

Potential therapeutic effects of pigment epithelium-derived factor for treatment of diabetic retinopathy

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Received: 2013-01-08 Accepted: 2013-03-10

Abstract

• **Diabetic retinopathy (DR), a major micro-vascular complication of diabetes, has emerged as a leading cause of visual impairment and blindness among working adults in the worldwide. The pathobiology of DR involves multiple molecular pathways and is characterized chronic neurovascular degeneration. Current approaches to prevent or to treat DR are still far from satisfactory. Therefore, it is important to develop new therapeutic strategies for the prevention and treatment to DR. Pigment epithelium-derived factor (PEDF), a 50-kDa secreted glycoprotein, has been described as a multi-functional protein. Some emerging evidences indicate that PEDF are able to target multiple pathways exerting neurotropic, neuroprotective, anti-angiogenic, antivasopermeability, anti-inflammation, anti-thrombogenic and anti-oxidative effects in DR. In this review, we addressed the functions of PEDF in different pathways, which could lead to potential therapeutics on the treatment to DR.**

• **KEYWORDS:** diabetic retinopathy; pigment epithelium derived factor; molecular therapeutics; pleiotropic functions

DOI:10.3980/j.issn.2222-3959.2013.02.22

Liu X, Chen HH, Zhang LW. Potential therapeutic effects of pigment epithelium-derived factor for treatment of diabetic retinopathy. *Int J Ophthalmol* 2013;6(2):221-227

INTRODUCTION

D **Diabetic Retinopathy** Diabetic Retinopathy (DR) as the major micro-vascular complication of diabetes, has emerged as a leading cause of visual impairment and blindness among workers in the developed country [1]. The

prevalence of DR continuously increases at an alarming rate with the rising incidence of diabetes in the world. A recent study of the prevalence of diabetes suggested that the diabetes has become a major public health problem among Chinese adults [2]. Current approaches to prevent or to treat DR include systemic controlling of blood pressure, blood glucose and lipids, as well as local treatment such as retinal photocoagulation, pars plana vitrectomy and administration of anti-vascular endothelial growth factor (VEGF) agent [3]. However, the effects of those treatments are limited because of either lacking of good efficacy or the considerable side effects. Therefore, it is important to develop new therapeutic strategies for the prevention and treatment to DR.

PEDF As a non-inhibitory member of the serpin (serine protease inhibitors) super-family, PEDF is a 50-kDa secreted glycoprotein containing 418 amino acids, which was first identified in 1989 from the conditioned medium of cultured human fetal retinal pigment epithelial cells and described as neuronal differentiating activity in human retinoblastoma cells [4-9]. A recent study indicates that the PEDF concentration in plasma is significantly higher in diabetic patients, especially in the eyes with PDR, when compared with the PEDF level in the aqueous and vitreous. Therefore, Ogata *et al* [10] suggested that high levels of PEDF in the plasma might be related to the progression of DR. However, most previous clinical evidences have demonstrated that reduction of aqueous and vitreous PEDF level are positively correlated with the severity of diabetic macular edema (DME) and PDR in patient with DR [11-14]. In another word, severe DME might be ascribed to the low level of PEDF. In addition, the concentration of PEDF in epiretinal membranes (ERMs) of diabetic patients is lower than that of non-diabetic patients [15]. Discrepancy among those study results on the concentration of PEDF indicate that the serum PEDF level may increase as a counteractive system to prevent the vascular damages in the metabolic syndrome [16]. But in most clinical reports PEDF levels in aqueous or vitreous are decreased in patients with diabetes, especially with PDR, which suggests that a loss of PEDF in the human eye may contribute to the development and progression of PDR [11]. Therefore, Yoshida *et al* [12], suggested that PEDF might be used as a therapeutic target for oxidative stress-involved eye diseases, especially PDR. Furthermore, animal studies also confirmed the therapeutic

effect of PEDF administration for retinal neovascularization in a model of diabetes-like retinopathy by overexpressing IGF-1 or oxygen-induced retinopathy (OIR) [17,18]. Evidence showed that PEDF targets multiple pathways involving both early and late phases in the pathological process of DR. As a multi-functional protein, PEDF contributes to promotion of pericyte survival and inhibition of vascular leakage and angiogenesis in DR. Therefore, PEDF might be used as a new therapeutic candidate for the treatment of DR. Here, we reviewed the possible molecular mechanisms of the drug action of PEDF.

