Oxygen Sensing in Retinal Health and Disease

Clemens A.K. Lange^{a, b} James W.B. Bainbridge^a

^aNIHR Biomedical Research Centre for Ophthalmology at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK; ^bUniversity Eye Hospital Freiburg, Freiburg, Germany

Key Words

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Abstract

The retina has a uniquely high metabolic demand for oxygen that is normally met by a highly efficient vascular supply. Oxygen plays an essential role in oxidative phosphorylation as an electron acceptor in the mitochondrial respiratory chain in the synthesis of adenosine triphosphate required to support the metabolic demand, including that of the visual cycle. Maintenance of normal retinal function depends on a continuous supply of oxygen and on the capability to detect and respond rapidly to local oxygen deficiency (hypoxia). The functional reserve of oxygen is small and retinal hypoxia can cause neuroretinal dysfunction and degeneration that lead directly to vision loss. Local oxygen sensing mechanisms control adaptive responses that can help protect against ischaemic injury. In the retina, powerful oxygen sensing mechanisms rapidly detect alterations in intracellular oxygen tension and respond with adaptive changes that redress the balance between oxygen supply and demand. These responses include rapid changes in blood flow, protective metabolic adaptations and angiogenesis. In the eye, however, the angiogenic response to hypoxia is typically associated with oedema, haemorrhage and fibrosis that can exacerbate hypoxic neuroretinal injury, causing severe vision loss. This aberrant response is the target of novel thera-

pies including inhibitors of vascular endothelial growth factor. However, non-specific angiostatic agents fail to consider appropriate beneficial adaptive responses to hypoxia, and risk compromising neuroprotective mechanisms. In this review, we discuss the current understanding of retinal oxygenation and oxygen sensing in health and disease, focusing on the central role of hypoxia-inducible transcription factors, and suggest that therapeutic strategies may be improved by considering more targeted interventions.

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Retinal Oxygenation

The vascular supply to the retina uniquely comprises 2 independent and physiologically different circulations (fig. 1). The inner retina in humans is nourished by superficial and deep vascular plexi derived from the central retinal artery, and the outer retina is supplied by choroidal circulation deep to the retinal pigment epithelium (RPE) and its ('Bruch's') basement membrane [1]. The retinal vasculature has a relatively low blood flow and a high oxygen extraction ratio (8 ml oxygen per 100 ml) compared to other tissues, resulting in a high arteriovenous oxygen saturation difference and a low venous oxygen tension [2, 3]. In contrast to the choroid, blood flow in the inner retinal vasculature is autoregulated; both retinal vascular endothelial cells and non-endothelial cells re-

Fig. 1. Histology and corresponding schematic highlighting the vascular supply of the human retina. IS/OS = Inner and outer segments; ONL = outer nuclear layer; OPL = outer plexiform layer; INL = inner nuclear layer; IPL = inner plexiform layer; GCL = ganglion cell layer; NFL = nerve fibre layer.

lease factors, including nitric oxide and endothelin, that modulate arterial tone, blood flow and oxygen delivery (for a detailed review, see Pournaras et al. [4]). The choroidal vasculature arises from long and short posterior ciliary arteries that pierce the sclera around the optic nerve to form 3 vascular layers that support the outer retina including photoreceptor cells [5]. The choroidal circulation has 20-fold greater blood flow than the retinal circulation and a very low rate of oxygen extraction resulting in a low arteriovenous difference and a high reserve of oxygen transport capacity [6]. Choroidal vessels are surrounded by smooth muscle cells and controlled by a perivascular nervous plexus comprising both divisions of the autonomic nervous system [7, 8].

The profile of oxygen tension across the retina has been investigated using polarographic electrodes in several species including guinea pigs [9], cats [10], rats [11] and macaques [12]. Retinal oxygen tension is highest at the level of the choroid and falls steeply between the choriocapillaris and the outer plexiform layer under light-adapted conditions. Mathematical modelling suggests that oxygen consumption is maximal in inner segments of photoreceptor cells, amounting to 15–20 ml per 100 g of tissue per minute in the dark, which is believed to reflect the metabolic demand of tightly packed mitochondria in this region [13]. In darkness, the partial pressure of oxygen in the outer retina is considerably lower, reaching 0 mm Hg at the outer nuclear layer, while the partial pressure in the choroid is unchanged [10, 12]. This finding reflects the increased oxygen consumption by photoreceptors in darkness, which is required for adenosine

triphosphate (ATP) production for maintenance of the dark current, in which Na^+ ions are actively transported extracellularly by a Na^+/K^+ ATPase pump [14]. The low oxygen tension in the outer retina in darkness results in a reversed oxygen gradient and oxygen diffusion from the inner retinal circulation to the outer retina (for a detailed review, see Wangsa-Wirawan and Linsenmeier [15]). In the cat, oxygen tension peaks in the inner and outer plexiform layers correspond to the superficial and deep retinal vascular plexi, respectively. The mean pO_2 in the inner retina of the cat during dark adaptation is 18.5 mm Hg [16], which is consistent with measurements of preretinal oxygen tension in cats of 18.9 mm Hg [17]. Although no difference in oxygen consumption has been demonstrated between darkness and steady illumination [18, 19], the oxygen tension in the inner retina is slightly reduced in light adaptation compared with darkness [16].

