Hypoxia and its Emerging Therapeutics in Neurodegenerative, Inflammatory and Renal Diseases

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Abstract

Hypoxia is a common underlying condition of many disease states. Hypoxia can occur with ischemia, a lack of blood flow to tissues, or independent of ischemia as in acute lung injury, anemia, and carbon monoxide poisoning. Hypoxia may be observed in patients with diseases such as obstructive sleep apnea, cerebrovascular diseases, systemic hypertension, cardiovascular diseases, chronic obstructive pulmonary disease (COPD), pulmonary hypertension and congestive heart failure (CHF), inflammatory disease states, and acute and chronic renal diseases. In the past decade, research has shown hypoxic signaling to be involved in a range of responses from adaptation of the body to reduced oxygen to pathogenesis of disease. Hypoxic signaling intermediates orchestrate a whole host of responses from angiogenesis, glycolysis, and erythropoiesis to inflammation and remodeling, which could be beneficial or harmful to the hosting organ. The length of exposure to low oxygen pressure as well as the existing signaling pathways within different cells dictates their benefit or disadvantage from hypoxic signaling. Therefore, activation or inhibition of hypoxic intermediates could serve as novel therapeutic strategies. In this chapter, we review the role of hypoxic signaling in neurodegenerative, inflammatory, and renal disease states and the emerging therapeutic approaches involving hypoxic signaling.

Keywords: hypoxia, hypoxia-inducible factor, neurodegenerative disease, Parkinson's disease, Alzheimer's disease, ischemia/reperfusion, inflammation, epigenetics, micro-RNA, inflammatory bowel disease, rheumatoid arthritis, acute kidney injury, chronic kidney disease, erythropoiesis, anemia, allograft rejection

1. Hypoxia and neurodegenerative diseases

1.1. Introduction

Neurodegenerative diseases are defined by the progressive loss of specific neuronal cell population and protein misfolding and aggregate. Reduced oxygen supply has been detected during the aging process as well as the pathogenesis of neurodegenerative diseases. Besides, diseases associated with a lowering of systemic oxygen levels predispose individuals to neurodegenerative diseases. Although the connection between hypoxia and neurodegeneration has been well established, the exact role of hypoxia in neurodegenerative diseases has yet to be elucidated.

This section summarizes current identified clues linking hypoxia to the onset and progression of neurodegenerative diseases, including neurotoxic effects, altered signaling transduction and protein expression, and abnormal epigenetic modification. Furthermore, the following discussion emphasizes on the detrimental impacts of cerebral oxygen deficiency on three major neurodegenerative diseases: Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS).

1.1.1. Hypoxia and Alzheimer's disease

AD is characterized by progressive impairments in memory and cognitive function. The hallmark features of AD are extracellular plaques whose major components are amyloid β peptide (Aβ) and intracellular neurofibrillary tangles constituted by hyperphosphorylated tau protein. Other changes identified in AD brains are loss of synapses and neurons, proliferation of reactive astrocytes, and microglial activation. The incidence of AD in the United States is 11% among the population aged over 65 years and approximately 32% among those 85 years and older (Alzheimer's Association, 2015) [1]. Apparently, aging is the most significant risk factor for AD, since the risk of developing AD doubles every 5 years after the age of 65 years. Other factors, including environmental neurotoxins/metals, gene mutations, susceptibility polymorphisms, cardiovascular diseases, traumatic brain injury, and ischemia/hypoxia, also potentially prompt the development of AD.

Although the exact mechanisms and triggers initiating AD remain unclear, both clinical and preclinical studies suggest that hypoxia should be considered as an important risk factor in AD pathogenesis. Chronic cerebral hypoperfusion and glucose hypometabolism appearing decades before cognitive dysfunction promote the initiation and progression of cognitive decline and AD [2]. Patients after cerebral hypoxia or ischemia are more susceptible to developing dementia. Cerebral blood flow (CBF) reduction decreases the synthesis of proteins necessary for memory and learning and contributes likely to neuritic injury, neuronal death, and the onset and progression of dementia [3]. Correspondingly, significantly reduced resting CBF is distinguished in AD patients and is also present in the early stages of AD pathogenesis [4].

Generally, hypoxia modifies Aβ production and tau phosphorylation at numerous points (**Figure 1**). Aβ is a cleavage product generated through the sequential actions of β- and γ-

secretases on amyloid precursor protein (APP). Hypoxia can stimulate Aβ generation and senile plaque formation in AD through increasing the expression of β- and γ-secretases along with the localization of γ-secretase from cell body to axon [5]. Furthermore, hypoxia elevates the levels of APP and presenilin-1 (PS-1), a main component of γ-secretase complex, in vivo [6]. The expression of neprilysin (NEP), an enzyme responsible for Aβ degeneration, is reduced during hypoxia [7]. Rats exposed to hypoxic stress display tau hyperphosphorylation in the hippocampus as well as memory deficit, and Aβ-induced tau phosphorylation is raised through calpain upon hypoxia exposure [8, 9]. The activity of protein phosphatase 2A (PP2A) is compromised in AD and is believed to be a cause of tau neurofibrillary. Brain hypoxia generates an acidic environment that promotes the cleavage of I_2^{PP2A}, a potent inhibitor of PP2A, by activating asparaginyl endopeptidase, thus giving rise to tau hyperphosphorylation [10].
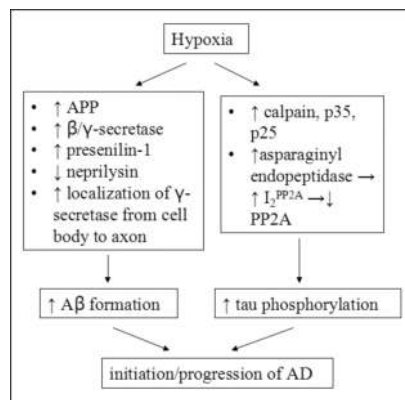
Figure 1. The molecular mechanisms of hypoxic predisposition to AD.

1.1.2. Hypoxia and Parkinson's disease

The clinical features of PD include classical motor symptoms (bradykinesia, rigidity, postural instability, resting tremor) and non-motor symptoms (dementia, sleep disorder, depression, autonomic dysfunction), resulting from a continuous degeneration and loss of dopaminergic neurons in the substantia nigra (SN) and the presence of intracytoplasmic proteinaceous inclusions called Lewy bodies (LB) [11].

α-Synuclein (α-syn), a major constituent of LB, is the pathological hallmark of PD. Hypoxic brain injury is a potential cause of PD, as it enhances α-synuclein expression and aggregation [12]. ATP13A2 (PARK9) mutations have been found in postmortem PD patients, declaring its relevance to PD pathogenesis [13]. Although the exact molecular mechanism remains un-known, it turns out that hypoxia upregulates ATP13A2 transcription via HIF-1 alpha (HIF-1α) in dopaminergic cells [14]. Hypoxia changes the localization of intracellular hemo-globin whose overexpression is correlated with an increased risk of PD [15]. In addition,

subnormal sensitivity to hypoxia has been noticed in PD patients even at an early stage of diseases, probably leading to the exacerbation of respiratory failure in PD [16].

1.1.3. Hypoxia and amyotrophic lateral sclerosis

ALS, also known as Lou Gehrig's disease, is a progressive and fetal disease resulted from damaged motor neurons in the spinal cord, brain stem, and motor cortex. The incidence rate of ALS worldwide is estimated to be 2 in 100,000 people, and in the United States, about 5000 persons are diagnosed with ALS every year [17]. ALS risk is influenced by physical activity, smoking habit, type of diet, and exposure to agriculture chemicals and heavy metals. Occupations that may cause intermittent hypoxia, such as fire fighter, double the risk of ALS, and genetic impairment in reaction to hypoxia predisposes motor neuron to death [18].

Hypoxia is not only a causative factor of ALS but also accelerates the progression of ALS. Motor neurons under hypoxic conditions fail to survive and undergo degeneration [19]. SOD1^{G93A} mutant mice, an ALS animal model, have experienced aggravation in motor neuronal loss, neuromuscular weakness and possibly cognitive deficiency, with higher level of oxidative stress and inflammation after chronic intermittent hypoxia [20]. Chronic sustained hypoxic condition induces the activation of apoptosis-related genes such as caspase 3, apoptosis-inducing factor (AIF), and cytochrome C in motor neurons from the spinal cord of ALS mice, facilitating the progression of ALS [21].