PLEIOTROPIC FUNCTIONS OF PEDF ON DR

PEDF working as a neurotropic and neuroprotective factor

It is known that the most striking histopathological feature of DR is vascular abnormalities [19]. Several studies indicate that retinal neuro-degeneration are also involved in the pathogenesis of DR and precede the onset of visible vascular change. Clinical study on fibrovascular tissue of diabetic patients showed that the immunoreactivity of PEDF in gliotic Müller cells decreased when compared with that of non-diabetic patients. This finding suggested that PEDF might work as a neuroprotector to prevent the neuroretinal damage of DR [20].

A study on streptozotocin (STZ) induced diabetic rats showed that intravenous administration of PEDF proteins, can significantly decrease the amplitude of electroretinogram and reduce the glial fibrillary acidic protein expression in Müller cells [21]. In addition, the application of PEDF can also contribute to the decrease of the activities of NADPH-oxidase. These findings indicate that PEDF might prevent neuronal derangements by inhibiting the generation of NADPH-oxidase-driven oxidative stress in the early phase of DR [22]. Furthermore, the extracellular glutamate levels of retina regulated by Müller cells, could excess in high glucose conditions [23,24]. Glutamate excitotoxicity might be implicated in the neuroretinal damage through the activation of N-methyl-D-aspartate (NMDA) receptors and the subsequent triggering in a cascade of apoptotic pathway [23]. Glutamine synthetase (GS) as a glial-specific enzyme can significantly affect the uptake of extracellular glutamate in the neural retina by catalyzing the amidation reaction of glutamate to glutamine [24]. Recent studies have demonstrated that PEDF could prevent from the glutamate-mediated neurodegeneration by increasing the expression of GS. As an anti-inflammatory factor, PEDF was identified to inhibit the action of IL-1 β (interleukin-1 β) in the retina of STZ-induced diabetic rat and protect retinal Müller cells under high glucose condition [25, 26]. Furthermore, the transporter of Müller cells helps to maintain the extracellular glutamate at low concentration in retina [27]. In experimental diabetes, activity of the high-affinity L-glutamate/L-aspartate transporter (GLAST) in Müller cells,

can be reduced through the mechanism of oxidative stress [27]. A recent *in vitro* study has revealed that the glutamate uptake of cells would significantly be decreased by using siRNA to silence PEDF expression in Müller cell and GLAST expression in the normal glucose condition. On the other hand, PEDF can act as an anti-oxidative agent to protect against down-regulation of GLAST expression in retinal Müller cell under high glucose conditions [28]. Therefore, PEDF plays an important role in maintaining the normal microenvironment in retina.

PEDF working as an antioxidative factor One of the earliest histopathological features of DR is the dropout of pericytes from the retinal capillaries. Dysfunction and death of hyperglycemia-induced endothelial cells caused by Loss of pericytes leads to the formation of acellular capillaries in the diabetic retina. Pericyte in retina plays a key role in the maintenance of vascular homeostasis and alleviation of oxidative stress, which could be induced by the loss and dysfunction of pericytes [29]. In cultured retinal pericytes, PEDF can exert an antioxidative effect by inhibiting AGE (advanced glycation end products)-induced ROS (reactive oxygen species) generation and subsequently decreased pericytes apoptosis [30,31]. The PEDF inhibitory effect, which is dependent on the modulation *via* Src phosphorylation at Y419, can be disrupted through the Src pathway by a pharmacologic inhibitor or Src mutant approaches [31]. Furthermore, P13K/Akt, an essential pathway for cell survival, is also involved in PEDF protective and survival effect in pericytes [32]. Using the same cells, PEDF has been shown to prevent from pericyte apoptosis in DR induced under high glucose/H₂O₂ condition. In addition, PEDF, through its anti-oxidative properties, can prevent the increased ratio of angiotensin-2 to angiotensin-1 mRNA level that could lead to the disturbance of pericyte-endothelial cell interaction [33]. Furthermore, in HUVEC (Human Umbilical Vein Endothelial Cells), angiotensin II is able to significantly induce the activation of redox-sensitive nuclear transcription factor- κ B (NF- κ B) and subsequently affect the expression of monocyte chemoattractant protein-1 (MCP-1) [34]. Both of the proteins are potent factors of vascular inflammation and atherosclerosis, which can be inhibited by PEDF *via* blocking NADPH-oxidase-mediated ROS generation [34]. It is known that the interaction between AGEs and their receptors (RAGE) can elicit the generation of ROS and subsequently induce the activation of NF- κ B [35]. Previous studies indicated that NF- κ B could act as pro-inflammatory and pro-apoptotic factor in the pathogenesis of DR. Moreover, AGEs up-regulate the mRNA levels of RAGE by promoting the intracellular ROS generation [35]. A recent *in vitro* study indicated that RAGE gene expression was suppressed in diabetic or AGE-treated rats by blocking the

