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Special Commentary

A future hope for the treatment of diabetic retinopathy, manipulating hypoxia-inducible factor-1 alpha pathway

The pathologic growth of new blood vessels is the common final pathway in proliferative diabetic retinopathy (PDR), and often leads to a catastrophic loss of vision due to vitreous hemorrhage and/or tractional retinal detachment. In diabetic retinopathy, hypoxia appears to be the primary stimulus to neovascularization by upregulating the production of angiogenic stimulators, and by reducing the production of angiogenic inhibitors, there by disturbing the balance between the positive and negative regulators of angiogenesis. Vascular endothelial growth factor (VEGF) and its cognate receptors are critical mediators of angiogenesis, mediating endothelial cell proliferation, migration, and tube formation [1]. Recently, pigment epithelium-derived factor (PEDF) has been shown to be a highly effective inhibitor of angiogenesis as it specifically inhibits the migration of endothelial cells. It was also shown that PEDF contributes to most of the antiangiogenic activity in the vitreous [2]. The elevated intraocular levels of the angiogenic VEGF [3,4] and decreased intraocular levels of the antiangiogenic PEDF [5] in patients with PDR have previously been demonstrated. The data support the concept that induction of angiogenesis in PDR requires not only elevation of angiogenic growth factors such as VEGF but also a decrease in angiogenesis inhibitors such as PEDF. In addition, strong evidence indicates that chronic, low-grade subclinical inflammation is implicated in the pathogenesis of diabetic retinopathy [6].

All the hypoxia-dependent events in cells appear to share a common denominator: the hypoxia-inducible factor (HIF)-1, which is a heterodimeric transcription factor. HIF-1 is composed of HIF-1α and HIF-1ß subunits, which are both members of the basic helix-loop-helix-PAS family of proteins. Whereas, the ß-subunit protein is constitutively expressed, the stability of the α-subunit and its transcriptional activity are precisely controlled by the intracellular oxygen concentration. Under normoxia, the level of HIF-1α protein is kept low through rapid ubiquitylation, and subsequent proteasomal degradation. In cells under hypoxia, the ubiquitylation and subsequent degradation of HIF-1α protein is suppressed, resulting in accumulation of the protein to form an active complex with HIF-1ß [7–9]. Under hypoxic conditions, HIF-1 triggers the activation of a large number of genes encoding proteins that regulate angiogenesis, such as VEGF, erythropoietin (EPO) and angiopoietins (Angs) [10–13]. Because hypoxia, a central pathogenic stimulus in PDR, induces HIF-1α that can induce the angiogenic molecules VEGF, Epo and Angs, we investigated the expression and distribution of these proteins in PDR fibrovascular epiretinal membranes. In addition, we studied the expression of the angiogenic inhibitor PEDF and the correlation between the number of leukocytes and the expression of angiogenic factors in PDR epiretinal membranes. The levels of vascularization and proliferative activity in epiretinal membranes were determined by immunodetection of the panendothelial marker CD34, and the proliferating cell marker Ki-67.

In this immunohistochemical study of PDR epiretinal fibrovascular membranes, there were four important findings [14]: The first finding with PDR membranes showed immunoreactivity for the transcriptional regulator HIF-1α (Fig. 1A) and its target angiogenic factors Ang-2 (Fig. 1B) and VEGF (Fig. 1C) on vascular endothelial, whereas there was no immunoreactivity for Epo, Ang-1, and PEDF. The second finding was significant correlations between the number of blood vessels expressing the panendothelial marker CD34 and the number of cells expressing the proliferating cell markerKi-67 and the number of blood vessels expressing Ang-2 and VEGF. Third, the number of blood vessels expressing Ang-2 and VEGF in membranes from patients with active PDR were significantly higher than that in the membranes from patients with inactive PDR, and the number of blood vessels expressing Ang-2 and VEGF correlated significantly. Fourth, significant correlations between the number of leukocytes expressing the leukocyte common antigen CD45 and the number of blood vessels expressing Ang-2 and VEGF. Taken together, our findings suggest that selective expression of HIF-1α, Ang-2 and VEGF, but not Ang-1 and PEDF, in diabetic epiretinal membranes may favour aberrant neovascularization and endothelial abnormalities.