1.2. The mechanism of hypoxia-induced injury in neural cells

Cellular and molecular pathways underlying hypoxia-induced neurotoxicity and cell death are multifaceted and complex, including a number of cross-talked mechanisms. Ensuing hypoxia stimulates the production and release of proteins mediating oxidative stress, inflammation, apoptosis, mitochondrial metabolism, metal homeostasis, synaptic transmission, and autophagy, contributing to neuronal death (**Figure 2**).
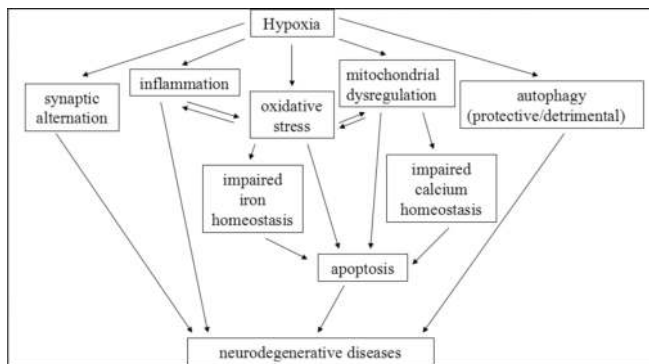
Figure 2. Different pathogenic mechanisms linking hypoxia to neurodegenerative diseases.

1.2.1. Hypoxia-promoted oxidative stress

Oxidative stress has been implicated in hypoxic injury and neurodegenerative diseases. It occurs due to the disruption of oxidative balance and excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide (O_2^-), and the highly reactive hydroxyl radicals ($\cdot$OH) [22]. The production of ROS and RNS is increased under hypoxic condition, probably because there is no acceptor for the electrons available. During hypoxic events, high levels of free radicals are produced through mitochondrial complex III, and the antioxidant status is depleted, thus leading to oxidative damage of vital cellular components. For instance, neuroblastoma cells exposed to hypoxia have augmented production of free radicals accompanied by a concomitant decrease in reduced glutathione (GSH) content, glutathione reductase (GR), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities, further inciting apoptotic death [22].

Increased oxidative stress is believed to be associated with neurological disorders and classical neuropathy. Reduced antioxidant capacity is a trait of AD. The activation of NO/NOS signaling system by cerebral ischemia in aged rats triggers hippocampal Aβ production through β-secretase 1 (BACE1) pathway, implying RNS is a bridge linking hypoxia to AD [23]. In retinal ganglion cells (RGEs) derived from rats, hypoxia exposure triggers Aβ formation, intracellular ROS accumulation, and following cell death, suggesting the involvement of Aβ in hypoxia-induced retinal degeneration in AD [24]. In PD, the promotion of ROS formation is highly correlated to mutant α-syn phosphorylation at serine 129 (Ser129), possibly preceding cell degeneration [25]. Agents with antioxidant property ameliorate neurodegenerative situation, including natural compounds and iron chelators.

1.2.2. Hypoxia-altered ionic homeostasis

Impaired cellular homeostasis of metals can be triggered by hypoxic conditions, resulting in neurodegeneration through various mechanisms, such as oxidative stress, inflammation, and aberrant expression of metalloproteins.

Calcium dyshomeostasis is a fundamental mechanism in the pathogenesis of neurodegenerative diseases. The interaction between γ-aminobutyric acid (GABA) and calcium-dependent neurotransmission as well as calcium-dependent neuronal metabolism also reveals the role of Ca^{2+} in neuronal degeneration. Ca^{2+} acts as an intracellular messenger, controlling not only transsynaptic signal transmission but also cellular metabolism by reaching the mitochondria [26]. Hypoxia can disrupt Ca^{2+} entry and signaling in various cell types. In hypoxic human neuroblastoma cells, the storage of intracellular Ca^{2+}, Na^+/Ca^{2+} exchange, and capacitative Ca^{2+} entry are boosted, indicating adaptive cellular remodeling in response to prolonged hypoxia [27]. Similarly, chronic hypoxia enhances capacitative Ca^{2+} entry and mitochondria Ca^{2+} content in the primary culture of rat type-I cortical astrocytes [28]. In terms of AD, chronic hypoxia potentiates posttranscriptional trafficking of L-type Ca^{2+} channels that may result from the interaction between Aβ and Ca^{2+} channel subunit [29].

Iron can be released from storage protein in the brain under hypoxic circumstances, and disruption of intracellular free iron homeostasis is an early event upon hypoxic stimulation in oligodendrocytes that contain enriched iron and ferritin [30]. Progressive hypoxia dramatically activates the synthesis of ferritin, a major iron-binding protein, in oligodendrocytes, and this induction may require ROS formation as it can be enhanced by co-treatment with H_2O_2 [31]. Intracellular free iron has neurotoxic effects. Iron promotes Aβ aggregation in vitro [32], and iron-Aβ interaction exhibits toxic effects through ROS [33]. Iron also binds to tau, but interestingly, its effect on tau relies on the oxidation state. Fe^{3+} induces the aggregation of hyperphosphorylated tau and reduces the phosphorylation of tau, whereas Fe^{2+} exerts an opposite action [34]. As for PD, abnormal accumulation of iron results in α-syn aggregation by promoting its synthesis and inhibiting its degradation [35].

1.2.3. Hypoxia-disrupted mitochondrial functions

The consequences of mitochondrial dysfunction cover oxidative stress, intracellular Ca^{2+} dysregulation, apoptosis, and metabolic failure, aggravating the deleterious effect.

Respiratory chain reprogramming is the first stage in the development of hypoxia-triggered mitochondrial disorders, converting complex I electron transport chain (ETC) to complex II succinate oxidation. The activation of succinate is regarded as a protective and compensatory mechanism in response to oxygen shortage and preserves the aerobic energy production [36]. Otherwise, the dysregulation of complex I during oxygen deficiency may lead neurons to acute degeneration, characterized by decreased membrane potential, loss of ATP, and respiration disorders caused by abnormal oxidation of nicotinamide adenine dinucleotide (NADH) [37]. The study of mitochondrial genes informs that hypoxia upregulates genes involved in glycolytic pathways, indicating a shift in energy production from oxidative phosphorylation to glycolysis, which converts glucose to pyruvate and eventually lactate. This shift is supported by the observation of elevated brain extracellular lactate concentration in traumatic brain injury (TBI) patients. A cerebral microdialysis study discloses that the neurons in TBI patient are unable to utilize lactate produced by astrocyte through tricarboxylic acid (TCA) cycle, leading to increased lactate/pyruvate ratio [38]. In addition, the ketogenic capacity of cultured astroglia and neurons is augmented under hypoxia, probably because of the susceptibility of pyruvate dehydrogenase to oxygen deprivation [39].

Many rare mitochondrial diseases are actually models of neurodegeneration, such as Leber's hereditary optic neuropathy (LHON) and autosomal dominant optic atrophy (ADOA), and abnormal mitochondrial function has been discovered in several age-related neurodegenerative diseases. Suppression of complex I potentiates tau phosphorylation, pointing out the role of mitochondrial dysfunction in the formation of tangles in AD [40]. During prolonged exposure to hypoxia, ROS production, Aβ accumulation, and Ca^{2+} dyshomeostasis are enhanced through regulation on ETC [41]. The SN of PD patients has reduced activity of mitochondrial complex I, and inhibitors of complex I produce neurological changes similar to PD [42].

1.2.4. Hypoxia-mediated apoptotic cascades

Cerebral hypoxia results in increased activities of caspase-9, caspase-8, and caspase-3 in the cerebral cortex of newborn piglets and enhances cytochrome C expression and caspase-3 activity followed by the induction of apoptosis in neuroblastoma cells. NO induced by hypoxia exerts proapoptotic property through elevating the expression of proteins such as Bax and Bad, leading to APAF-1 activation and consequential activation of caspase-9 and caspase-3, and, on the other hand, through downregulating antiapoptotic proteins of the B-cell lymphoma-2 (Bcl-2) family [22, 43] . Exposure of primary neuron cells from ALS mice to chronic sustained hypoxia results in enhanced cellular apoptosis, suggesting hypoxia could accelerate ALS via neuronal apoptosis [21]. Angiogenin (ANG) is a potent inducer of neovascularization and is responsive to hypoxia. Silence of ANG promotes hypoxic injury-induced motor neuron apoptosis, while exogenous overexpression of ANG has an antiapoptotic function. Mutation of ANG has been identified in ALS patients, proposing the importance of ANG in ALS pathogenesis [44].

Blockage of apoptosis can be neuroprotective. Rasagiline and its derivatives, a group of highly potent irreversible monoamine oxidase (MAO) B inhibitor, exert their anti-Parkinson feature by preventing apoptotic cascades. They activate Bcl-2 and protein kinase C (PKC) and inhibit proapoptosis FAS and Bax against neuronal apoptosis [45]. Treatment of 0.5% isoflurane, an inhaled anesthetic, attenuates caspase-3 activation, BACE upregulation, and Bcl-2 reduction caused by hypoxia in H4 human neuroglioma cells, hinting the neuroprotective effect of isoflurane in AD [46].

1.2.5. Hypoxia-modified synaptic signaling

Synaptic transmission in the central nervous system (CNS) is extremely sensitive to hypoxia, since it requires 30–50% of cerebral oxygen. Decrease in synaptic efficacy occurs very early during hypoxia and is possibly the first response of neurons to ischemic insult.