activation of superoxide-mediated NF- κ B after administration of PEDF^[36].

PEDF working as a factor of antivasopermeability

Vascular permeability in the retina plays a key role in the maintenance of vascular homeostasis. Increase of vascular permeability and leakage might enhance the development and progression of DR^[37]. Clinical evidence has shown that lower vitreous concentration of PEDF is related to higher retinal vascular hyperpermeability and aggravation of DME^[11, 12]. Therefore, some scientists pointed out that down-regulation of PEDF expression might lead to severe DME^[11]. Studies *in vitro* and *in vivo* have demonstrated that PEDF has an antivasopermeability effect by counteracting the biological actions of VEGF^[38, 40]. However, the precise mechanism of its protective effect on blood-retinal barrier (BRB) function in DR still remains unknown. AGEs, BRB breakdown and diabetes-induced retinal vascular hyperpermeability can be prevented by administration of PEDF, which inhibits the generation of NADPH oxidase-driven oxidative stress and the expression of down-regulated VEGF in rats^[26,39]. Those results indicated that the blockage on AGE-ROS-VEGF pathway might imply one of the protective mechanisms on the antipermeability effects of PEDF in DR. Furthermore, intravitreal injection of PEDF in STZ-induced diabetic rats significantly reduced the vascular permeability, which is correlated with the decrease of retinal VEGF and VEGF receptor-2 (VEGFR-2), as well as the down-regulated expression of inflammatory factors [such as MCP-1, tumor necrosis factor- α and intercellular adhesion molecule-1 (ICAM-1)]. This suggests that the decrease of vascular permeability might be mediated by PEDF through its anti-inflammatory activity^[11, 41].

The coordinated opening and closing of tight junctions (TJs) and adherent junctions (AJs) play important roles in vascular endothelial cell-cell junctions^[42]. β -catenin is a key component of the AJs transmembrane complexes. Under normal condition, free cytosolic β -catenin is phosphorylated by binding to glycogen synthetase kinase 3 (GSK3), and then GSK3-mediated phosphorylation triggers β -catenin ubiquitylation and degradation^[43]. The block of GSK3-mediated phosphorylation will lead to the accumulation of β -catenin. Subsequently, β -catenin will be transferred into nucleus as a transcription factor to enhance the expression of urokinase plasminogen activator receptor (uPAR). The accumulation of uPAR will lead to the activation of the pro-uPA on the cell surface. Activation of uPA will cause the cleavage of plasminogen into the active plasmin, which then in turn activates the matrix metalloproteinases (MMPs)^[44]. Several studies have shown that the activation of uPA/uPAR systems is involved in VEGF-induced vascular

hyperpermeability in endothelial cell under high glucose concentration and in the retina of STZ-induced diabetic rat^[45, 46]. In hypoxia-exposed retinal capillary endothelial cells, VEGF-induced paracellular permeability can be blocked by PEDF *via* the MAPK/glycogen synthase kinase (GSK)/ β -catenin signaling pathway, which consequently inhibit the activation of uPA and its receptor system^[47]. In addition, it has been shown that VEGFR2-induced vascular permeability and angiogenesis in retinal microvascular endothelial cells can be regulated by γ -secretase through cleavage and translocation of the C-terminal domain of the full length VEGFR-1^[54]. A recent study has shown that VEGF-induced vascular permeability can be blocked by PEDF, which prevents the dissociation of endothelial junctions by blocking the γ -secretase in cultured micro-vascular endothelial cell and in mouse retinal vasculature^[48]. The relationship among those pathways is summarized in Figure 1.