Direct measurement of oxygen tension within the human retina has not been reported. However, preretinal oxygen tension is considered a valid indicator of inner retinal oxygen tension [20] and can be measured safely in humans. The mean oxygen tension at the inner limiting membrane in human subjects having surgery for idiopathic epiretinal membrane or full-thickness macular hole, conditions in which retinal oxygenation is believed to be normal, ranges between 9.8 and 15.0 mm Hg depending on the site of measurement [21, 22].

Several lines of evidence demonstrated hypoxia in retinal vascular disease. Inner retinal oxygen tension is reduced by half in cats with diabetes [23], and hypoxia is evident on magnetic resonance imaging [24] and immu-

nohistochemistry using the oxygen-dependent chemical probe pimonidazole in rodent models of diabetes [25]. Oxygen tension measured using an optical oxygen probe in humans with diabetes is reduced in the mid-vitreous, which is believed to reflect global oxygenation of the retina [21, 26]. Indirect evidence of the role of hypoxia in early diabetic retinopathy includes hyperoxia-induced reversal of electroretinographic changes (early oscillatory potential reduction) [27], contrast sensitivity deficit [28] and macular oedema [29]. Experimental retinal vascular occlusion in animal models results in inner retinal inner hypoxia [30–33] and dysfunction [34] demonstrated by reduced b-wave amplitude on ERG [35]. In humans, central retinal vein occlusion is associated with reduced mid-vitreous oxygen levels [22]; while venular oxygen saturation is reduced, arteriolar saturation can be relatively spared [36].

Evidence also suggests that oxygen availability is involved in the pathogenesis of age-related macular degeneration (AMD). The aetiology of AMD involves a complex interaction of genetic susceptibility, oxidative stress, environmental influences and inflammatory processes (for a detailed review, see Ding et al. [37]). Outer retinal ischaemia and hypoxia have been implicated in the progression of the disease [38]. Individuals with AMD are more likely to have reduced choroidal perfusion [39–41] or watershed perfusion defects [42]. Thickening of Bruch's membrane, which increases the distance between the retina and the choroid, may further limit oxygen availability [38, 43].

Oxygen Sensing in the Retina

Retinal hypoxia can be caused by systemic cardiorespiratory failure or vascular insufficiency affecting the carotid or ophthalmic vessels or the retinal and/or choroidal circulations. Systemic hypoxia is sensed by chemoreceptors located near the respiratory centres in the brainstem medulla, and in the aortic and carotid body located on the aortic arch and at the bifurcation of the common carotid artery, respectively. The mechanisms of hypoxia sensing in chemoreceptors may involve hypoxia-induced depolarization of mitochondrial membranes or suppression of K^+ channels, resulting in membrane depolarization, calcium influx and secretion of neurotransmitters such as adenosine and dopamine [44]. The release of these factors activates efferent nerve fibres in the glossopharyngeal (carotid body) or the vagus nerve (aortic bodies) projecting to the nucleus tractus solitarii in the

medulla. In close coordination with other brainstem mechanisms including baroreceptor stimulation, activation of sympathetic efferent pathways induces adaptive cardiorespiratory responses to hypoxia that include increased respiratory and cardiac rates, and modulation of regional blood flow by changes in vascular tone (for a detailed review, see Weir et al. [45]).

In addition to these systemic and regional responses, several mechanisms mediate adaptive responses to hypoxia within tissues at the cellular level to help ensure that vascular supply meets the local demand. Tissue sensing of oxygen availability in the retina is a fundamental biological process critical for appropriate adaptation to changing environments and physiological conditions and is also implicated in the pathogenesis of blinding retinal disorders including AMD, diabetic retinopathy and vaso-occlusive retinal disease. The retina responds rapidly to acute hypoxia by promoting blood flow and to sustained hypoxia by inducing expression of genes directing molecular and cellular responses that protect against hypoxic injury and redress an imbalance in supply and demand by controlling angiogenesis and metabolic requirement. Mechanisms of oxygen sensing in the retina include acute changes in transmembrane ion permeability, generation of vaso-active molecules, and stabilization of key transcription factors orchestrate multiple adaptive pathways to maintain oxygen homeostasis.

Oxygen-Dependent Ion Channels

Evidence for the likely role of hypoxia-dependent changes in ion permeability in the retina is based principally on the investigation of microvasculature of the central nervous system. The resting membrane potential and the contractile status in smooth muscle cells and pericytes is dependent upon the balance of K^+ , Ca^{2+} and Cl^- channel activity. Oxygen-sensitive ion channels are believed to function as oxygen sensors and contribute to relaxation of vascular smooth muscle under hypoxic conditions. These channels may be stimulated directly by low oxygen or indirectly by changes secondary to hypoxia, including reduced ATP levels, increased adenosine diphosphate levels, and reduced intracellular pH [46]. ATP-gated K^+ channels (K_{ATP}) in smooth muscle cells may serve as a critical link between hypoxia and vasodilatation in cerebral arterioles during hypoxia. Under normoxic conditions, K_{ATP} channels are inhibited by intracellular ATP. During hypoxia, a lack of ATP is accompanied by increased transmembrane K^+ flux, cell

hyperpolarization, closure of voltage-dependent Ca^{2+} channels and a reduction in intracellular Ca^{2+} levels resulting in vasorelaxation and dilatation [47, 48]. Furthermore, hypoxia reduces Ca^{2+} flux in smooth muscle cells by a direct effect on Ca^{2+} channels or indirectly by changes in ATP, pH, Ca^{2+} and Mg^{2+} [49–51]. The extent to which such changes in ion permeability upon hypoxia also occur in retinal and choroidal arteriolar smooth muscle cells and pericytes is not currently well defined.