Oxygen-sensitive ion channels and voltage-gated Ca^{2+} and K^+ channel are activated in response to hypoxia, bringing about changes in excitation and inhibition of neuronal and glial cells [47]. Under hypoxic circumstance, there is an accumulation of adenosine in the extracellular space, due to the increased catabolism of adenosine triphosphate (ATP) into adenosine monophosphate (AMP) [48]. Adenosine is a neurotransmitter inhibiting synaptic transmission, and its effect is mediated by adenosine A1 receptor. The mechanism is that receptor activation stimulates inwardly rectifying K^+ channels, substantially inhibiting Ca^{2+} channels, phospholipase C activation, and the release of neurotransmitters including glutamate, dopamine, serotonin, and acetylcholine [49].

P2Y1 receptor is a G-protein-coupled ATP receptor activated by ATP released from neurons and astrocytes during neuronal activity or under pathophysiological conditions such as hypoxia, brain injury, and AD [50]. Emerging evidence shows that P2Y1 receptor obstructs the release of neurotransmitters and modulates synaptic plasticity in the brain, especially in the prefrontal cortex, hippocampus, and cerebellum, leading to impaired cognitive process [50]. P2Y1 receptors are localized with AD features such as neurofibrillary tangles and neuritic

plaques, suggesting the altered distribution of P2Y1 in AD brains [51]. Astrocytic hyperactivity consisting of single-cell transients and Ca^{2+} waves has been observed around Aβ plaques. P2Y1 receptors are strongly expressed by reactive astrocytes, and blockade of P2Y1 receptors can reduce astrocytic hyperactivity back to normal [52].

1.2.6. Hypoxia and autophagy

In general, autophagy is regarded as a survival mechanism, but under severe hypoxia/ischemia, autophagy may cause self-digestion and eventual cell death due to its overactivation [53]. The morphological characteristics of autophagic-programmed cell death have been observed in both mice and rats with cerebral ischemia [54, 55].

Enormous studies indicate autophagy dysfunction in AD. Autophagic vacuoles (AVs) are significantly accumulated in the brain of AD patients compared to normal brain, possibly leading to lysosomal enzyme dysfunction [56]. The cross talk between autophagy and tau aggregation indicates the change of autophagic function in the pathogenesis of AD. Autophagy initially degrades tau to protect neurons; however, hyperphosphorylation of tau results in autophagic dysfunction, which substantially exacerbates AD via inducing tau aggregation [57, 58]. Remarkably, hypoxia induces autophagic activation through AMPK-mTOR signaling, resulting in more Aβ production and AD aggravation in vitro [56].

Defective autophagy has been implicated in PD [59], and several mutations in PD are strongly relevant to autophagy dysregulation, such as PTEN-induced putative kinase 1 (PINK1) [60]. Autophagy in ALS prevents neurons from degeneration, and inhibition of autophagy aggravates motor neuron viability, since the aggregates composed of intermediate filaments and insoluble forms of proteins can be cleared by autophagy pathway [61].

1.3. The role of hypoxia-sensitive transcription factors in neurodegenerative diseases

Several transcription factors are responsive to hypoxia and subsequently alter gene expression and cellular activity. The signaling pathways relevant to these transcription factors have been indicated in the development of neurodegenerative diseases. Therefore, these transcription factors may provide a link between hypoxic environment and neurodegeneration. The following discussion will include HIF-1, the most well-studied hypoxia-inducible gene, and two other redox-sensitive transcription factors, nuclear factor-kappa B (NF-κB) and NF-E2-related factor 2 (Nrf2).

1.3.1. Hypoxia-inducible factor-1

Hypoxia-inducible factor-1 (HIF-1) is a transcriptional activator involved in oxygen hemostasis, regulating the expression of genes and the activation of signaling pathways that participate in angiogenesis, erythropoiesis, neovascularization, iron metabolism, glucose metabolism, cell proliferation, apoptosis, and cell cycle control (**Figure 3**).
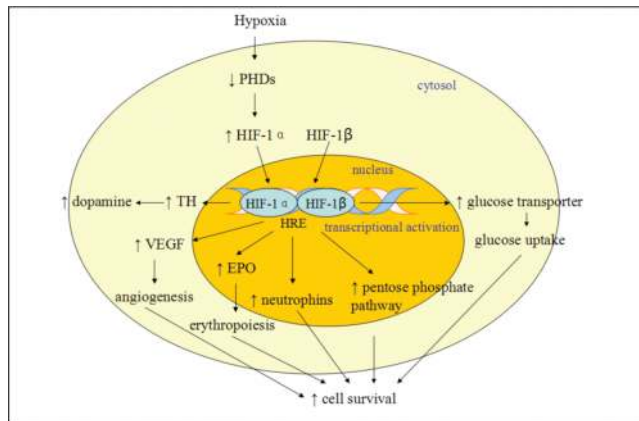
Figure 3. The neuroprotective role of HIF-1α activation in hypoxia.

In AD, HIF-1α upregulates neuronal glucose transporters such as GLUT-1 and GLUT-3 and facilitates glucose uptake, thus providing increased oxygen supply to hypoxic tissues [62]. It also contributes to cell survival by inducing the key enzymes in pentose phosphate pathway, including glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [63]. HIF-1α also connects hypoxia to amyloidogenic processing of APP through transcriptionally upregulating BACE1 and eventually increases Aβ formation [64].

The protective role of HIF-1 in PD has been demonstrated by its ability to increase dopamine synthesis and dopaminergic neuron growth. Tyrosine hydroxylase (TH) is the rate-limiting enzyme of dopamine synthesis in dopaminergic neurons, and interestingly, it contains an HRE [65]. HIF-1 elevated in response to hypoxia increases TH expression in rat brain stem, and HIF-1α conditional knockout mice exhibit reduced expression of TH and aldehyde dehydrogenase in SN [57]. HIF-1 activation may defend against dysregulation of brain iron homeostasis and mitochondria in PD. Iron accumulation has been observed in the SN of PD patients and is considered as a culprit of ROS generation and intracellular α-syn aggregation [66]. Moreover, the neurotransmitter dopamine is a metal reductant that reduces the oxidation state of metals such as Fe^{3+} and subsequently results in elevated oxidative stress [67]. Defer-oxamine (DFO), an iron chelator, prevents neurotoxicity in MPTP-treated mice through upregulation of HIF-1α protein expression, leading to declined expression of proteins such as α-syn, divalent metal transporter with iron-responsive element (DMT1 + IRE) and transferrin receptor (TFR), and elevated expression of HIF-1 target genes, including TH, vascular endo-thelial growth factor (VEGF), and growth associated protein 43 (GAP43) [68].

HIF-1 activation during hypoxia should be beneficial to ALS. HIF-1-VEGF pathway can induce angiogenesis and increase blood supply to motor neurons. VEGF overexpression delays motor neuron loss and impairment in SOD1^{G93A} mutant mice and prolongs the survival of mice [69]. Deletion of HRE in VEGF promoter region abolishes hypoxia-increased VEGF expression, causing motor neuron degeneration [70]. Additionally, HIF-1-erythropoietin (EPO) pathway

is suggested to be a new therapeutic target for ALS. EPO treatment in SOD1^{G93A} mice postpones the onset and progression of motor deterioration and modulates the immune-inflammatory response through reducing the levels of pro-inflammatory cytokines and enhancing the expression of anti-inflammatory cytokines [71, 72]. However, both above pathways are impaired in ALS. The level of VEGF is low in the CSF of early ALS patients, and likewise, the expression of VEGF in the CSF from hypoxemic ALS patients is lower than that in the CSF from normoxemic ALS patients [73, 74]. EPO protein level is declined in the surrounding glial cells of SOD1^{G93A} mice, and in the anterior horn cells (AHCs) from SOD1^{G93A} mice, impaired cytoplasmic-nuclear transport of HIF-1α has appeared since the presymptomatic stage, indicating the abnormality in HIF-1 pathway might precede motor neuron degradation [75, 76].

The well-studied group of agents targeting HIF-1 is iron chelators. The neuroprotective and neurorestorative activities of M30, an iron chelator with brain-selective monoamine oxidase (MAO) AB inhibitory function, share a same pathway, the activation of HIF-1, in different neurodegenerative diseases. M30 elevates HIF-1 to regulate neurotrophins BDNF, GDNF, VEGF, and EPO in PD, and meanwhile, it delays the onset of ALS in SOD1^{G93A} mutant mice through HIF-1 upregulation [77, 78]. In APP/PS1 AD mice model, M30 treatment upregulates HIF-1α in the frontal cortex, resulting in the beneficial modulation of target glycolytic gene expression, such as aldolase A, enolase-1, and GLUT-1 [79].

