element to oxygen sensing. Mol Cell 2008; 32(6): 838–848.

49 Anderson SA, Nizzi CP, Chang YI, Deck KM, Schmidt PJ, Galy B, Damnernsawad A, Broman AT, Kendziorski C, Hentze MW, Fleming MD, Zhang J, Eisenstein RS. The IRP1-HIF-2alpha axis coordinates iron and oxygen sensing with erythropoiesis and iron absorption. Cell Metab 2013; 17(2): 282–290.

50 Ghosh MC, Zhang DL, Jeong SY, Kovtunovych G, Ollivierre-Wilson H, Noguchi A, Tu T, Senecal T, Robinson G, Crooks DR, Tong WH, Ramaswamy K, Singh A, Graham BB, Tuder RM, Yu ZX, Eckhaus M, Lee J, Springer DA, Rouault TA. Deletion of iron regulatory protein 1 causes polycythemia and pulmonary hypertension in mice through translational derepression of HIF2alpha. Cell Metab 2013; 17(2): 271–281.

51 Koh MY, Darnay BG, Powis G. Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1alpha, leading to its oxygen-independent degradation. Mol Cell Biol 2008; 28(23): 7081–7095.

52 Luo W, Zhong J, Chang R, Hu H, Pandey A, Semenza GL. Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1alpha but Not HIF-2alpha. J Biol Chem 2010; 285(6): 3651–3663.

53 Richard DE, Berra E, Gothie E, Roux D, Pouyssegur J. p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. J Biol Chem 1999; 274(46): 32631–32637.

54 Sodhi A, Montaner S, Patel V, Zohar M, Bais C, Mesri EA, Gutkind JS. The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1alpha. Cancer Res 2000; 60(17): 4873–4880.

55 Hur E, Chang KY, Lee E, Lee SK, Park H. Mitogen-activated protein kinase kinase inhibitor PD98059 blocks the trans-activation but not the stabilization or DNA binding ability of hypoxia-inducible factor-1alpha. Mol Pharmacol 2001; 59(5): 1216–1224.

56 Peyssonnaux C, Nizet V, Johnson RS. Role of the hypoxia inducible factors HIF in iron metabolism. Cell Cycle 2008; 7(1): 28–32.

57 Shah YM, Xie L. Hypoxia-inducible factors link iron homeostasis and erythropoiesis. Gastroenterology 2014; 146(3): 630–642.

58 Vidal SM, Malo D, Vogan K, Skamene E, Gros P. Natural resistance to infection with intracellular parasites: isolation of a candidate for Bcg. Cell 1993; 73(3): 469–485.

59 Templeton DM, Liu Y. Genetic regulation of cell function in response to iron overload or chelation. Biochim Biophys Acta 2003; 1619(2): 113–124.

60 Chaloupka R, Courville P, Veyrier F, Knudsen B, Tompkins TA, Cellier MF. Identification of functional amino acids in the Nramp family by a combination of evolutionary analysis and biophysical studies of metal and proton cotransport *in vivo*. Biochemistry 2005; 44(2): 726–733.

61 Skjorringe T, Burkhart A, Johnsen KB, Moos T. Divalent metal transporter 1 (DMT1) in the brain: implications for a role in iron transport at the blood-brain barrier, and neuronal and glial pathology. Front Mol Neurosci 2015; 8: 19.

62 Di Lorenzo F. Iron and Parkinson's disease. Neuro Endocrinol Lett 2015; 36(1): 24–27.

63 Hubert N, Hentze MW. Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: implications for regulation and cellular function. Proc Natl Acad Sci U S A 2002; 99(19): 12345–12350.

64 Lee PL, Gelbart T, West C, Halloran C, Beutler E. The human Nramp2 gene: characterization of the gene structure, alternative splicing, promoter region and polymorphisms. Blood Cells Mol Dis 1998; 24(2): 199–215.

65 Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 1997; 388(6641): 482–488.

66 Wardrop SL, Richardson DR. The effect of intracellular iron concentration and nitrogen monoxide on Nramp2 expression and non-transferrin-bound iron uptake. Eur J Biochem 1999; 263(1): 41–49.

67 Jiang H, Song N, Xu H, Zhang S, Wang J, Xie J. Up-regulation of divalent metal transporter 1 in 6-hydroxydopamine intoxication is IRE/IRP dependent. Cell Res 2010; 20(3): 345–356.