Oxygen-Dependent Vaso-Active Mediators

Various vaso-active compounds including nitric oxide, hydrogen oxide, K^+ , adenosine, GABA, adrenomedullin (ADM), excitatory amino acids and others have been suggested as potential mediators of hypoxia-induced vasodilatation. Nitric oxide contributes to hypoxia-induced retinal vasodilatation and increased retinal blood flow in the cat [52]. Furthermore, hypoxia may induce glial-mediated secretion of a 'retinal relaxing factor', independent of nitric oxide, that induces retinal vasodilatation possibly by activating a membrane-bound Ca^{2+} /ATPase [53]. The direct link between hypoxia and the secretion of these factors has not yet been identified but may depend on changes in ATP levels and ion currents in hypoxic glial cells (for a detailed review, see Pournaras et al. [4]).

Oxygen-Dependent Transcription Factors

Hypoxia-inducible transcription factors (HIFs) are heterodimeric transcription factors, each composed of 1 of the 3 oxygen-sensitive HIF- α subunits (HIF-1 α , HIF-2 α and HIF-3 α) and the oxygen-insensitive and constitutively expressed HIF- β subunit (ARNT). In normoxic conditions, HIFs are continuously and rapidly hydroxylated by 3 different catalyzing prolyl-4-hydroxylase isoforms (PHD 1–3) in a reaction that has an absolute requirement for molecular oxygen as a cosubstrate [54]. Hydroxylation of proline residues changes the conformation of HIF-1 α and increases the affinity of the von Hippel-Lindau (VHL) protein and other factors including elongin B and elongin C [55], Cullin 2 [56] and RBX1 [57], which results in proteasomal proteolysis of HIF- α subunits by the ubiquitin-proteasome pathway [58, 59]. In addition, HIF- α subunits are hydroxylated at a specific asparaginyl residue by another member of the 2-oxoglutarate-dependent oxygenase superfamily, which was originally identified as a protein that binds HIF and was

named factor inhibiting HIF [60]. Asparaginyl hydroxylation abrogates the interaction between HIF-1 and the transcriptional co-activator p300/CBP and provides a second oxygen-regulated mechanism by which HIF- α molecules that escape the prolyl hydroxylation/degradation pathway are prevented from activating gene transcription.

Under hypoxic conditions, in contrast, prolyl hydroxylation is suppressed and HIF- α subunits escape VHL protein-mediated degradation (fig. 2). Consequently, the α unit accumulates to high levels and is translocated to the nucleus where it dimerizes with the β unit. The HIF-1 α /HIF-1 β dimer binds to p300/CBP and activates the hypoxia response element in various hypoxia-responsive target genes. This triggers the transcription of many genes that facilitate adaption to low oxygen conditions including those encoding vascular endothelial growth factor (VEGF) [61], inducible nitric oxide synthase [62], erythropoietin (EPO) [63], endothelin-1 [64] and many others (for a detailed review, see Sharp and Bernaudin [65]). Although the increase of HIF-1 α in hypoxia is primarily controlled by the inhibition of the VHL degradation pathway, hypoxia also promotes translation of HIF-1 α by stabilization of HIF mRNA in certain cell types [66]. Although originally identified as hypoxia-inducible factors, HIFs can also be activated in normoxia, for example in response to inflammatory processes [67].

Localization and Target Genes of HIF- α Isoforms

HIF-1 α and HIF-2 α are differentially expressed in different cells or tissues, resulting in activation of different target genes with non-overlapping functions. HIF-1 α is expressed ubiquitously and participates in most of the chronic cellular responses to low oxygen levels. HIF-2 α was originally thought to be restricted to endothelial cells and therefore initially named EPAS [68] but is more widely expressed than previously believed and is detected in non-endothelially derived tissues including lung, kidney, olfactory epithelium and adrenal gland [69]. Both *Hif1a* [70] and *Hif2a* knockout mice [68] die in midgestation with multiple cardiovascular malformations and mesenchymal cell death, showing that each gene has critical non-redundant functions. Although HIF-1 α and HIF-2 α subunits are structurally similar in their dimerization domains and DNA binding sites, they differ in their transactivation domains, which results in specific target gene regulation [71]. Whereas HIF-1 α specifically regulates glycolytic genes [72, 73] as well as carbonic dehydrase-9

Fig. 2. Oxygen-dependent regulation and target genes of HIF-1 α . In normoxia, HIF is hydroxylated by ‘factor inhibiting HIF’ (FIH-1) and prolyl hydroxylase enzymes (PHD 1–3). In addition, ARD1 acetylates lysine (L) 532 in HIF-1 α to facilitate binding to the von Hippel-Lindau (VHL) protein. VHL binding to HIF serves as an E3 ubiquitin ligase to polyubiquitylate HIF-1 α and target HIF-1 α

for degradation in the proteasome. Under hypoxic conditions, hydroxylation of HIF does not occur, which prevents VHL binding. HIF-1 α accumulates in the cytosol and is translocated to the nucleus where it dimerizes with HIF-1 β and activates hypoxia response element (HRE) in various hypoxia-responsive target genes.

