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Editorial

Hypoxia Induces Activity of both Efflux and Influx Transporters in Retinal Epithelial Cells

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Introduction

A continuous oxygen supply is pivotal for tissue development, regeneration, proper maintenance, and homeostasis of the body. During oxidative phosphorylation oxygen (O_2) is necessary for the cells to produce ATP. This process produces higher energy than glycolysis. O_2 is an essential element for cell survival, membrane transport, regulation of cell signaling, and gene expression. Deficiency of O2 (hypoxia ≈1%) occurs when cellular O_2 supply from the blood is insufficient than demand. O_2 is supplied through the circulation by erythrocytes and its production is regulated by hormone/ growth factor called erythropoietin (EPO), the product of liver and kidney. EPO supply is inversely proportional to blood O_2 level. During hypoxia EPO is stimulated promoting red blood cells production enhancing O_2 transport capacity. Also, response to reduced tissue O_2 level is mediated by specific transcriptional regulator called hypoxia-inducible factor-1 (HIF-1). HIF-1A gene is encoded on 14q21-q24 human chromosome. The Heterodimeric HIF-1 comprises an O_2 dependent nuclear subunit (HIF1-α) and a constitutively expressed O_2 independent subunit HIF1-β. The HIF1-β is also recognized as aryl-hydrocarbon receptor nuclear translocator (ARNT). Both subunits belong to basic helix-loop-helix (bHLH-PAS) proteins family [1]. HIF-1 can induce transcription of more than 60 genes including vascular endothelial growth factor (VEGF) and erythropoietin resulting in angiogenesis and erythropoiesis respectively [2]. In normoxic conditions, HIF1-α synthesized *de novo* is degenerated by 26S proteasome, whereas in hypoxia this HIF1-α is stabilized by joining the HIF1-β resulting in transcriptional activation of several genes. These target genes are involved in regulation of cell survival, angiogenesis, cellular proliferation, invasion, genetic instability, metastasis, immortalization, radiation and chemotherapy resistance, and stem cell maintenance [3].

It is well established fact that hypoxia mediates a number of vascular retinal diseases such as age related macular degeneration (AMD), diabetic retinopathy (DR), macular degeneration (MD), glaucoma, retinopathy of prematurity ROP), retinitis pigmentosa and von Hippel-Lindau (VHL) disease. Retina is a highly light sensitive organ and very sensitive O_2 tension. O_2 is required as an electron acceptor during oxidative phosphorylation for ATP production. Thus lack of O_2 or hypoxia may elicit adaptive changes. Under normoxic condition HIF1-α is hydroxylated by prolyl hydroxylases (PHD1-3) and promptly degraded by proteasomalubiquitination. During hypoxia hydroxylation is inhibited due to inactive PHDs resulting in translocation of HIF1-α to the nucleus. Then HIF1-α heterodimerizes with HIF1-β, recruits various co-activators and tides to hypoxia response element sequence (RCGTG) and consequently activate numerous target genes such as VEGF, EPO, GLUT-1, angiopoietin-2, endothelin-1, heme oxygenease-1, nitric oxide synthase, platelet derived growth factor-B and stromal derived growth factor-1 [3].

Efflux transporters: Hypoxia caused retinal cells secrete various growth factors which induce angiogenesis, fibro vascular tissue generation, retinal ablation and vision loss. Despite of several interventions developed for these retinal diseases, currently available therapeutic agents are not very effective, possibly because most of these therapeutic agents target only one factor at a time or sub-therapeutic concentrations reach to retinal tissue due to its barriers. Drug disposition is primarily controlled by drug transporters and receptors. Our laboratory has evaluated expressions of several drug transporters including influx and efflux transporters in response to hypoxia. These

studies were performed in-vitro using two retinal cell lines (D407 and ARPE-19). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis suggests that hypoxia induces multidrug resistance gene (MDR1) overexpression in D407 cell just after 12h as well as 72h exposure. Interestingly, the expression of multidrug associated protein (MRP2) in hypoxic cells only up regulates from 6 to24h. Further continuous hypoxia exposure to 48h or 72h, MRP2 expression diminishes. A similar trend appears for breast cancer resistance protein (BCRP). The induction of MRP2 and BCRP genes at hypoxic state is transient. Overexpression of all these efflux transporters is indeed influenced by HIF1-α which also overexpresses in D407 cells at hypoxia. In fact the promoter region of BCRP is in close proximity to the HIF1-α response element which may responsible for the regulation of BCRP. BCRP plays a significant role in heme and folate homeostasis which is essential for cell survival under hypoxic state. Also, overexpression of these efflux transporters plays a significant role for cell survival in malignant or disease condition or from toxic therapeutic agents [4].

Influx transporters: Alongside efflux transporters we have investigated several influx transporters after exposed to hypoxia. Enhanced expression of $B^{(0,+)}$, FR- α and SMVT after hypoxic exposure to ARPE19 cells indicates that these influx transporters are also vulnerable O_2 deficiency. Besides these, peptide transporter (PEPT-1 and PEPTI-2) and organic cationic transporters (OCT-1 and OCT-2) are investigated at hypoxic conditions. In contrast to $B^{(0,+)}$, FR- α and SMVT, the expression of PEPT-1 and PEPTI-2 remains unaltered, while expression of OCT-1 and OCT-2 shows to diminish following 24 h O_2 deficiency. Evaluation of functional aspect of both efflux and influx transporters in O_2 deficiency condition suggests that lower accumulation of 3H-Digoxin, 3H-Lopinavir and 3H-Abacavir resulting higher efflux by of MDR1, MRP2 and BCRP respectively due to the overexpression while rise in cellular accumulation of 14C-Arginine, 3H-Folic acid and 3H-Biotin is the result of more activity of influx transporters $B^{(0,+)}$, FR- α and SMVT respectively. These studies clearly suggest that overexpression of both influx and influx transporters is the result of activation of HIF1-α and these transporters definitely play a pivotal role in retinal cell survival at hypoxic exposure [4].

Clinical impact: So far more than 100 HIF target genes have been identified and a number of genes play critical role mediating angiogenesis, a major pathological condition underlying sight-threatening diseases including AMD, DR, ROP, and neovascular glaucoma. Currently available anti-VEGF therapy for treatment of ocular neovascular diseases includes pegapanib (Macugen, Valeant Pharmaceuticals, USA), bevacizumab (Avastin, Genentech Inc., USA), ranibizumab (Lucentis, Genentech Inc., USA), aflibercept (Eylea, Regeneron Pharmaceutical Inc., USA). Activation of HIF pathway is the leading cause of angiogenesis. Inhibiting the signaling mechanism of HIF1-α may have tremendous therapeutic value. We have shown that HIV protease inhibitor ritonavir can inhibit HIF1-α induction and resulting overexpression of VEGF in retinal cells exposed to hypoxia. Ritonavir mediated HIF1-α inhibition in retinal cells is concentration dependent and 20μM ritonavir produces maximum inhibition of both HIF1-α and VEGF. Hypoxia induced cell proliferation is completely abolished in choroid-retinal cells (RF/6A) following 10 μM and 20 μM of ritonavir and remains control value at normoxia. Ritonavir at 20μM concentration does not show any cytotoxicity. This study may form a platform for the application of ritonavir in neovascular ocular diseases [5].

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