68 Junxia X, Hong J, Wenfang C, Ming Q. Dopamine release rather than content in the caudate putamen is associated with behavioral changes in the iron rat model of Parkinson's disease. Exp Neurol 2003; 182(2): 483–489.

69 Donovan A, Lima CA, Pinkus JL, Pinkus GS, Zon LI, Robine S, Andrews NC. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab 2005; 1(3): 191–200.

70 Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, Fleming MD, Andrews NC, Zon LI. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 2000; 403(6771): 776–781.

71 McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, Miret S, Bomford A, Peters TJ, Farzaneh F, Hediger MA, Hentze MW, Simpson RJ. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell 2000; 5(2): 299–309.

72 Abboud S, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 2000; 275(26): 19906–19912.

73 Harris ZL, Durley AP, Man TK, Gitlin JD. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. Proc Natl Acad Sci U S A 1999; 96(19): 10812–10817.

74 Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, Gitschier J, Anderson GJ. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat Genet 1999; 21(2): 195–199.

75 De Domenico I, Ward DM, di Patti MC, Jeong SY, David S, Musci G, Kaplan J. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J 2007; 26(12): 2823–2831.

76 Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306(5704): 2090–2093.

77 Fleming MD. The regulation of hepcidin and its effects on systemic and cellular iron metabolism. Hematology Am Soc Hematol Educ Program 2008: 151–158.

78 Taylor M, Qu A, Anderson ER, Matsubara T, Martin A, Gonzalez FJ, Shah YM. Hypoxia-inducible factor-2alpha mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. Gastroenterology 2011; 140(7): 2044–2055.

79 Nicolas G, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, Vaulont S. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proc Natl Acad Sci U S A 2001; 98(15): 8780–8785.

80 Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, Sirito M, Sawadogo M, Kahn A, Vaulont S. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc Natl Acad Sci U S A 2002; 99(7): 4596–4601.

81 Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, Beaumont C, Kahn A, Vaulont S. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest 2002; 110(7): 1037–1044.

82 Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, Loreal O. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 2001; 276(11): 7811–7819.

83 Lesbordes-Brion JC, Viatte L, Bennoun M, Lou DQ, Ramey G, Houbron C, Hamard G, Kahn A, Vaulont S. Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. Blood 2006; 108(4): 1402–1405.

84 Wang J, Jiang H, Xie JX. Ferroportin1 and hephaestin are involved in the nigral iron accumulation of 6-OHDA-lesioned rats. Eur J Neurosci 2007; 25(9): 2766–2772.

85 Song N, Wang J, Jiang H, Xie J. Ferroportin 1 but not hephaestin contributes to iron accumulation in a cell model of Parkinson's disease. Free Radic Biol Med 2010; 48(2): 332–341.

86 Pelizzoni I, Zacchetti D, Smith CP, Grohovaz F, Codazzi F. Expression of divalent metal transporter 1 in primary hippocampal neurons: reconsidering its role in non-transferrin-bound iron influx. J Neurochem 2012; 120(2): 269–278.

87 Ke Y, Chang YZ, Duan XL, Du JR, Zhu L, Wang K, Yang XD, Ho KP, Qian ZM. Age-dependent and iron-independent expression of two mRNA isoforms of divalent metal transporter 1 in rat brain. Neurobiol Aging 2005; 26(5): 739–748.

88 Moos T, Skjoerringe T, Gosk S, Morgan EH. Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involve-

ment of divalent metal transporter 1. J Neurochem 2006; 98(6): 1946–1958.

89 Song N, Jiang H, Wang J, Xie JX. Divalent metal transporter 1 up-regulation is involved in the 6-hydroxydopamine-induced ferrous iron influx. J Neurosci Res 2007; 85(14): 3118–3126.

90 Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001; 276(11): 7806–7810.

91 Krause A, Neitz S, Magert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, Adermann K. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett 2000; 480(2–3): 147–150.

92 De Domenico I, Ward DM, Langelier C, Vaughn MB, Nemeth E, Sundquist WI, Ganz T, Musci G, Kaplan J. The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol Biol Cell 2007; 18(7): 2569–2578.