Taken together, HIF-1 is a key player protecting neuron cells against hypoxia and oxidative stress, as well as a reasonable therapeutic target against major neurodegenerative diseases, since its participation in the pathogenesis of neurodegeneration has been well identified.

1.3.2. Nuclear factor-kappa B

Nuclear factor-kappa B (NF-κB) is analogous to HIF-1 in structure, function, and mechanism of activation and plays a critical role in inflammation, immune response, synaptic transmission, neuronal plasticity, and apoptosis [80]. In resting state, NF-κB is complexed with the inhibitory subunit I-κB; however, under physiological or pharmacological stimulus such as oxidative stress, I-kappa B (I-κB) is degraded, leading to translocation of NF-κB from cytoplasm to nucleus to modulate gene transcription. NF-κB and I-κB proteins comprise a growing family of structurally related transcription factors, and functional NF-κB complexes are present in generally all cell types in the nervous system, such as neurons, astrocytes, microglia, and oligodendrocytes [81, 82]. In neurons, the most common variants consist of p50, p65/RelA, and I-κB subunits.

As a redox-sensitive transcription factor, the mobilization and upregulation of NF-κB have been reported in hypoxia and ischemia-reperfusion damage. Hypoxic-ischemic brain damage (HIBD) upregulates the expression of NF-κB and the NO content in rat cortex cells, suggesting the involvement of NF-κB/nNOS pathway during the recovery of HIBD-induced neuron damage [83]. The role of NF-κB in neonatal HIBD depends on the duration of hypoxia. Early activation of NF-κB is detrimental, and at that time point, treatment of NF-κB inhibitor, TAT-NBD, exhibits significant therapeutic outcomes, whereas late NF-κB activation enhances antiapoptotic pathway and contributes to endogenous neuroprotection [84]. The overall effect

of NF-κB activation seems to facilitate ischemic neuronal degeneration, but still, the effect can be either neuroprotective or deleterious depending on the cell type and the strength of signal [85]. The suppression of NF-κB or I-κB in neuron can reduce infarct size after stroke, and the inhibition of NF-κB caused by Ginkgolide B has protective effects on ischemic stroke [86, 87].

NF-κB activation has been observed in neurons and astroglia of brain sections from AD patients but only in cells surrounding early plaques, suggesting that the induction of NF-κB activity by Aβ is partially responsible for the aberrant gene expression in diseased nervous tissue [88]. In addition, intraperitoneal injection of sodium hydrosulfide (NaHS), a donor of H_2S whose level is reduced in the hippocampus of Aβ-injected rats, inhibits MAPK/NF-κB pathway and dramatically mitigates cognitive decline and neuroinflammation [83]. Another novel drug for AD, Gx-50, exerts anti-inflammatory effects against Aβ-triggered microglial overactivation in AD mice model via inhibition of NF-κB signaling [89].

Increased NF-κB activation has been reported in dopaminergic neurons of SN from PD patients, as well as in astrocytes of spinal cords from ALS patients [90]. Compounds inhibiting NF-κB translocation in microglia such as vinyl sulfone compound (VSC2) downregulate the expression of inducible NOS (iNOS) and TNF-α, leading to anti-inflammatory and antioxidant events in PD animal model [91]. NF-κB is also involved in microglia-induced motor neuron death in ALS. Deletion of NF-κB signaling in microglia rescues motor neuron from microglia-mediated death and extends survival in ALS mice by impairing pro-inflammatory microglial activation [92].

Collectively, NF-κB is responsive to the injury of nervous system in both acute and chronic neurodegenerative conditions. Agents suppressing NF-κB activation have been tested in animal models of neurodegenerative conditions, but their usage should be considered cautiously because of the involvement of NF-κB in learning and memory.

1.3.3. NF-E2-related factor 2

NF-E2-related factor 2 (Nrf2) is a basic leucine zipper (bZIP) transcription factor that is ubiquitously expressed in a wide range of tissues and cell types. It heterodimerizes with small Maf or Jun proteins and binds to the antioxidant response element (ARE) in the promoter region of target genes in response to oxidative stress [93]. Nrf2 knockout mice are susceptible to oxidative stress and neurodegeneration without obvious phenotypic defects [94].

The upregulation of Nrf2 exerts neuroprotective action during hypoxia/ischemia. Hypoxia preconditioning on rat brain against severe hypoxia or ischemia insult is through upregulating Nrf2 and HO-1 expression and alleviating oxidative stress damage [95]. rhEPO administration in ischemic rat activates Keap-Nrf2/ARE pathway to decrease H_2O_2 concentration and to protect brain tissue [96]. Similarly, in oxygen-deficient astrocytes, sulfiredoxin-1, an endogenous antioxidant protein, ameliorates oxidative stress via Nrf2/ARE pathway to prevent the brain from ischemic injury [97].

The expression level of Nrf2 is significantly decreased in the hippocampal neurons from AD patients [98]. The beneficial effect of Nrf2 upregulation in AD is evidenced by the finding that Nrf2 is able to induce NDP52, an autophagy adaptor protein, which facilitates the clearance

of phosphorylated tau in neurons [99]. Examination of postmortem brain samples from PD patients reveals that NQO1 and p62 whose expression is associated with Nrf2 are partly sequestered in LB, demonstrating the impaired Nrf2 signaling in PD, and pharmacological activation of Nrf2 defends PD by protecting nigral dopaminergic neurons against α-syn toxicity and decreasing astrocytosis and microgliosis [100]. Correspondingly, in ALS mice model, WN1316, a novel acylaminoimidazole, boosts the activity of Nrf2 to protect motor neurons against oxidative injury and repress glial inflammation, microgliosis, and astrocytosis [101].

The Nrf2 signaling pathway is an attractive therapeutic target for neurodegenerative diseases, and thus, the chemopreventive agents aiming at Nrf2 might be suitable candidates against the development and progression of neurodegeneration.

1.4. Epigenetic modification

Epigenetics is the study of heritable and nonheritable changes in gene expression without changes to the underlying DNA sequence. Currently, at least three systems, DNA methylation, histone medication, and noncoding RNA (ncRNA)-associated gene silencing, are identified in epigenetic changes. A large body of evidence documents that hypoxia triggers epigenetic alternation that contributes to the initiation and aggravation of neurodegeneration.

1.4.1. Modification of DNA and histone

DNA methylation and histone modification are two important epigenetic mechanisms altering the transcription of genes. The methylation of CpG island in the promoter region results in the silence of gene expression, whereas demethylation undergoes the opposite direction. The posttranslational modification (PTM) of histone includes acetylation, methylation, and phosphorylation that are regulated by pairs of enzymes, impacting gene expression via altering chromatin structure or recruiting histone modifiers.

Short-term hypoxia causes long-lasting changes in genomic DNA methylation in hippocampal neuronal cells and subsequent alternation in the expression of a number of genes participating in neural growth and development [102]. Chronic hypoxia-mediated downregulation of NEP in mouse primary cortical and hippocampal neurons is through G9a histone methyltransferase and histone deacetylase 1 (HDAC1) other than methylation of gene promoter [103]. Cultured astrocytes under ischemia-hypoxia (IH) condition show hypermethylation of global DNA and hypoacetylation of histone H3/H4, manifesting epigenetic reprogramming induced by hypoxia [104]. Chronic hypoxia exaggerated the neuropathology and cognitive impairment in AD mice through decreasing the expression of DNA methyltransferase 3b (DNMT3b) to prevent the methylation of γ-secretase promoter [105].

Epigenetic modifications are reversible that make it a promising candidate for therapy. Valproic acid is a neuroprotective agent showing HDAC inhibitory property. It prevents the decrease of H3-Ace in the NEP promoter regions in prenatal hypoxia-induced AD neuropathology, upregulating NEP to improve learning deficits and decrease Aβ level [106].

1.5. Conclusion

This section reviews the major consequences of hypoxia in the CNS and the contribution of individual consequence to the pathogenesis of several neurodegenerative diseases. However, the cross-link among these consequences and how they may predispose hypoxic patients to neurodegeneration remain to be determined, as well as the communication between neurons and glia in response to hypoxic environment. Different types of hypoxia, acute, chronic, sustained, or intermittent, may vary in terms of their effects on neural cells. Therefore, further investigation is required. The prevention of hypoxic condition is clearly helpful for the reduction of neurodegeneration, and the molecules targeted by hypoxia provide therapeutic strategies and interventions against common neurodegenerative diseases.

2. Hypoxia and the inflammatory diseases

2.1. Introduction

Inflammatory diseases are pathological conditions associated with local or systemic activation and persistent activity of inflammatory mediators, leading to cellular, tissue, or organ damage. The inflammatory cascade leads to increased vascular leakage, recruitment of leukocytes, increased generation and secretion of local and systemic inflammatory cytokines and chemokines, and activation and proliferation of innate and adaptive immune cell members. Ultimately, the inflammatory response leads to destruction of target molecules as well as their hosting cells and tissues, which could lead to pathological conditions such as inflammatory bowel disease and rheumatoid arthritis.