[74] and *BNIP3* [75], HIF-2 α exclusively regulates other factors such as the transcription factor Oct-4, cyclin D1, and TGF- α [76]. Other hypoxia-inducible genes, such as VEGF, facilitated glucose transporter-1, adipose differentiation-related protein, and ADM are regulated by both HIF-1 α and HIF-2 α [72]. The role of HIF-3 α is poorly understood but may regulate the activity of other HIF- α isoforms as a dominant negative regulation of HIF-mediated control of gene expression [77].

HIF Activation in the Retina

Hypoxia-sensing mechanisms and HIFs are critical for normal retinal vascular development. During ocular development, Hif1 α co-localization with Vegf in ganglion cells and cells along the inner border of the neuroblastic layer of mice is consistent with a role in hypoxia, Vegf expression and physiological angiogenesis [78, 79]. Conditional inactivation of *Hif1a* in the peripheral retina results in arrested development of the intermediate vascu-

lar plexus [80], whereas overactivation of *Hifs* in the neuroretina leads to dysmorphic retinal vascular development and persistence of embryonic vascular structures into adulthood [78, 81]. In healthy human and rat retina, HIF-1 α is detectable in the ganglion cell layer, inner nuclear layer, and outer nuclear layer suggesting that active HIF-1 signalling occurs constitutively and has a physiological role in the human retina [82].

There is accumulating evidence that HIFs also play a critical role in the pathogenesis of hypoxia-associated retinal vascular disease. During retinal hypoxia both HIF-1 α and HIF-2 α are stabilized and the downstream molecules VEGF and EPO upregulated in the inner retina [83–85]. HIF-1 α is strongly activated in the ganglion cell and the inner nuclear layer, whereas HIF-2 α activation is restricted to a discrete subset of cells within the inner nuclear layer, most likely Muller glia, and to occasional astrocytes in the nerve fibre layer [84]. HIF- α activation in Muller cells appears to be important in the development of pathological retinal neovascularization. Conditional knockout of *Hif1a* in Muller cells results in reduced Vegf expression and reduced preretinal neovascularization in oxygen-induced retinopathy [86]. Overexpression of Hif-2 α , but not Hif-1 α , in astrocytes induces Vegf and Epo expression in a mouse model of retinal hypoxia and the loss of Vegf or Hif-2 α , but not Hif-1 α , reduces pathological retinal neovascularization [87]. These findings underline the importance of HIFs in the development of retinal neovascularization and suggest distinct roles for HIF-1 and HIF-2 in retinal cell subtypes.

Substantial evidence indicates an important role for HIF in diabetic retinopathy. Hif-1 α is upregulated by hyperglycaemia in rodents [86, 88], and Hif-2 α is upregulated in the retina of mice with diabetes [89]. In humans, the concentrations of both HIF-1 α and VEGF are increased in the vitreous of patients with proliferative diabetic retinopathy and correlate with disease activity [90]. Other HIF-1 α -dependent downstream molecules such as EPO [91], soluble VEGF receptor [92] and connective tissue growth factor (CTGF) [93] are increased in the vitreous of subjects with proliferative diabetic retinopathy. Furthermore, immunohistochemistry studies demonstrated a strong staining for HIF-1 α on surgically excised fibrovascular membranes from subjects with proliferative diabetic retinopathy [94, 95]. These findings strongly suggest that HIFs are involved in the pathogenesis and progression of diabetic retinopathy by promoting adaptive responses to tissue hypoxia including the secretion of pro-angiogenic factors such as EPO, CTGF and VEGF. However, HIF-1 α can be stabilized in normoxia, for ex-

ample by nitric oxide and reactive oxygen species in the inflammatory processes [96]. In advanced proliferative diabetic retinopathy, Lange et al. [21] identified no clear correlation between mid-vitreous hypoxia and VEGF levels, suggesting that other factors such as inflammation may contribute to retinal cytokine expression in this context. In central retinal vein occlusion, hypoxia and vitreous upregulation of VEGF [97] and EPO [98] are consistent with retinal activation of the HIF pathway.

Hypoxia-induced HIF activation may also promote choroidal neovascularization in AMD. Stefansson et al. [38] have suggested that hypoxia-induced expression of VEGF might paradoxically exacerbate hypoxia by inducing accumulation of subretinal fluid, with or without choroidal neovascularization and associated subretinal haemorrhage. HIF-1 and -2 expression is evident in RPE and choroidal neovascularization membranes [99, 100], with activation of HIF-2 α particularly prominent [100]. Activation of both Hif-1 α and Hif-2 α in the RPE by tissue-specific inactivation of *Vhl* leads to neovascularization in the outer retina with chorioretinal anastomosis in the mouse. Chorioretinal anastomosis develops despite additional inactivation of Hif-1 α in these animals, suggesting an important role for Hif-2 α in the RPE in choroidal neovascularization [101].

