Roles of Hypoxia Response in Retinal Development and Pathophysiology

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The hypoxia response is a fundamental phenomenon mainly regulated by hypoxia-inducible factors (HIFs). For more than a decade, we have investigated and revealed the roles of the hypoxia response in the development, physiology, and pathophysiology of the retina by generating and utilizing cell-type-specific conditional knockout mice. To investigate the functions of genes related to the hypoxia response in cells composing the retina, we generated various mouse lines that lack HIFs and/or related genes specifically in retinal neurons, astrocytes, myeloid cells, or retinal pigment epithelium cells. We found that these genes in the different types of retinal cells contribute in various ways to the homeostasis of ocular vascular and visual function. We hypothesized that the activation of HIFs is likely involved in the development and progress of retinal diseases, and we subsequently confirmed the pathological roles of HIFs in animal models of neovascular and atrophic ocular diseases. Currently, anti-vascular endothelial growth factor (anti-VEGF) therapy is a first-line treatment widely used for neovascular retinal diseases. However, alternative or additional targets are now required because several recent large-scale clinical trials and animal studies, including our own research, have indicated that VEGF antagonism may induce retinal vascular and neuronal degeneration. We have identified and confirmed a microRNA as a candidate for an alternative target against neovascular retinal diseases, and we are now working to establish a novel HIF inhibitor for clinical use based on the disease mechanism that we identified. (DOI: 10.2302/kjm.2017-0002-IR; Keio J Med 67 (1): 1–9, March 2018)

Keywords: retina, hypoxia-inducible factor (HIF), vascular endothelial growth factor (VEGF), von Hippel-Lindau protein (PVHL), microRNA

Introduction

Hypoxia-inducible factors (HIFs) are transcriptional factors that induce various genes required for cell survival under hypoxia, such as those involved in angiogenesis, erythropoiesis, energy metabolism, and inflammation. HIFs are composed with α and β subunits, and there exist in mammals at least three types of α subunits: HIF-1α, HIF-2α, and HIF-3α (Fig. 1). In the past decade, the roles of the hypoxia response in the development, physiology, and pathophysiology of the retina have been investigated and revealed by utilizing cell-type-specific conditional knockout mice. Although clinical studies have been performed and new surgical instruments and procedures have been developed, several problems remain unresolved. For example, photoreceptors basically cannot be regenerated once they are lost in the degenerated retina in conditions such as retinal detachment, retinopathies, and retinitis pigmentosa. Antiangiogenic therapies have enabled some neovascular retinal diseases to be cured, however, such treatments potentially may induce atrophic retinal degeneration. Therefore, among the disease mech-
Fig. 1 Schematic of the VHL E3 ligase complex and its target HIF-α. (Left panel) HIF-α fate and function in the context of proteasomal degradation ubiquitylated by the VHL E3 ligase complex under normoxia. Under conditions of oxygen sufficiency, prolyl hydroxylase (PHD) constitutively hydroxylates HIF-α on a specific prolyl residue. Prolyl hydroxylation opens an HIF-α recognition site in the VHL complex that targets HIF-α for degradation by the 26S proteasome. (Right panel) Hypoxia-induced transcriptional activity of HIF-α. Inhibition of hydroxylation by the lack of oxygen promotes the stabilization of HIF-α, its transport to the nucleus, and its dimerization with ARNT (also known as HIF-1β). The resulting heterodimer joins with nuclear hypoxia response element (HRE) and a co-activator to transcribe genes for cell survival under hypoxia such as VEGF. FIH, Factor Inhibiting HIF-1α; 2-OG, 2-oxoglutarate; Ub, ubiquitin; ARNT, aryl hydrocarbon receptor nuclear translocator; CBP, CREB-binding protein.

Organisms identified in the retina, neural degeneration was the main focus, and basic research in this area was conducted. On the other hand, the discovery of adult stem cells and activation of endogenous neuronal progenitors in the retina had also attracted attention and had raised the possibility that neuroprotection, and ultimately neuronal regeneration, may be possible. Furthermore, it was reported that a hypoxic environment and the function of HIFs are important for the maintenance of neuronal undifferentiation and proliferation, and that hypoxic preconditioning or pharmacological HIF activation may suppress pathological phenotypes of experimental retinal degeneration and cerebral infarction. Based on this background, we hypothesized that constitutive HIF activation may protect the retina from various disease states. Consequently, we undertook the generation of a mouse line that lacks the von Hippel-Lindau gene (Vhl, a negative regulator of HIFs) specifically in the retinal neuron. These retinal-specific Vhl knockout mice showed ragged retinae that looked similar to those at the end stage of diabetic retinopathy (Fig. 2). This completely contrary result to the initial hypothesis was surprising, and it motivated us to continue research into the biological hypoxia response to the present time. In this review article, our research findings about the roles of the hypoxia response in the development and pathophysiology of the retina are described, and the possibility of HIF antagonism for clinical application is discussed.