93 Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta 2012; 1823(9): 1434–1443.

94 Finberg KE. Regulation of systemic iron homeostasis. Curr Opin Hematol 2013; 20(3): 208–214.

95 Meyron-Holtz EG, Ghosh MC, Iwai K, LaVaute T, Brazzolotto X, Berger UV, Land W, Ollivierre-Wilson H, Grinberg A, Love P, Rouault TA. Genetic ablations of iron regulatory proteins 1 and 2 reveal why iron regulatory protein 2 dominates iron homeostasis. EMBO J 2004; 23(2): 386–395.

96 Galy B, Ferring-Appel D, Kaden S, Grone HJ, Hentze MW. Iron regulatory proteins are essential for intestinal function and control key iron absorption molecules in the duodenum. Cell Metab 2008; 7(1): 79–85.

97 Wallander ML, Leibold EA, Eisenstein RS. Molecular control of vertebrate iron homeostasis by iron regulatory proteins. Biochim Biophys Acta 2006; 1763(7): 668–689.

98 Sanchez M, Galy B, Schwanhaeusser B, Blake J, Bahr-Ivacevic T, Benes V, Selbach M, Muckenthaler MU, Hentze MW. Iron regulatory protein-1 and -2: transcriptome-wide definition of binding mRNAs and shaping of the cellular proteome by iron regulatory proteins. Blood 2011; 118(22): e168–e179.

99 Muckenthaler MU, Galy B, Hentze MW. Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. Annu Rev Nutr 2008; 28: 197–213.

100 Hentze MW, Kuhn LC. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. Proc Natl Acad Sci U S A 1996; 93(16): 8175–8182.

101 Anderson ER, Shah YM. Iron homeostasis in the liver. Compr Physiol 2013; 3(1): 315–330.

102 Casey JL, Koeller DM, Ramin VC, Klausner RD, Harford JB. Iron regulation of transferrin receptor mRNA levels requires iron-responsive elements and a rapid turnover determinant in the 3' untranslated region of the mRNA. EMBO J 1989; 8(12): 3693–3699.

103 Cheng CM, Wang D, Cao X, Luo QQ, Lu YP, Zhu L. Iron regulatory protein 1 suppresses hypoxia-induced iron uptake proteins expression and decreases iron levels in HepG2 cells. J Cell Biochem 2015; 116(9): 1919–1931.

104 Gulec S, Anderson GJ, Collins JF. Mechanistic and regulatory aspects of intestinal iron absorption. Am J Physiol Gastrointest Liver Physiol 2014; 307(4): G397–G409.

105 Yang X, Xu MM, Wang J, Xie JX. Effect of estrogen on iron metabolism in mammals. Acta Physiol Sin (生理学报) 2016; 68(5): 637–643.

106 Rolfs A, Kvietikova I, Gassmann M, Wenger RH. Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. J Biol Chem 1997; 272(32): 20055–20062.

107 Tacchini L, Bianchi L, Bernelli-Zazzera A, Cairo G. Transferrin receptor induction by hypoxia. HIF-1-mediated transcriptional activation and cell-specific post-transcriptional regulation. J Biol Chem 1999; 274(34): 24142–24146.

108 Aisen P, Brown EB. Structure and function of transferrin. Prog Hematol 1975; 9: 25–56.

109 Mastrogiannaki M, Matak P, Keith B, Simon MC, Vaulont S, Peyssonnaux C. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. J Clin Invest 2009; 119(5): 1159–1166.

110 McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, Mudaly M, Richardson C, Barlow D, Bomford A, Peters TJ, Raja KB, Shirali S, Hediger MA, Farzaneh F, Simpson RJ. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science 2001; 291(5509): 1755–1759.

111 Knutson MD, Oukka M, Koss LM, Aydemir F, Wessling-Resnick M. Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin. Proc Natl Acad Sci U S A 2005; 102(5): 1324–1328.

112 Yang L, Fan M, Du F, Gong Q, Bi ZG, Zhu ZJ, Zhu LL, Ke Y. Hypoxic preconditioning increases iron transport rate in astrocytes. Biochim Biophys Acta 2012; 1822(4): 500–508.

113 Matsushima K, Hakim AM. Transient forebrain ischemia protects against subsequent focal cerebral ischemia without changing cerebral perfusion. Stroke 1995; 26(6): 1047–1052.