Hypoxia and inflammation have been extensively studied, and the two conditions seem to have a complex interrelated relationship. In general, hypoxia induces the inflammatory response via activation of cytokines and inflammatory cells, while inflammatory states are complemented with severe hypoxia and induction of hypoxic signaling intermediates [107, 108]. A key mediator of hypoxic signaling in inflammation is HIF-1. Aside from low oxygen tension, recent evidence shows that various oxygen-independent pathways regulate HIF-1α transcription and translation under normoxia. For example, endogenous nitric oxide has been shown to stabilize HIF-1α under normoxia [109–111]. Angiotensin II is another factor that increases HIF-1α transcription and translation under normoxia, and angiotensin receptor blockade has shown to independently reduce HIF-1α levels under hypoxic injury [112, 113]. Other nonhypoxic HIF-1 regulatory molecules are via growth factors, thrombin, bacterial lipopolysaccharide (LPS), interleukins, and tumor necrosis factor-α (TNF-α) [114]. In general transcriptional and translational regulation of HIF-1α occurring as a secondary mode of HIF-1 regulation may aggravate or hinder the hypoxic response of the protein.

It has been noted that during hypoxemic states the levels of inflammatory cytokines such as IL-1, IL6, and TNF-α increase in serum [107, 115, 116]. Activation of macrophages and other innate and adaptive immune cell members is also shown to be induced by HIF-1 under hypoxia via activation of Toll-like receptor (TLR) signaling [117, 118]. Likewise, ischemia reperfusion

is associated with recruitment of polymorphonuclear (PMN) leukocytes and vascular leakage [116, 119, 120]. This response is shown to be mediated via several endothelial cell surface glycoproteins and receptors and secondary activation of signaling via HIF-1–induced adenosine generation and NF-κB [116, 119].

It is noteworthy that ischemia and hypoxia are observed in inflamed tissues due to occlusion of blood flow via inflammatory cells [108]. As a result, signaling via inflammatory intermediates has been shown to potentiate hypoxic signaling via HIF-1. Macrophages in specific have been shown to release cytokines that stabilize and increase the activity of HIF-1 [111, 121]. Ultimately, transcriptional activation of factors such as VEGF by HIF-1 seems to increase angiogenesis and blood flow restoration to the site of inflammation.

Activation of HIF-1 further assures energy supply and survival of myeloid cells as well as bactericidal capacity of macrophages [122, 123]. Among the signaling pathways induced by HIF-1 in macrophages are mediators such as NF-κB, TNF-α, and nitric oxide that play key roles in the inflammatory capacity of the myeloid cells [111, 121, 123]. Interestingly, HIF-1α stabilization in turn positively regulates the production of inflammatory cytokines such as TNF-α, and therefore, through a positive feedback mechanism, inflammation and hypoxic signaling potentiate one another [123]. In the following sections, detailed mechanisms of this interaction will be discussed. Furthermore, the role of hypoxia and HIF molecules in arthritic and inflammatory bowel disease (IBD) pathophysiology and potential therapeutic targets relating to hypoxic signaling will be examined.

2.2. Hypoxic signaling and key inflammatory intermediates

2.2.1. TNF-α

TNF-α is a key mediator of the inflammatory response. It has been shown that HIF-1a stabilization and DNA-binding activity are enhanced by TNF-α [111]. Interaction of TNF-α and HIF-1 is rather complex. Physiologically, the stabilization of HIF-1a by TNF-α is thought to be mediated by activated macrophages [121]. Accumulation of HIF-1α via the TNF-α is via a mechanism independent from hypoxic accumulation or transcriptional activation of HIF-1α. Several studies have investigated the mechanism of HIF-1α stabilization via TNF-α, and among such mechanisms, NF-κB signaling seems to be the key mediator of this process [124, 125]. Studies by Zhou et al. have shown that TNF-α leads to accumulation of ubiquitinated form of HIF-1α, which is normally one of HIF-1α degradation steps. This interaction was mediated through increased NF-κB transcription [124]. They also noted that transfection of cells with p50/p65 members of NF-κB family leads to normoxic accumulation of HIF-1α in the absence of TNF-α [124]. Interestingly it has also been shown that reactive oxygen species (ROS) such as H_2O_2 or SO^- interfere with TNF-α–mediated accumulation of HIF-1α [126]. Aside from protein accumulation, additional studies have shown increased translation of HIF-1α via TNF-α that is also mediated via NF-κB through upregulation of an antiapoptotic protein Bcl-2 [127].

2.2.2. Nuclear factor-kappa β

NF-κB is a family of transcription factors involved in development, proliferation, survival, and antimicrobial response of innate and adaptive immune system cells. Numerous extensive studies have been conducted to elucidate the very complex role of NF-κB in the immune response [128]. The NF-κB family is composed of five related transcription factors, which can form homodimers or heterodimer complexes with DNA-binding activity. These identified members are p50, p52, RelA (p65), RelB, and c-Rel [128]. NF-κB complexes are inactive in the cytoplasm and are bound to an inhibitory protein called I-κB. Once NF-κB signaling is activated, the I-κB proteins are degraded, which then allow the transcription factors to translocate to the nucleus [128]. In the innate immune response, NF-κB is activated secondary to Toll-like receptor (TLR) activation. Toll-like receptors are pattern recognition receptors (PRR), which help immune cells recognize and combat pathogenic components. There are 11 identified mammalian TLRs with various coupled signaling pathways. TLRs are expressed in the cytosol as well as on the plasma membrane of immune cells [128]. Upon ligand binding, TLR signaling leads to recruitment of specific adaptor proteins and second messenger molecules, which in turn activate several transcription factors. Among such signaling pathways are mediators that result in degradation of I-κB proteins and activation of NF-κB [128]. NF-κB in turn induces gene expression of cytokines and other proteins involved in bactericidal activity against pathogens. NF-κB activation and signaling are also involved in adaptive immunity. T-cell and B-cell receptor activation and signaling activate NF-κB, which in turn activates antiapoptotic proteins and increases transcription of cytokines that ensure survival, proliferation, and differentiation of B and T cells [128].

2.2.3. Hypoxia and the cross talk between HIF-1 and NF-κB

It has been shown that NF-κB is directly activated under hypoxic conditions [129, 130]. Although the mechanism of NF-κB activation under hypoxia remains to be an extensive area of research, it has been shown that I-κB tyrosine residues are phosphorylated under hypoxia [129]. More recent studies suggest phosphorylation and inactivation of I-κB under hypoxia occur secondary to activation of transforming growth factor beta-activated kinase-1 (TAK1) and I-kappa B kinase (IKK) complex, primarily responsible for in I-κB degradation resulting in NF-κB activation [130–133]. Additionally, it has been shown that O_2-dependent prolyl hydroxylases (PHDs) that are involved in HIF-1 inactivation also play a role in proline hydroxylation of IKKβ and NF-κB repression [133]. Thus, during hypoxia loss of PHD activity would activate NF-κB.

Although hypoxic activation of NF-κB is to be better understood, a large body of convincing evidence shows a critical role for NF-κB in induction of HIF-1. Activation of NF-κB leads to induction of HIF-1$α$ gene expression and basal HIF-1$α$ mRNA, and protein levels are dependent upon NF-κB subunit expression levels [134, 135]. Several studies have explored the mechanism of regulation of HIF-1 by NF-κB [124, 127, 134, 136, 137]. It has been shown that NF-κB induces expression and increases protein levels of HIF-1$α$ both in hypoxia and normoxia [124, 134, 137]. Indeed, certain studies suggest that HIF-1$α$ gene expression under hypoxia is dependent upon intact NF-κB signaling pathway [134, 137]. These studies also

provide mechanistic evidence into the regulation of HIF-1α gene expression via binding of several NF-κB subunits to the HIF-1α promoter region [134, 135]. Thus, secondary to direct activation of HIF-1 under hypoxic conditions, interaction of NF-κB additionally contributes to this process by increasing basal levels of HIF-1α protein.

Respective regulation of NF-κB by HIF-1 has also been reported in the literature [114, 138, 139]. These studies suggest direct activation of NF-κB via HIF-1 signaling in inflammatory cells. Among suggested mechanisms are increased expression of TLR2 and TLR6 leading to activation of NF-κB, hyperphosphorylation of IKKβ, and phosphorylation of serine residues of p65 subunit of NF-κB leading to its translocation to nucleus and transcriptional activity [117, 138, 139].

Overall, hypoxia and signaling via NF-κB and HIF-1 are closely linked and, respectively, regulate one another to enhance the inflammatory response.