Hypoxia Response in Retinal Development

As research progressed, we found that retinal neuron-specific Vhl knockout mice already have significant phenotypes in the developmental stage, especially in terms of the vascular system. The ocular vasculature consists of three types of circulatory system: the retinal, choroidal, and hyaloidal vascular system, with the hyaloidal vascular system existing in the vitreous cavity only during the developmental stage. The hyaloidal vascular system regresses and the retinal vascular system takes over the
blood supply in the early developmental period. This circulatory transition from the embryo to the adult is important because congenital ocular diseases such as persistent hyperplastic primary vitreous (PHPV) may occur if the transition process is not completed appropriately. The hyaloidal vascular system starts to regress simultaneously with the extension of the retinal vasculature. The regression is normally complete between 23 and 28 weeks of gestation in humans. PHPV is a condition in which the embryonic hyaloidal vasculature persists in the vitreous cavity and suppresses the normal development of various parts of the eye, resulting in severe visual disturbance. This raises the question of why hyaloidal vascular regression occurs concomitantly with the extension of the retinal vascular. To answer this question, we focused on the changes in expression of hypoxia response components in the retina. We found that HIF-α expression decreases in retinal cells along with increasing oxygen tension according to the establishment of the retinal vasculature. Furthermore, we generated conditional Vhl knockout mice (specifically in retinal neurons or astrocytes), and found that these mice exhibited phenotypes resembling human PHPV because of the disturbance of the proper hypoxia response (Fig. 3). These findings suggested that the appropriate cellular hypoxia response during retinal development is important for the ocular circulatory transition from the embryo to the adult.

The molecular mechanism of retinal vascular development has been well documented. The rodent retinal vasculature starts to extend from the optic nerve head after birth, and researchers utilize the rodent retina as an experimental system to observe physiological in vivo vascular development. Vascular endothelial growth factor (VEGF) plays an important role in angiogenesis, and the gene encoding VEGF is one of the most representative targets of the HIF transcriptional factor. The Vegf gene is strongly detected in the avascular area of the developing murine retina. Vegf gene expression is immediately suppressed in the area covered by the vasculature. To investigate the regulatory system for retinal vascular development, we generated retinal neuron-specific Hif-1α knockout mice. These mice showed a delay in retinal vascular development because of a decreased number of endothelial tip cells with fewer filopodial extensions located at the leading edge of the spreading vasculature. Amacrine and horizontal cell-specific Hif-1α knockout mice also showed a decreased density of vascular plexus in the intermediate retinal layer where these cells are located. In contrast, we found that deletion of Vegf, Hif-1α, or Hif-2α specifically in astrocyte glia (which forms as a template for the retinal vasculature) did not induce phenotypic change during retinal vascular development although another group reported a dissimilar phenotype of glia-specific Hif-2α knockout mice utilizing a different Cre transgenic mouse line. Our findings indicated that the hypoxia response in neurons, rather than in glia, is important during physiological retinal vascular development and acts to control Vegf expression.
Hypoxia Response in Retinal Pathophysiology

Pathological neovascularization is the most common pathophysiology in various retinal diseases, such as diabetic retinopathy, retinopathy of prematurity, retinal vein occlusion, and wet-type age-related macular degeneration. Various animal models of ocular neovascularization have clarified the roles of VEGF in pathological neovascularization.\textsuperscript{26–29} For the last decade, anti-VEGF agents have been developed and approved for clinical use against these neovascular ocular diseases.\textsuperscript{30–32} Now, the role of VEGF in the progress of retinal diseases is widely recognized in the ophthalmological field.\textsuperscript{33–35} Surprisingly, we found that \textit{Vegf} gene deletion induced in retinal pigment epithelium (RPE) cells in the adult mature retina in mice rapidly led to degeneration of the choriocapillaris and the cone photoreceptors, whereas \textit{Hif-1\alpha} and/or \textit{Hif-2\alpha} knockout mice exhibited no such phenotype\textsuperscript{34} (Fig. 4). This finding indicated that VEGF, but not HIFs, are required for retinal homeostasis to maintain the normal vascular structure. HIFs do not exist in the normal state under normoxia after tissue development is complete. However, VEGF expression, which is regulated by transcriptional factors other than HIFs in normal states, is required not only to maintain the vascular structure but also for neuronal activity. It is noteworthy that the development of geographic atrophy (GA), which is characterized as photoreceptor and RPE cell loss, has been reported during long-term anti-VEGF therapy in multiple clinical studies.\textsuperscript{36,37} For example, in a large clinical cohort, a GA
incidence of 38% was reported at 5 years after initial anti-VEGF treatment among patients who did not have GA at baseline.38 Although anti-VEGF therapy is currently the first-line treatment for neovascular retinal diseases, alternative or additional targets are now required in consideration of the need to maintain the physiological vascular and neuronal function.