Molecular and Cellular Adaptation to Retinal Hypoxia

Retinal hypoxia can cause neuroretinal dysfunction and degeneration that lead directly to vision loss. The oxygen sensing mechanisms described above control adaptive responses that can help protect against ischaemic injury. These responses comprise rapid compensatory changes in blood flow and the activation of molecular and cellular mechanisms that protect against hypoxic injury and redress hypoxia by controlling angiogenesis and metabolic demand (table 1).

Hypoxia-Induced Retinal Vasodilatation

The inner retinal vasculature responds promptly to acute hypoxia by increasing vessel diameter and blood flow and thereby oxygen supply to the inner retina [3, 16, 102, 103]. In humans, hypoxia leads to dilatation of retinal arterioles and venules by 8–9% within minutes [104]. Prolonged hypoxia, in humans at high altitude, can increase retinal vessel diameter by 24% [105]; retinal blood

flow can increase by 89% within 2 h and by 174% after 7 weeks [102]. The mechanisms that underlie hypoxia-induced vasodilatation involve changes in ion permeability in vascular smooth muscle cells and local generation of vaso-active mediators (for a detailed review, see Pour-naras et al. [4]). Choroidal blood flow is not significantly affected by moderate hypoxia suggesting that the choroidal circulation has excess capacity [106, 107]. However, a substantial drop in venous oxygen saturation, such as can occur at prolonged high altitude, can induce increased choroidal blood flow that promotes oxygen delivery to the outer retina [107].

Hypoxia-Induced Retinal Angiogenesis

A causative association between retinal hypoxia and the development of retinal neovascularization was first suggested by Michaelson et al. [108] and further explored by Wise [109], who speculated that a hypoxia-induced growth factor ‘factor X’ was responsible for inducing retinal neovascularization. Many candidate mediators have since been identified and VEGF has emerged as a particularly powerful pro-angiogenic growth factor [110]. VEGFs are a family of growth factors, expressed as a number of splice isoforms that vary in their length and affinity to heparin (for a detailed review, see Holmes and Zachary [111]). VEGFs bind to 2 high-affinity receptors, VEGFR1 [112] and VEGFR2 [113], promoting vascular permeability and proliferation of vascular endothelial cells in the eye [114], and facilitating the migration of vessels by inducing the production of matrix metalloproteinases [115]. VEGF signalling is essential for a normal development of the retinal and choroidal vasculature [116] and plays a central in the pathogenesis and progression of common disorders of the retina including diabetic retinopathy and AMD. Expression of VEGF is upregulated by activation of HIF-1 α in human retinal glial cells and RPE cells upon hypoxia [117, 118]. Hypoxia-induced upregulation of VEGF expression is inhibited by siRNA against HIF-1 α [117] and in retinal ischaemia by hyperoxia, presumably by promoting VHL-induced prolyl hydroxylation of HIF- α . In the retina, increased levels of HIF-1 α precede VEGF upregulation and correlate both spatially and temporally with the development of retinal neovascularization [84].

In addition to mediating upregulated expression of VEGF and VEGFR1, HIFs promote the expression of several other pro-angiogenic factors in hypoxia, including ADM [119] plasminogen activator inhibitor [120], CTGF

Table 1. Hypoxia-induced molecular mediators

Proteins	Species	Reference
Increased retinal activity upon hypoxia		
HIF-1 α	rat	182
HIF-2 α	mouse	183
NMDAR1	rat	182
NMDAR2D	rat	85
NMDAR2C	rat	85
Flk1	rat	85
GluR2	rat	182
GluR3	rat	182
VEGF	rat	184
IGF-I	rat	185
IGF-II	rat	185
Ang-2	rat	185
Nitric oxide	rat	182
iNOS	rat	182
eNOS	rat	182
nNOS	rat	182
iNOS	rat	182
Flk1	rat	85
EPO	rat	85
TNF- α	rat	186
TNF receptor 1	rat	186
IL-1 β ,	rat	186
IL-1 receptor 1	rat	186
MCP-1	rat	186
CCR2	rat	186
Decreased retinal activity upon hypoxia		
PEDF	rat	185

and tissue PAI [121]. ADM is a ubiquitously expressed peptide initially isolated from phaeochromocytoma [122] that protects endothelial cells against apoptosis (for a detailed review, see Ribatti et al. [123]). ADM is secreted from cultured human RPE in hypoxia [124] and associated with attenuation of ischaemia-induced injury [125]. CTGF controls growth, adhesion and survival of vascular endothelial cells (for a detailed review, see Perbal [126]). Plasminogen activator inhibitor is the principal inhibitor of fibrinolysis and has been associated with promoting angiogenesis (for a detail review, see Agirbasli [127]). Both CTGF and plasminogen activator-1 levels are up-regulated by hypoxia in cultured human RPE cells via HIF-1 α [121]. The in vivo function of these molecules in the retina is not well-defined and further studies will be necessary to elucidate their role in hypoxia-driven physiological and pathological angiogenesis.