2.3. Hypoxia and inflammatory bowel disease (IBD)

IBD is associated with loss of intestinal mucosal barrier, inflammation of mucosa, and increased incidence of bacterial infections [140]. IBD is categorized as ulcerative colitis (UC) and Crohn's disease (CD). Both conditions are associated with severe inflammation and breakdown of intestinal mucosal barrier and chronic gastrointestinal discomfort. Current therapeutic approaches to IBD include anti-inflammatory agents mostly targeted at TNF-α and immune cell members.

Hypoxia has been shown to be a critical component of inflammation in IBD. Surgical specimens of intestinal mucosa of IBD patients show increased expression of HIF-1 and HIF-2 [141]. Increased vascular proliferation and density has been noted in intestines of IBD patients secondary to hypoxia-induced VEGF activity [142]. Additionally microvascular abnormality and loss of endothelial nitric oxide production are seen in IBD mucosa [143].

The intestinal mucosa is exposed to fluctuating levels of oxygen. On the one hand, the intestinal lumen is nearly anoxic, and oxygen pressure is generally low on the luminal side of the mucosa. On the other hand, the rate of perfusion of the subendothelium is dependent upon meal intake, and PO_2 changes dramatically from high to low in between meals. The shift in oxygen tension in the mucosal layer renders it resistant to hypoxic states. This could be in part due to basal activity of hypoxic signaling intermediates such as HIF-1 in the intestinal mucosal. Indeed, HIF-1α–null mice in the intestinal epithelium show diminished mucosal protection and increased clinical symptoms in murine model of colitis [144]. HIF-1–induced epithelial protection is shown to be due to induction of several proteins such as mucin, p-glycoprotein, and ecto-5′-nucleotidase (CD73), an enzyme that converts AMP to adenosine (A_2B) receptor [140]. Adenosine production during hypoxia has shown to decrease vascular leakage and neutrophil accumulation and thus plays an anti-inflammatory role [120]. In a case-control cohort study, patients with polymorphisms in CD39, a vascular and immune cell ecto-nucleotidase that converts extracellular ATP and ADP to AMP, had increased susceptibility to Crohn's disease [145]. Therefore, HIF-1 signaling via adenosine is a key step in protection against IBD inflammation (**Figure 4**).
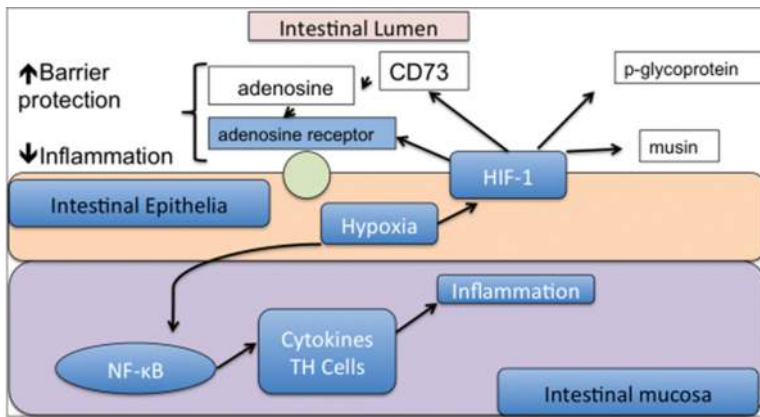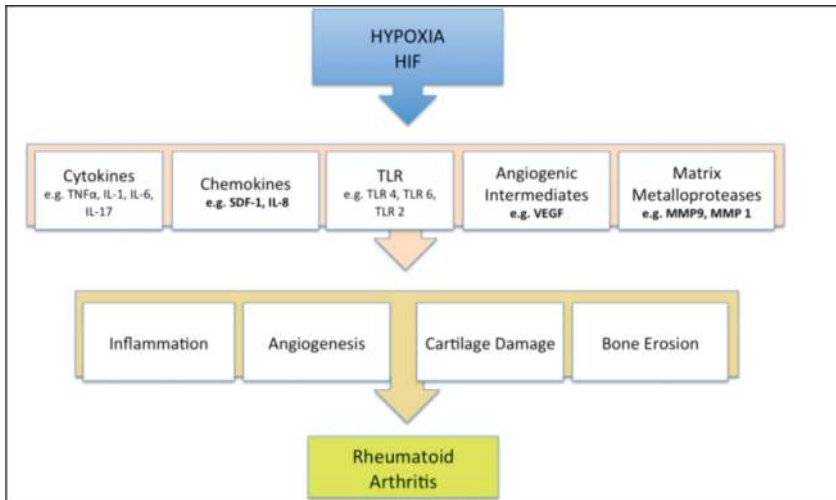
Figure 4. Hypoxia and IBD pathogenesis.

Aside from HIF-1, NF-κB is also involved in inflammatory events of IBD [146, 147]. Nuclear levels of NF-κB p65 have long been seen in lamina propria biopsies of patients with Crohn's disease [148]. Activation of NF-κB in mucosal macrophages leads to induction of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, which mediate mucosal tissue damage [149]. NF-κB activation in intestinal mucosa also plays a role in differentiation of T-helper cells, which also play a role in IBD inflammation (**Figure 4**) [149]. In addition to pro-inflammatory activity, some studies have shown a protective role for NF-κB [146]. Loss of β or γ subunits of the IKK complex leads to colitis and apoptosis of intestinal mucosa [150, 151]. Additionally, polymorphisms of TLR4 and TLR5, which are involved in NF-κB activation, have been strongly associated with IBD in canines [152]. The protective role of NF-κB in IBD is thought to be in terms of maintaining mucosal barrier and integrity. Overall, NF-κB seems to play a dual role in IBD.

Due to the protective role of HIF-1 in models of colitis, it has been proposed that induction of HIF-1 could serve as a potential therapeutic target for treatment of IBD. The common pharmacological method of HIF-1 induction is via inhibition of PHD enzymes, which break down the HIF-1α subunit in the presence of oxygen. In vitro pharmacological inhibition of PHD using 2-oxoglutarate analogs as co-substrates of PHDs or dimethyloxaloglycine, has shown to stabilize HIF-1α [153–155]. In these studies PHD inhibitors decreased clinical symptoms in murine models of colitis and thus present promising therapeutic targets for IBD [153, 155, 156]. As mentioned previously blockade of PHDs can also lead to NF-κB activation. Using PHD inhibitors has thus been suggested to have dual benefits in treatment of IBD.

NF-κB activity, however, is associated with increased inflammation, and therefore, inhibition of NF-κB has also been examined and shows promise in treatment of IBD [149]. Selective NF-κB inhibitors, antisense oligonucleotides against NF-κB, and targeting DNA-binding activity of NF-κB using decoy oligodeoxynucleotides have been among the strategies tested that have produced promising results in murine models of colitis and IBD [157, 158].

2.4. Hypoxia and rheumatoid arthritis (RA)

Rheumatoid arthritis is the most common type of inflammatory arthritis. As an autoimmune disorder, RA is characterized as inflammation of the synovium, loss of cartilage, and bone erosion leading to joint pain and dysfunction [159]. The synovial fluid in RA is infiltrated with fibroblasts, immune cells, and angiogenesis of new vasculature [159, 160]. Additionally, a key feature of synovial fluid in RA is hypoxia. It has been shown that the synovium of knee joints of RA patients has significantly less O_2 pressure than that of osteoarthritis (OA) patients [161]. Immunohistochemical analysis of synovial stromal cells and macrophages of RA- and OA-affected joints show significant increases in HIF1α and HIF2α expression compared to normal. Additionally, the levels of HIFs were directly correlated with VEGF expression in the stromal cell lining in these specimens [162]. Other studies have identified HIF-2α significantly upregulated in fibroblast-like synoviocytes of RA and associated IL-6 upregulation in these cells [163]. These and other similar studies imply HIF signaling as the orchestrator of synovial inflammation and secondary joint damage [159, 164, 165]. A large number of HIF-activated inflamamtory mediators have been identified in RA synovial fluid including but not limited to stromal cell–derived factor 1 (SDF-1), VEGF, TNF-α, IL-1β, and IL-8 [166]. Various TLRs are also expressed in synovial tissue and macrophages, which further activate NF-κB pathway and increase expression of other inflammatory proteins [167]. Not surprisingly, HIF-dependent pathways have also been implicated in TLR expressions in many tissues including synovial cells [117, 118]. Finally, recruitment of CXCR4+ lymphocytes and matrix metalloproteinases (MMPs) in the synovial fibroblasts involved in cartilage destruction has also shown to be HIF-1 mediated and NF-κB mediated [168, 169]. Overall, a large body of evidence implicates hypoxia and HIF signaling as a key underlying mechanism in pathogenesis of RA (**Figure 5**).

Figure 5. Hypoxia and pathogenesis of rheumatoid arthritis.