PEDF working as an anti -thrombogenic factor

Microvascular occlusion is the early stage of the pathological changes in the DR. The adhesion of leukocytes to the vascular endothelial cells plays an important role in initiating this process^[49, 50]. In spontaneously diabetic Torii (SDT) rats and STZ-induced diabetic rats, the number of leukocytes in the retinal vessels (leukostasis), accompanied by the increase of plasma level of PEDF and ICAM-1, is significantly decreased when compared to STZ-induced diabetic rats^[51]. Furthermore, the retinal microvascular leukostasis in diabetic and AGEs-induced rats can be inhibited by PEDF *via* blocking oxidative stress generation and ICAM-1 expression^[52]. Therefore, aiming at the regulation of ICAM-1 by PEDF might be the central mechanism about the inhibition of leukostasis in DR.

PEDF working as an anti -angiogenic factor

Neovascularization is the hallmark of PDR, which is a major cause of the vision loss in DR patients. By blocking the expression of VEGF and disturbing the VEGF-mediated pathways, PEDF might be the most-likely potential inhibitor of angiogenesis in the mammalian eyes^[41]. Previous studies have indicated that MAPK plays a role in vascular hyperpermeability induced by VEGF while the glucose concentration is high in the retina of diabetic rat^[53, 54]. A study on OIR and hypoxia-exposed retinal capillary endothelial cells has demonstrated that PEDF could partially down-regulate the VEGF expression by inhibiting the activation of MAPK and hypoxia-inducible factor-1^[55]. Meanwhile, PEDF could inhibit VEGF-induced phosphorylation of VEGF receptor-1, which play a critical role in the regulation of VEGF receptor-2 induced angiogenesis^[55, 56]. Furthermore, PEDF also could exert an antiangiogenic activity by binding to Wnt co-receptor with high affinity, and subsequently

