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Concerted inhibition of HIF-1α and -2α expression markedly suppresses angiogenesis in cultured RPE cells

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Abstract HIF-1α is known to play an important role in the induction of VEGF by hypoxia in retinal pigment epithelial (RPE) cells. However, the involvement of the other isoform, HIF-2α, in RPE cells remains unclear. Thus, the purpose of present study was to clarify the role of HIF-2α during induction of angiogenic genes in hypoxic RPE cells. When human RPE cells (ARPE-19) were cultured under hypoxic conditions, HIF-1α and HIF-2α proteins increased. This induced an increase in mRNA for VEGF, causing secretion of VEGF protein into the medium. This conditioned medium induced tube formation in human vascular endothelial cells (HUVEC). The increased expression of mRNA for VEGF in hypoxic RPE cells was partially inhibited by HIF-1α siRNA, but not by HIF-2α siRNA. However, co-transfection of HIF-1α siRNA and HIF-2α siRNA augmented downregulation of VEGF mRNA and protein in hypoxic RPE cells and inhibited formation of tube-like structures in HUVEC. GeneChip and PCR array analyses revealed that not only VEGF, but also expression of other angiogenic genes were synergistically downregulated by co-transfection of hypoxic RPE cells with HIF-1α and HIF-2α siRNAs. These findings suggest an important compensatory role for the HIF-2α isoform in the regulation of angiogenic gene expression. Thus, suppression of angiogenic genes for HIF-1α and HIF-2α may be a possible therapeutic strategy against retinal angiogenesis in Age-related macular degeneration (ARMD).

Keywords Hypoxia-inducible factor-1α and -2α · Retinal pigment epithelial cells · Hypoxia · Vascular endothelial growth factor · Age-related macular degeneration

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Introduction

Hypoxia-inducible factor (HIF) has been termed the "master switch" for control of cellular responses to low-oxygen conditions. Isoforms of HIFα (HIF-1α and HIF-2α) form highly conserved transcriptional complexes found in most oxygen-breathing species. Under physiological conditions, HIF-1α and HIF-2α are short-lived because they are hydroxylated by HIF prolylase, ubiquitinated, and degraded by the proteasome [1]. Under hypoxic conditions, HIF-1α and -2α become more stable and form heterodimers with similarly structured HIF-1β and -2β subunits. Formation of HIF α/β heterodimers induces transcription of more than 70 genes mediating angiogenesis, cell proliferation/survival, and glucose/iron metabolism [2]. Since these HIF-induced responses allow cell survival under low-oxygen conditions, HIF inhibitors are under active investigation as potential clinical drugs to combat diseases such as cancer.

Hypoxia/ischemia may also be major causes of age-related macular degeneration (ARMD), a leading cause of severe vision loss throughout the world in people over