As discussed above, hypoxia- and HIF-mediated signaling is highly pro-inflammatory and destructive in RA. The key approach to treatment of RA is thus inhibition of HIF signaling. Many HIF inhibitors have been tested in cancer that may show promise in treatment of RA [170]. The limiting factor in administering HIF inhibitors is pharmacokinetic availability of these compounds in the synovial space as well as specific targetting of joints rather than systemic therapy. Gene targetting of HIF molecules using antisense oligonucleotides targetting HIF-1α mRNA has also been tested, which may show efficacy in RA treatment [159]. Additional approaches including anti-VEGF antibodies or anti-VEGF receptor molecules have been tested in models of arthritis and have shown efficacy in delaying onset and severity of RA in animal models [159, 171]. These strategies remain to be clinically tested yet show great promise in novel therapeutics of RA.

2.5. Conclusion

Section 2 discussed the complex relationship between hypoxia and inflammatory process and highlighted the key intermediates and pathways involved in this relationship. The discovery of hypoxic-inflammatory pathways has led to a greater understanding of inflammatory and autoimmune diseases such as IBD and RA and the use of novel pharmacological approaches targetting HIF and hypoxic signaling intermediates in these conditions. So far, these agents have been mostly studied in cancer clinical trials. Additional clinical studies are needed to examine the safety and efficacy of new HIF-modulating agents in treatment of inflammatory disease states.

3. Hypoxia and renal diseases

3.1. Introduction

Approximately 26 million Americans have some evidence of chronic kidney disease (CKD) and are at risk to develop kidney failure. The number of Americans with end-stage renal diseases (ESRD) is expected to grow to 785,000 by 2020 (currently 485,000). The annual cost of treating ESRD is currently over $32 billion. It is estimated that healthcare system can save up to $18.5 to $60.6 billion by reducing rate of progression of chronic kidney disease (CKD) by 10–30% over the next decade.

In acute setting acute kidney injury (AKI) has been shown to be associated with bad outcome, for instance, mortality rate of hospitalized patients with AKI increases more than fourfold [172]. Due to high medical and economic impact of AKI and CKD, finding new therapeutic tools in treatment of CKD is becoming of an increasing importance.

Hypoxia-inducible factor (HIF) has become the focus of medical community as a putative target because its augmentation is likely to ease the burden of kidney disease. The following sections discuss the evidence regarding the role of HIF molecules in various kidney pathologies and potential therapeutic approaches with respect to the HIF system.

3.1.1. Pathophysiology

Kidneys have a rich blood supply. In fact human kidneys receive 20% of cardiac output, while they weigh less than 1% of the total body weight. However, renal medulla, physiologically, has low oxygen tension and hence is very sensitive to hypoxia.

Hypoxia is the final common pathway to irreversible renal damage and eventually ESRD [173]. Since Fine et al. introduced chronic hypoxia hypothesis for the first time, it has been confirmed in several studies [174]. Also, hypoxia plays a role in pathogenesis of AKI as well as transformation of AKI to CKD.

Three phases of cell damage have been recognized following hypoxic insult to kidneys (by ligation of a branch of renal artery) [175]:

- Phase I: 1–6 h post hypoxic damage; in this phase parenchymal cells still appear viable.

- Phase II or intermediate phase: 1–3 days following insult; in this phase tissue damage is completed.

- Phase III or late phase: after 3 days; when tissue repair and remodeling are initiated.

In order to survive hypoxemia or regional hypoxia, the kidneys adopt a set of sophisticated defense mechanisms, which include expression of HIF. HIF is the cornerstone of adaptation to hypoxia. This master regulator of the cellular response to hypoxia orchestrates several hundred target genes affecting metabolism, the cell cycle, and inflammation [176]. The hypoxia-inducible transcription factors have been extensively studied in the kidneys [177]. HIF-1α is mainly expressed in tubular cells, while HIF-2α is found in peritubular, interstitial, endothelial, and glomerular regions [178]. Likewise, PHD1 and PHD3 are mostly present in glomeruli, and PHD1, PHD2, and PHD3 express more in the distal tubules than in the proximal tubules [179].

Numerous studies have found critical roles for HIF molecules in hypoxic adaptation of the kidneys as well as pathophysiology of various kidney diseases [177]. Given the fact HIF is the key step in renal response to hypoxia targeting HIF and its regulatory mechanisms is a plausible approach to prevent and treat hypoxic insults to kidney. In quest for novel therapeutic tools for treatment and prevention of kidney diseases, HIF-related pathways have shown promising results.

3.2. HIF in acute kidney injury

AKI is defined by rapid decline in renal function. AKI has multitude of causes. One of the most common causes of AKI is ischemia as a result of decreased renal perfusion, which leads to acute tubular necrosis (ATN) [180]. With renal ischemia several mechanisms in small arterioles will perpetuate regional hypoxia (**Figure 6**); these mechanisms include:

a. Decreased generation of nitric oxide (vasodilator) by endothelial cells [181]

b. Enhanced reactivity to endogenous mediators of vasoconstriction [182]

c. Small vessel occlusion due to activation of coagulation system interaction between the endothelium and leukocytes [183]
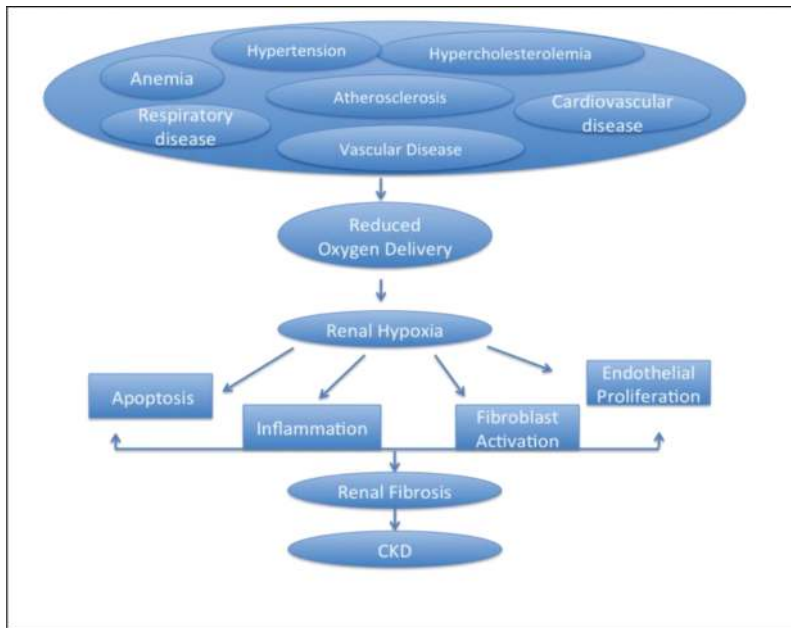
Figure 6. Diagram summarizing the interrelation between different factors causing hypoxia and CKD.

It has been shown that after renal ischemic attack, the number of capillaries in the medulla will decrease, which in turn leads to chronic ischemia, fibrosis, and progression to CKD [184]. Therefore, AKI is a risk factor for development of CKD. At the same time, patients with CKD have more incidence of AKI. In fact the most important risk factor of AKI is CKD [185]. AKI carries high risk of mortality; among patients older than 66 years with a first AKI hospitalization, the in-hospital mortality rate in 2013 was up to 14.4% (2015 USRDS Annual Data Report). Mortality rate in patients with AKI admitted to intensive care unit may surpass 50%. These data obviated the need for finding new therapies in AKI focused on renal hypoxia.

The key hypoxic intermediates mostly studied in animal models of AKI are HIF-1 and HIF-2. Rosenberger et al. showed that upregulation of HIF-1α occurs up to 7 days following ligation of a branch of the renal artery. HIF-2α expression has also been noted but to a lesser degree than HIF-1α and was confined to resident and infiltrating peritubular cells in the cortex [186]. Numerous studies have shown the involvement of HIF proteins in protection against acute renal injury [177]. Induction of HIF-1 or its target genes have shown to reduce injury secondary to various types of acute renal insult [187, 188].

3.2.1. HIF in contrast-induced nephropathy

The exact mechanism of contrast-induced nephropathy (CIN) remains elusive. Among possible mechanisms are renal vasoconstriction and decreased vasodilatation, which leads to tubular hypoxemia, decreased mitochondrial function and generation of reactive oxygen

species (ROS), increased prostaglandins, decreased NO levels, and increased oxygen consumption due to osmotic demand of contrast media on tubular Na/K ATPase, all of which lead to medullary cell damage [189, 190]. Clearly, a direct link with hypoxia and CIN exists. Reversible renal vasoconstriction has been demonstrated in animal studies [191]. In an animal study, Rosenberger et al. induced renal hypoxia by a combination of COX inhibition, radio-contrast material, and blockade of nitric oxide synthase. In this study generalized HIF induction (tubules, interstitium, and endothelial cells) initiated within minutes of regional renal hypoxia. They showed medullary thick ascending limb (TAL) of Henle had less HIF expression, which may explain the higher susceptibility of this region to hypoxia [175]. These findings render regional hypoxia a plausible cause for CIN pathophysiology and a potential role for preventative HIF induction therapy in this condition.