Accordingly, we generated RPE or astrocyte cell-specific Hif-2α knockout mice and observed a significant suppression of pathological choroidal and retinal angiogenesis in these mice, just as we did in conditional Vegf knockout mice.24,34,39 This finding indicates that these retinal cells produce angiogenic factors by the recognition of hypoxic conditions through HIF activation (Fig. 5). To regulate retinal pathological neovascularization more safely, HIF would be an ideal therapeutic target, considering the pathophysiology based on these findings.

Conclusion

The establishment of anti-VEGF therapy for ocular diseases has greatly contributed to the cure of some vision-threatening diseases, such as age-related macular degeneration. However, another target is now required because it has become clear that VEGF antagonism may induce retinal atrophy in some cases. We confirmed a microRNA as a candidate for the alternative target for neovascular retinal diseases40 (Fig. 6). Furthermore, we revealed that ectopic HIF activation induces not only pathological angiogenesis but also atrophic neuronal degeneration through a metabolic shift. This finding indicates that HIF antagonism could be applied to various pathological states in the retina39 (Fig. 7). In addition to hypoxia, HIF can be activated by various other stresses, such as inflammation and starvation; this condition is called “pseudo-hypoxia” because it is a hypoxia-like response despite adequate oxygen availability. Aging also leads to ectopic HIF accumulation, resulting in pseudo-hypoxia, and this state may be involved in the development and progression of various age-related diseases.41–44 Regulation of the hypoxia response could constitute a potential next-generation therapeutic target for ocular diseases and others. In our laboratory, we are working to establish a novel HIF inhibitor for clinical use based on the theory described in this article.

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Hif-2α deletion suppresses pathological angiogenesis in animal models of neovascular ocular diseases (based on the results from Kurihara et al.\textsuperscript{34})

(Upper panel) The volume of laser-induced choroidal neovascularization (CNV) is decreased in the VMD-Cre-mediated RPE-specific Hif-2α knockout mice. (Lower panel) Pathological angiogenesis (red) in an oxygen-induced retinopathy (OIR) model is reduced in the GFAP-Cre mediated astrocyte-specific Hif-2α knockout mice. Scale bar: 100μm. (Modified from Weidemann et al., \textit{Glia}, 2010;58(10)1177-1185, Fig. 5, and Kurihara et al., \textit{J Clin Invest}, 2012; 122(11):4213-4217, Fig. 3. Reproduced with permission from John Wiley and Sons, Copyright 2010, and the American Society for Clinical Investigation, Copyright 2012.)

microRNA-132 (miR132) specifically contributes to pathological angiogenesis (based on the results from Westenskow, Kurihara et al.\textsuperscript{40})

(1) The Ras pathway is activated in extending vascular tip cells by VEGF and other angiogenic factors. (2) Increased levels of Ras GTPase activating protein (RasGAP), which is an endogenous negative regulator of the Ras pathway, is induced by downregulation of miR132 in mature vascular endothelial cells. (3) Anti-miR132 increases RasGAP expression and suppresses the Ras pathway in endothelial cells independently from other types of angiogenic factors. This could constitute a new therapeutic approach against ocular and other neovascular diseases. Ang II, angiotensin II; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.
Fig. 7 Ectopic HIF activation in RPE cells led to both neovascular and atrophic retinal degeneration mimicking age-related macular degeneration (based on the results of Kurihara et al.39)

(A) Schematic depicting how dysregulated HIF degradation in the compromised VHL E3 ubiquitin ligase complex may result in increased transcription of HIF target genes in RPE cells. Ectopically stabilized HIFαs can alter the expression of angiogenesis genes and energy homeostasis gene networks. Increased glucose uptake is observed in RPE cells lacking the Vhl gene, and increased glycolysis and impaired tricarboxylic acid (TCA) cycle activities result in the generation of lipid by-products in mitochondria caused by defects in β-oxidation. (B) Schematic demonstrating how neighboring cells are affected by Vhl deletion in RPE cells. Increased levels of angiogenic factors secreted from Vhl knockout RPE cells increase the thickness of the choriocapillaris. The accumulation of material resembling human drusen may impair protein degradation even further, and accelerated glucose intake may promote glycogen accumulation. A shift from mitochondrial oxidative phosphorylation to aerobic glycolysis may provide the RPE with sufficient ATP, but provides less ATP to photoreceptors, thereby promoting photoreceptor degeneration independently from oxygen availability (pseudo-hypoxia). OS, outer segments; CNV, choroidal neovascularization; RPE, retinal pigment epithelium; BM, Bruch’s membrane; CC, choriocapillaris; HRE, hypoxia response element.
References


Conflicts of Interest

The author has no conflicts of interest to report.