HIF-1α mediates the angiogenic response to hypoxia by upregulating the expression of multiple angiogenic factors [10–13]. In a rabbit model of hind limb ischemia, administration of DNA plasmid encoding HIF-1α/VP16 increased angiogenesis and blood supply [15]. Recently, Matsuda et al. [16] demonstrated that HIF-1α DNA induced angiogenesis in a rat cerebral ischemia model. Overexpression of HIF-1α in tumor tissues has been correlated with increased tumor angiogenesis, aggressive tumor growth, and poor patient prognosis [17–20]. In the present study, we demonstrated that HIF-1α was specifically localized in vascular endothelial cells in PDR fibrovascular epiretinal membranes. Our observations are consistent with previous reports showing that exposure to hypoxia induced a significant increase of HIF-1α protein expression in vascular endothelial cells *in vitro* [21,22], and *in vivo* [23]. *In vitro* studies demonstrated that direct infection of vascular endothelial cells with Ad2/ HIF-1α/VP16 promoted endothelial cell proliferation and tube formation that was attributable to increased mRNA and protein levels of VEGF and Ang-2, but not Ang-1. It was also demonstrated that HIF-1 mediated the hypoxic upregulation of VEGF, and Ang-2 in vascular endothelial cells [13]. These findings suggest that HIF-1α is involved in angiogenesis in PDR epiretinal fibrovascular membranes. Further support that HIF-1α is associated with diabetes comes from Chavez et al. [24], who showed that the expression of HIF-1α and its target genes are increased in the nerves of diabetic rats.

In conclusion, the present data suggest production of HIF-1α and its target angiogenic factors VEGF and Ang-2 by vascular endothelial cells in diabetic fibrovascular epiretinal membranes and local autocrine action of these proteins in stimulating angiogenesis. Clinically,

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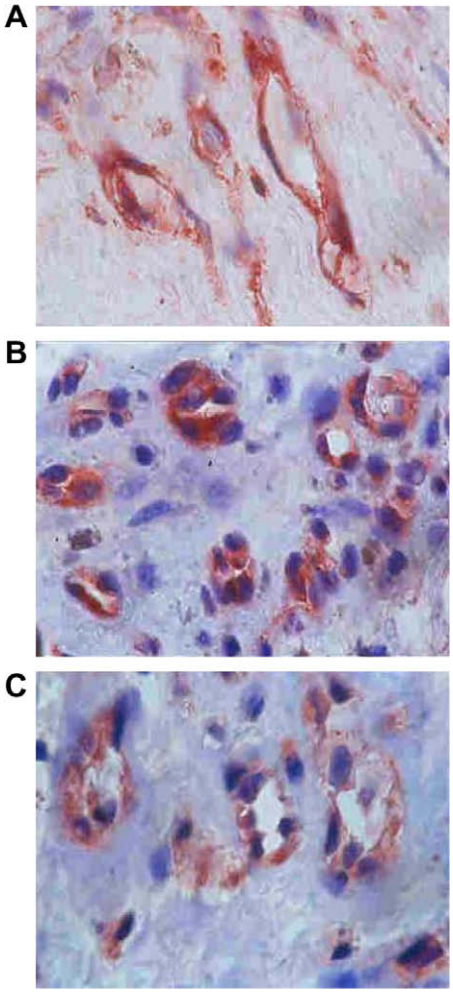

Fig. 1. Proliferative diabetic retinopathy epiretinal membranes. (A) Immunohistochemical staining for hypoxia-inducible factor-1α showing immunoreactivity on vascular endothelial cells (original magnification ×100). (B) Immunohistochemical staining for angiopoietin-2 showing immunoreactivity on vascular endothelial cells (original magnification ×100). (C) Immunohistochemical staining for vascular endothelial growth factor showing immunoreactivity on vascular endothelial cells (original magnification ×100).

manipulating the HIF-1α pathway in the treatment of diabetic retinopathy might be an attractive choice in comparison to targeting VEGF and other growth factor that localize downstream. It has been demonstrated that the HIF-1α pathway can be used as a therapeutic target for development of novel cancer therapeutics [25].

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