In the eye, the powerful angiogenic response to hypoxia is typically associated with oedema, haemorrhage

Table 2. HIF target genes

Role	HIF target genes	Ref.
Vasomotor control	ADM	119
	nitric oxide synthase 2 (eNOS)	187
	nitric oxide synthase 3 (iNOS)	62
	α_{1B} -adrenergic receptor	188
	endothelin 1 (ET1)	189
	tyrosin hydroxylase	190
	atrial natriuretic peptide	191
Angio-genesis	VEGF	61
	FLT1 (VEGF receptor 1, VEGFR1)	192
	plasminogen activator inhibitor-1	120
	apelin	193
Hormonal regulation/erythropoiesis	CTGF	194
	EPO	141
Iron metabolism	transferrin	195
	transferrin receptor	196
	ceruloplasmin	197
Cell cycle control	DMT1	198
	p21 (WAF1/CIP1)	199
	insulin-like growth factor 2 (IGF2)	200
	IGFBP1, IGFBP2, IGFBP3	201
	endoglin	202
	Wilms' tumour suppressor	203
	α -fetoprotein (negative regulation)	204
Cell death	calcitonin-receptor-like receptor	205
	NIP3, NIX	206
	P53	207
Cell migration	CXCR4	208
Energy metabolism	glucose transporters 1 and 3 (GLUT1, GLUT3)	133
	prolyl-4-hydroxylase α_1	209
	phosphofructokinase	210
	lactate dehydrogenase A	210
	aldolases A and C	210
	pyruvate kinase M	210
	enolase 1	210
	hexokinases 1 and 2	70
	Max interactor	211
	pyruvate dehydrogenase kinase	212
	monocarboxylate transporter 4	213
	cytochrome oxidase isoform 2	214
	glyceraldehyde phosphate dehydrogenase	215
	6-phospho-2-kinase/fructose 2,6 bisphosphatase	216
Miscellaneous	plasminogen activator inhibitor (PAI1) 38	120
	transforming growth factor β 3 (TGF β 3)	217
	haem oxygenase 1	218
	adenylate kinase 3	219
	phosphoglycerate kinase 1	210
	carbonic anhydrase 9	220
	leptin	221
	Rab20	222

and fibrosis that can exacerbate hypoxic neuroretinal injury, causing severe vision loss [128]. In ischaemic retinal diseases such as diabetic retinopathy, retinal neovascularization typically fails to revascularize the ischaemic neuroretina appropriately, and instead is paradoxically misdirected toward the vitreous and into the iridocorneal angle leading to vitreous haemorrhage, tractional retinal detachment, aqueous outflow obstruction and glaucoma. The reason for this misdirection is not well understood but may depend on nitric oxide signalling, which is increased in ischaemic retinal vascular disease [129, 130]. Sennlaub et al. [131] demonstrated that inducible nitric oxide synthase is expressed in the ischaemic mouse retina and inhibits its revascularization at least in part by downregulation of VEGF receptor 2. More recent evidence suggests that the neuronal guidance cue semaphorin 3A (Sema3A), which is secreted by hypoxic neurons, repels neovessels toward the vitreous. Silencing of Sema3A expression enhances normal vascular regeneration within the ischaemic retina, restores metabolic supply, diminishes aberrant neovascularization and preserves neuroretinal function [132]. In AMD, VEGF-induced choroidal neovascularization, which may be the result of both hypoxic and inflammatory activation of HIFs, typically exacerbates retinal injury with exudation, haemorrhage and fibrosis.

Hypoxia-Induced Retinal Metabolic Adaption

A critical adaptation to hypoxia is the ability of cells to utilize glucose by means other than mitochondrial oxidative phosphorylation, for which oxygen is an absolute requirement. Hypoxic activation of HIF-1 α shifts energy production from mitochondrial to glycolytic sources by inducing a wide range of genes involved in glucose metabolism (table 2) including those such as glucose transporter-1 that promote glucose transport into the hypoxic cell [133, 134]. In addition, HIF-1 α promotes expression of enzymes responsible for the glycolysis of intracellular glucose such as aldolase A [70], hexokinases 1 and 2 and phosphofructokinase 1 [135, 136]. Under normoxic conditions, pyruvate can be metabolized by oxygen-dependent mitochondrial oxidative phosphorylation to generate ATP efficiently. In hypoxia, however, pyruvate is converted by lactate dehydrogenase to lactate with less efficient generation of ATP. Hypoxic activation of HIF-1 α also downregulates mitochondrial function and oxidative phosphorylation by promoting the expression of genes such as pyruvate dehydrogenase kinase 1 [137] and

MAX interactor 1 [138], ultimately leading to reduced oxygen demand and consumption. The effect of these mechanisms is to compensate for hypoxia by reducing the oxygen demand while maintaining a supply of energy. In the retina, hypoxia induces an increase in extracellular concentration of lactate and increased utilization of glucose [139]. Lactate and glucose accumulates in the vitreous in proliferative diabetic retinopathy [140] and induction of hypoxia-responsive genes by cobalt chloride increases the expression of Glut1 and Glut3 in the neuroretina. Taken together, these findings are consistent with a shift in energy production from mitochondrial phosphorylation to anaerobic glycolysis induced by hypoxia in the retina.