3.2.2. Ischemic acute kidney injury

Ischemic injury in thick ascending limb of Henle is believed to play a pivotal role in pathogenesis of AKI due to regional renal low oxygen tension. Activation of HIF-1 has shown to be protective in models of ischemia-reperfusion injury. Schley and his colleagues showed that deletion of the von Hippel-Lindau (VHL) protein in thick ascending limb (TAL) of Henle preserved its function following ischemia-reperfusion. Notably, this study demonstrated better recovery in VHL-knocked-out animals by showing higher number of proliferating cells [192]. Furthermore, preconditional activation of HIF-1 via carbon monoxide or PHD1 inhibitor has shown to ameliorate the degree of renal ischemic damage in rat models of ischemia-reperfusion injury [188]. Others have shown activation of HIF-1 via cobalt chloride leads to reduction of tubulointerstitial damage secondary to acute renal injury in rats [187].

3.3. HIF in chronic kidney disease (CKD)

Chronic renal hypoxia causes apoptosis and also differentiation of tubular cells to myofibroblasts. Under hypoxic condition renal fibroblasts will also get activated. These together will lead to progressive renal failure and eventually ESRD. Glomerulosclerosis as a result of chronic high blood pressure or high blood sugar can also cause tubular ischemia by impairing tubular perfusion.

Several pharmacological means of reducing renal hypoxia are already widely available for use in daily clinical practice. Treatment with erythropoietin (EPO)-stimulating agents has been shown to slow down the progression of CKD [193]. Renin-angiotensin system (RAS) blockade can also be protective against hypoxia. RAS blockade will improve perfusion of peritubular capillaries by decreasing tone of efferent arteriols in parent glomerulus [194]. Yu et al. studied the effect of HIF activation (via a nonselective PHD inhibitor, L-mimosine) in rats with CKD. Animals underwent subtotal nephrectomy. In this study they demonstrated HIF activation can have different (beneficial or deleterious) effects on renal tissue. It was also shown that function of remnant kidney is also dependent upon the timing of HIF activation. Early activation of HIF in CKD caused increased fibrosis (rise in mRNA of collagen type III) and inflammation, while late activation of HIF showed anti-fibrotic effects [195].

3.3.1. HIF in diabetic nephropathy

Diabetic kidney disease (diabetic nephropathy (DN)) is the leading cause of ESRD. Hypergly-cemia and resultant hyperfiltration will increase renal oxygen consumption. Eighty percent of the total renal oxygen consumption is related to sodium-potassium pump in cortical proximal tubule. Diabetes causes decreased renal oxygen tension by increasing oxygen consumption. Inoue et al. by using diffusion-weighted (DW) and blood oxygen level-dependent (BOLD) MRI showed tissue hypoxia in diabetic kidneys [196]. Palm et al. also demonstrated lower paren-chymal oxygen tension along with higher oxygen consumption in diabetic rats [197]. In the setting of hypoxia, paradoxically, the activity of HIF-1α seems to be decreased or altered in diabetic rat kidneys [198, 199]. Polymorphism of pro582ser in HIF-1α gene, which results in altered response of HIF-1α to low oxygen, is associated with increased incidence of diabetic nephropathy in diabetic patients [199]. It appears from this evidence that HIF-1α-protective activity in the kidney is compromised in the setting of diabetes. This is further supported by the finding that pharmacologic activation of HIF pathway decreases renal damage in diabetic rats by decreasing proteinuria, improving tubulointerstitial damage and normalizing glomer-ular hyperfiltration [200]. There is thus indication for the use of HIF-1–activating approaches in prevention of diabetic nephropathy.

3.4. HIF in anemia of kidney disease

HIF plays a role in anemia of CKD and ESRD. Erythropoietin is secreted from human kidneys after birth. The kidney accounts for ~90% of the total EPO production in the adult human [201]. Renal erythropoietin-producing cells are fibroblasts in peritubular capillaries in the cortex and outer medulla [202].

Kidneys are the perfect choice to be responsible for erythropoietin secretion due to their regional low oxygen tension. Any minute changes in renal oxygen tension will lead to adjustments of serum hematocrit. In subcellular level HIF binds to the EPO enhancer, the hypoxia-responsive element, and activates the transcription of EPO. Renal EPO synthesis is regulated by HIF-2 [203]. HIF-2 exerts its multipronged effect by increasing EPO production, increasing iron absorption, and also increasing maturation of erythroid progenitors in the bone marrow. Studies indicate that in ESRD patients erythropoietin concentration is below normal due to dysfunctional EPO-producing cells (not due to cell death) [204]. Erythropoietin-producing cells in renal fibrosis remain alive and preserve their plasticity: although the exact mechanism of erythropoietin production in ESRD remains elusive, it is possible plasticity of erythropoietin-producing cells plays a role when signals for HIF pathway are augmented. Pathways to stabilize or even augment HIF response will mimic the state of hypoxia, which will lead to erythropoietin production; this is considered a novel therapeutic tool in our armamentarium to treat anemia of CKD. HIF stabilizers inhibit PHDs, which will subsequently cause accumulation of HIF, and as a result erythropoietin production ensues.

In 2010 a phase 1 clinical trial revealed PHD inhibitor (FG-2216) led to increased EPO produc-tion and plasma EPO levels in patients with ESRD [205]. In a phase 2-b study of nondialysis-dependent patients with chronic kidney disease, related anemia treatment with an oral PHD

inhibitor (Roxadustat) was shown to increase EPO level and correct anemia. Clinical response was independent of iron intake (oral or IV) [206].

3.5. HIF in renal transplant

As of January 2016, there are 100,791 people waiting for renal transplants in the United States. Every 14 min a patient is added to the kidney transplant waiting list. In 2012, the probability of 1-year graft survival was 92% and 97% for deceased and living donor kidney transplant recipients, respectively. The estimated US average charges for a kidney transplant in 2011 is $262,900. This data emphasizes on the need for exploring new ways to save and preserve more allografts.

In the process of renal transplantation, harvested organ is subjected to hypoxia. Hypoxia-reperfusion occurs during organ procurement, preservation, and after implantation. Ischemia-Reperfusion injury (IRI) has prognostic implications for the allograft and kidney recipient. As mentioned before HIF has been shown to be a renoprotective agent and may alter transplantation outcome.

Conde et al. found HIF-1α increases in human proximal tubular cells (in vitro) after hypoxia and also during reoxygenation period. A similar biphasic pattern was observed in IRI model in SD rats (en vivo). The en vivo part of the study proved that HIF-1α induction during reperfusion phase was required for survival of proximal tubule cells and expedited recovery. Conde and his colleagues also studied human allograft biopsies (7–15 days post-transplant): HIF-1α expression was more robust in proximal tubule cells with minimal ischemic damage. Again, this finding indicate a protective role of HIF in IRI. AN interesting finding in this study was demonstration of the role of Akt/mTOR signaling in HIF-1α induction. Using rapamycin (mTOR inhibitor) during reoxygenation period prevented HIF-1α stabilization [207].

Renal ischemia-reperfusion injury is an important factor in determination of the fate of a renal allograft. Immunological response is potentiated under ischemia-reperfusion. CD4+ T cells play the main role in pathogenesis of IRI and natural killer (NK) cells are part of the immediate response to IRI. Regulatory family differentially affect the immune response to the of HIF affect allograft's during ischemia-reperfusion. While HIF-1α plays a crucial role in T-cell survival and function , HIF-2α has a protective function in T-cell mediated renal IRI [208]. In an animal study, Zhang et al. showed the role of HIF-2α in mitigating NK T-cell–mediated cytotoxicity in IRI. In this study HIF-2α and adenosine A2A receptor (adora2a) worked in concert with each other (so-called hypoxia-adenosinergic immunosuppression) to restrict NK T-cell activation [209]. This finding is of clinical importance as pharmacologic activation of HIF-2α can potentially limit allograft IRI and subsequently improve the outcome of renal transplantation.

3.6. Conclusion

The overwhelming clinical and economical impact of renal disease and the limited therapeutic options available have placed a great demand on finding additional therapeutic ap-

proaches. The evidence discussed in this section suggests a widespread role of hypoxia and HIF signaling in a range of acute and chronic renal diseases and a clear indication for HIF-targeted therapies. It appears that HIF-1 activity is protective in acute renal injury, while prolonged activity of HIF-1 may lead to worsened outcomes in CKD. The protective versus deleterious roles of HIF-1 thus complicate the use of HIF-1–targeted approaches. On the other hand, HIF-2 therapies may be more promising especially in terms of anemia of kidney disease and renal allograft rejection. In either case, additional clinical research is needed in the use and efficacy of both HIF-1 and HIF-2 therapies in prevention or treatment of various renal diseases.

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