114 Vannucci RC, Towfighi J, Vannucci SJ. Hypoxic preconditioning and hypoxic-ischemic brain damage in the immature rat: pathologic and metabolic correlates. J Neurochem 1998; 71(3): 1215–1220.

115 Gidday JM, Shah AR, Maceren RG, Wang Q, Pelligrino DA, Holtzman DM, Park TS. Nitric oxide mediates cerebral ischemic tolerance in a neonatal rat model of hypoxic preconditioning. J Cereb Blood Flow Metab 1999; 19(3): 331–340.

116 Wagner BP, Nedelcu J, Martin E. Delayed postischemic hypothermia improves long-term behavioral outcome after cerebral hypoxia-ischemia in neonatal rats. Pediatr Res 2002; 51(3): 354–360.

117 Wu LY, Ding AS, Zhao T, Ma ZM, Wang FZ, Fan M. Underlying mechanism of hypoxic preconditioning decreasing apoptosis induced by anoxia in cultured hippocampal neurons. Neurosignals 2005; 14(3): 109–116.

118 Du F, Zhu L, Qian ZM, Wu XM, Yung WH, Ke Y. Hyperthermic preconditioning protects astrocytes from ischemia/reperfusion injury by up-regulation of HIF-1 alpha expression and binding activity. Biochim Biophys Acta 2010; 1802(11): 1048–1053.

119 Bianchi L, Tacchini L, Cairo G. HIF-1-mediated activation of transferrin receptor gene transcription by iron chelation. Nucleic Acids Res 1999; 27(21): 4223–4227.

120 Tacchini L, Fusar Poli D, Bernelli-Zazzera A, Cairo G. Transferrin receptor gene expression and transferrin-bound iron uptake are increased during postischemic rat liver reperfusion. Hepatology 2002; 36(1): 103–111.

121 Weinreb O, Mandel S, Youdim MB, Amit T. Targeting dysregulation of brain iron homeostasis in Parkinson's disease by iron chelators. Free Radic Biol Med 2013; 62: 52–64.

122 Guo C, Hao LJ, Yang ZH, Chai R, Zhang S, Gu Y, Gao HL, Zhong ML, Wang T, Li JY, Wang ZY. Deferoxamine-mediated up-regulation of HIF-1alpha prevents dopaminergic neuronal death via the activation of MAPK family proteins in MPTP-treated mice. Exp Neurol 2016; 280: 13–23.

123 Lim J, Kim HI, Bang Y, Seol W, Choi HS, Choi HJ. Hypoxia-inducible factor-1alpha upregulates tyrosine hydroxylase and dopamine transporter by nuclear receptor ERRgamma in SH-SY5Y cells. Neuroreport 2015; 26(6): 380–386.

124 Wang J, Pantopoulos K. Regulation of cellular iron metabolism. Biochem J 2011; 434(3): 365–381.

125 Wilkinson N, Pantopoulos K. IRP1 regulates erythropoiesis and systemic iron homeostasis by controlling HIF2alpha mRNA translation. Blood 2013; 122(9): 1658–1668.

126 Kapitsinou PP, Liu Q, Unger TL, Rha J, Davidoff O, Keith B, Epstein JA, Moores SL, Erickson-Miller CL, Haase VH. Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. Blood 2010; 116(16): 3039–3048.

127 Galy B, Ferring D, Hentze MW. Generation of conditional alleles of the murine Iron Regulatory Protein (IRP)-1 and -2 genes. Genesis 2005; 43(4): 181–188.

128 Frazer DM, Wilkins SJ, Becker EM, Vulpe CD, McKie AT, Trinder D, Anderson GJ. Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. Gastroenterology 2002; 123(3): 835–844.

129 Luo QQ, Wang D, Yu MY, Zhu L. Effect of hypoxia on the expression of iron regulatory proteins 1 and the mechanisms involved. IUBMB Life 2011; 63(2): 120–128.

130 Luo QQ, Qian ZM, Zhou YF, Zhang MW, Wang D, Zhu L, Ke Y. Expression of iron regulatory protein 1 is regulated not only by HIF-1 but also pCREB under hypoxia. Int J Biol Sci 2016; 12(10): 1191–1202.