Hypoxia-Induced Retinal Neuroprotection

Accumulating evidence indicates that HIF-dependent expression of EPO can protect the retina against injury. EPO is a glycoprotein produced in hepatocytes during development and by renal interstitial fibroblasts in the adult, in an oxygen-dependent manner controlled by HIF-1 α [141], and acts as a hormone to regulate production of red blood cells in the bone marrow by preventing apoptosis of erythroid progenitor cells. In addition to its known function of stimulating haematopoiesis, EPO is expressed in many other adult tissues, including the spleen, the brain and the retina [142] indicating a paracrine function. EPO and its receptor are expressed in the human neuroretina and to a significantly higher extent in the retinal pigment epithelium [143]. Hypoxia-induced stabilization of both HIF-1 α and HIF-2 α is associated with increased EPO expression in the eye [84, 144]. Activation of EPO receptors, which are expressed in photoreceptors [144] and in the ganglion cell layer in rodents [145], lead to autophosphorylation and activation of multiple signal transduction pathways including the Jak/STAT5 and AKT pathways that have multiple effects on gene transcription, caspase activation and inflammation (for a detailed review, see Li et al. [146]). EPO protects neuronal cells in vitro and in vivo by reducing nitric oxide-induced free radical damage [147]. Endogenous EPO is implicated in hypoxic preconditioning that protects photoreceptors against light-induced apoptosis [144, 148] and can protect the retina against ischaemia-induced dysfunction [84]. Systemic EPO supplementation protects ganglion cells against ischaemia [148] and can protect the retinal vasculature against oxygen-induced vasoobliteration [149]. Reduction in local EPO by inhibition

of HIF-1 α using YC-1 is associated with inhibition of pathological retinal neovascularization [150]. Taken together, these findings indicate that EPO can promote protective adaptive responses to hypoxia in the retina and suggest that it has potential value for therapeutic intervention in retinal ischaemia. However, since EPO supplementation can exacerbate pathological retinal neovascularization [149], the possibility of adverse effects will require careful evaluation.

Therapeutic Intervention for Retinal Hypoxia

Hypoxia resulting from disorders of retinal perfusion should be addressed clinically by appropriately managing specific underlying causes. For example, endarterectomy is conventional management for carotid stenosis, and optimal management of blood glucose blood pressure (and perfusion pressure) can slow progression of retinal vascular disease [151, 152]. Experimental interventions for retinal vascular occlusions include radial optic neurotomy for central retinal vein occlusion, adventitial sheathotomy for branch retinal vein occlusion, and embolectomy for branch retinal artery occlusion. In addition to redressing perfusion deficits, the availability of oxygen to the retina may be enhanced by increasing the supply or by reducing the demand for oxygen itself. Increased oxygen delivery may be achieved by inhalation of hyperbaric or supplemental oxygen. Hyperbaric oxygen treatment is reported to ameliorate the blood-retinal barrier breakdown in diabetic retinopathy in rats [153]. Supplemental inhaled oxygen for 3 months was associated with improved diabetic macular oedema [29]. While these interventions are unlikely to provide practical treatment options, the findings provide further evidence of the importance of hypoxia in diabetic retinopathy.

Improving Inner Retinal Oxygenation by Panretinal Photocoagulation

Panretinal photocoagulation (PRP) promotes regression of pathological retinal neovascularization and is a conventional treatment for proliferative retinopathies associated with chronic ischaemia. PRP causes thermal destruction of retinal pigment epithelial cells and adjacent photoreceptors, which are replaced by glial cells with relatively low oxygen consumption [154, 155]. Reduced local consumption of oxygen at sites of photocoagulation is believed to promote oxygen diffusion from the choroidal

circulation to the inner retina and to relieve inner retinal hypoxia locally [156]. Evidence supporting this hypothesis has been described in the cat [157], the pig [158], the rabbit [159], and in the human [160]. Application of PRP is followed by vascular constriction consistent with autoregulatory adaption to locally increased oxygenation [161] and with reduction in VEGF and VEGF receptor levels in the vitreous [110, 162]. Although PRP offers a powerful technique to protect central vision against the consequences of retinal neovascularization, conventional photocoagulation strategies cause predictable adverse impact on peripheral vision, contrast sensitivity and night vision, and safer alternative approaches are highly desirable.

Improving Inner Retinal Oxygenation by Vitrectomy

Vitrectomy can reduce retinal neovascularization and macular oedema [163, 164] while increasing the rate of cataract formation and iris neovascularization [165, 166]. These clinical observations may be explained by changes in ocular oxygenation associated with the promotion of fluid currents in the vitreous cavity [156]. The vitreous gel has a relatively low oxygen tension, possibly maintained by a high ascorbate content that acts as an oxygen sink to protect the lens from oxidative damage [167]. The removal of vitreous gel and its replacement with aqueous facilitates diffusion of both oxygen and growth factors. Vitrectomy results in increased oxygen tension in the mid-vitreous and is associated with accelerated development of nuclear sclerotic lens opacity [26, 26]. Vitrectomy may promote diffusion of oxygen to the retina from the anterior segment [32], and possibly from better-perfused areas of the retina to areas of retinal hypoxia [21]. Conversely, increased clearance of hypoxia-induced vaso-active growth factors such as VEGF from the eye following vitrectomy may account for iris neovascularization and neovascular glaucoma (for a detailed review, see Stefansson [168]).

Improving Inner Retinal Oxygenation by Maintaining Light Adaptation

Arden et al. [169] have suggested that inner retinal hypoxia in diabetes may be aggravated by dark adaptation because the metabolically demanding dark current of photoreceptor cells in the outer retina reduces availability of oxygen to the inner retina. They further postulated

that progression of diabetic retinopathy may be controlled by minimizing dark adaptation to promote retinal oxygen availability. Preliminary reports suggest that application of trans-lid retinal illumination during sleep to one eye of subjects with non-proliferative diabetic is associated with improved outcome [170].

Therapeutic Targeting of HIF Signalling

As an alternative to improving retinal oxygen supply, the retina may be protected against the effects of local hypoxia by manipulating adaptive responses so as to inhibit harmful consequences and, potentially, to promote beneficial adaptive responses. Intra-ocular delivery of anti-VEGF antibodies can effectively control pathological neovascularization and oedema. Clinical trials of VEGF inhibitors have demonstrated a significant benefit in AMD [171] and considerable potential in diabetic retinopathy [172]. While these results demonstrate the enormous potential of local anti-angiogenic therapy for retinal vascular disease, there are considerable limitations. The short half-lives of VEGF antibodies in the eye necessitate multiple repeated intra-ocular injections that are not cost effective and cumulatively increase the risk of sight-threatening local adverse effects. Furthermore, anti-VEGF therapy has been associated with systemic adverse effects including non-ocular haemorrhage and stroke [173]. While non-specific VEGF inhibitors target aberrant angiogenesis, they fail to address the underlying hypoxia and to consider appropriate endogenous compensatory responses including neuroprotective mechanisms and appropriate vascular remodelling. While anti-VEGF therapies are valuable for targeting neovascular disease, future therapeutic strategies should consider how to protect and promote appropriate retinal responses to hypoxia. Improved understanding of oxygen sensing and the HIF pathway is likely to help identify new therapeutic opportunities to promote neuroprotection and appropriate vascular remodelling, while protecting against hypoxic/oxidative stress and pathological angiogenesis.

Based on its central role as a master regulator of responses to hypoxia/ischaemia, HIF is a potentially relevant target for treating vascular disease in the eye. HIF signalling can be manipulated by interfering with its expression using siRNA, by direct protein inhibition using small inhibitors, or by inhibition or activation of its degradation by interfering with VHL or PHD activity. Systemic stabilization of Hif by inhibition of prolyl hydroxylases

can protect against oxygen-induced retinopathy [174]. This finding is consistent with an observed protective effect of Epo supplementation on vascular and neuronal survival in the early phase of this model [149]. Furthermore, HIF-1 α stabilization by pyruvate is neuroprotective in a model of light-induced retinal degeneration [175]. Systemic stabilization of HIF in the proliferative stage of the oxygen-induced retinopathy model, however, exacerbates pathological neovascularization [174]. Systemic administration of digoxin was reported to reduce HIF-1 α levels in the proliferative stage and to suppress retinal and choroidal neovascularization [176]. Intra-ocular administration might minimize the potential for systemic side effects of HIF inhibitors [177, 178]. Intravitreal injection of anti-HIF-1 α short hairpin RNAs in nanoparticles inhibits experimental choroidal neovascularization [179]. Overexpression of VHL reduces HIF-1 α levels and inhibits intra-ocular angiogenesis in laser-induced retinal vein occlusion in monkeys [180]. Furthermore, a small molecule inhibitor of HIF-1 α , which is currently undergoing clinical trials in cancer treatment [181], improves physiological revascularization, and reduces pathological neovascularization in the retina of mice [150].

Activation of HIF signalling, for example by prolyl hydroxylase inhibition, offers a potential therapeutic option to improve ischaemic tolerance in retinal vascular disease by upregulating beneficial HIF-dependent processes such as metabolic adaptation, neuroprotection and appropriate revascularization [174]. Conversely, inactivation of HIF signalling, for example by controlled activation of PHDs, may control pathological neovascularization in retinal ischaemia. Given the complexity of the HIF signalling system, in which multiple PHD and HIF isoforms regulate the transcription of numerous genes that intersect with multiple other signalling pathways, successful intervention is likely to depend on careful molecular targeting and timing of intervention. Whether or

not targeting of PHDs can be sufficiently selective to avoid non-therapeutic effects, retaining the pleiotropy that distinguishes PHD inhibition or activation from therapeutic targeting of specific downstream HIF-regulated gene products will require careful investigation. Target specificity may be improved by defining the cellular distribution of the PHD isoforms and their interaction with HIF isoforms in the retina, with a view to cell-specific targeting.

Conclusion

Sophisticated molecular processes have evolved to help ensure that tissue oxygen availability meets local demand. In this review, we summarize the current understanding of oxygen sensing mechanisms in retinal health and disease. In the retina, powerful molecular mechanisms mediate responses to alterations in oxygen availability with adaptive changes in blood flow, metabolism and angiogenesis. In the eye, the angiogenic response to hypoxia is aberrant and can exacerbate hypoxic neuroretinal injury, leading to severe vision loss. A key response is mediated and coordinated by the transcription factor HIF-1 that is central to embryonic development and disorders of retinal ischaemia. Manipulation of this response by activation or inhibition of HIFs or PHDs offer potential therapeutic approaches to promote appropriate adaptive mechanisms and protect against inappropriate effects that exacerbate ischaemic injury. Although the molecular mechanisms of HIF- α isoforms and PHDs have been the subject of extensive investigation, their roles in the retina appear to be contextual with significant cellular specificity. Detailed investigation of these mechanisms in the retina will help determine whether targeted intervention upstream in this pathway can take advantage of pleiotropic downstream effects without significant risk of adverse consequences.

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