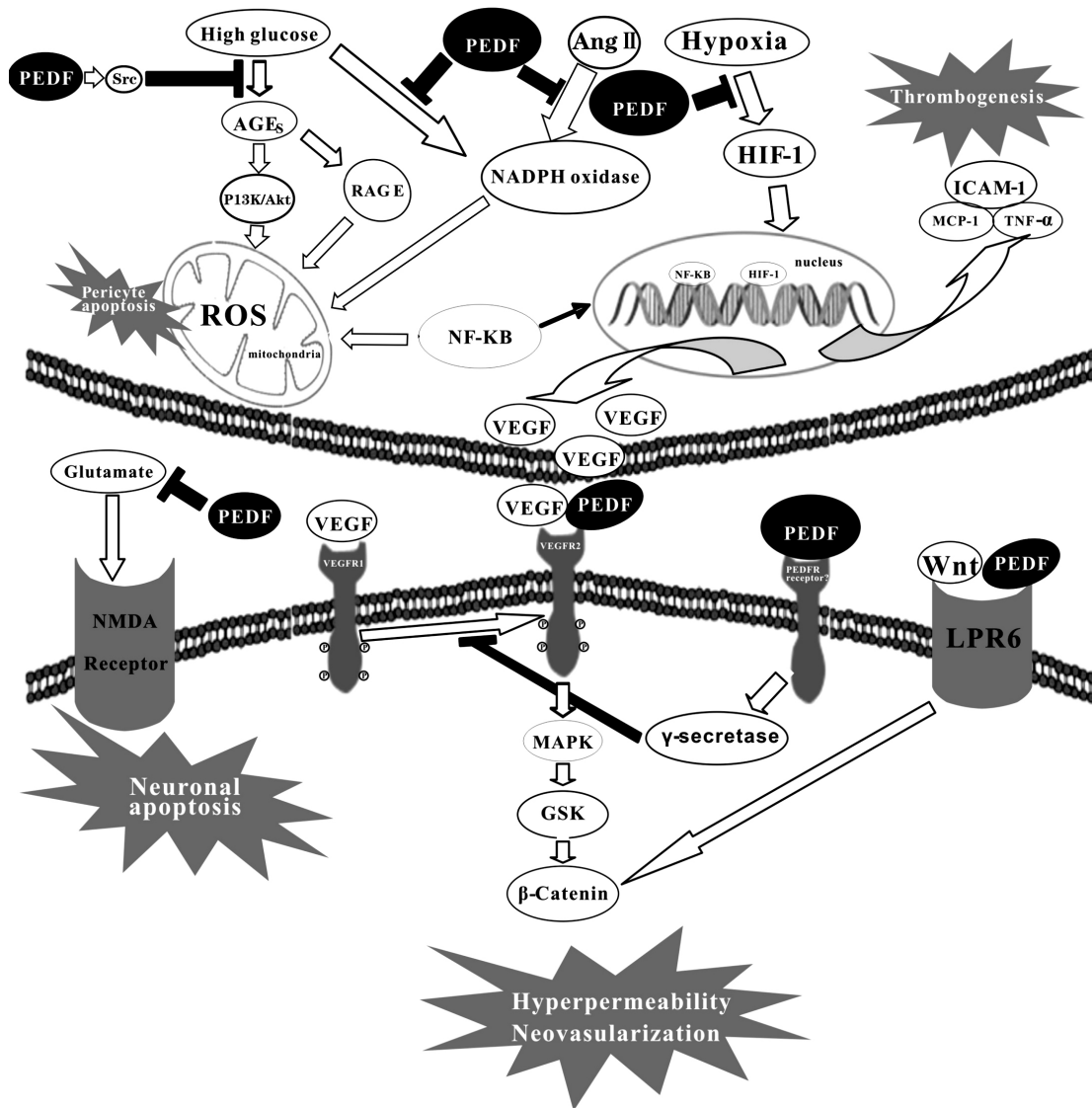


Figure 1 The summary of PEDF targets multiple pathways exerting pleiotropic functions in the pathology of diabetic retinopathy VEGF: Vascular endothelial growth factor; PEDF: Pigment epithelium derived factor; HIF-1: Hypoxia-inducible factor-1; AGEs: Advanced glycation end products; RAGE: AGE receptor; ICAM: Intercellular adhesion molecular; TNF- α : Tumor necrosis factor- α ; MCP-1: Monocyte chemoattractant protein-1; ROS: Reactive oxygen species; NF- κ B: Nuclear transcription factor- κ B; NMDA receptor, N-metil-D-aspartame receptors; GS: Glutamine synthetase; MAPK: Mitogen-activated protein kinase; GSK: Glycogen synthase kinase; Ang II: Angiotensin II.

blocking the Wnt/ β -catenin pathway activation and then down-regulating the VEGF expression [57]. The activation of Wnt pathway found in the retina of human with DR and in the retina of STZ-induced diabetic rats has been shown to regulate the angiogenic factors such as VEGF [58]. Those findings suggest that PEDF may exert an antiangiogenic effect by inhibiting the expression of VEGF at the transcriptional level and disturbing the VEGF-mediated pathways addressed above.

CLINICAL –TRANSLATIONAL PERSPECTIVE AND CURRENT CHALLENGES

As described above, PEDF was involved in multiple pathways which exert pleiotropic functions in DR (Figure 1). Furthermore, PEDF are of vital importance in some other ways. Firstly, PEDF might be the most-likely potential

inhibitor of pathological angiogenesis in the mammalian eyes [41]. Secondly, over-expression of PEDF in developing retina exerted no marked or permanent effects on the normal pattern of retinal vessel development [59]. Moreover, investigation on phase I clinical trials concerning the age-related macular degeneration indicated that intravitreal injection of adenoviral vector-delivered PEDF had a possible dose-dependent anti-angiogenic effect, without serious adverse side effect and toxicities [60]. In that study, data demonstrated that intravitreal injection of PEDF of 10^{9.5} particle units (PU) in subjects with advanced neovascular AMD patients is safe and generally well tolerated. In addition, the dose up to 10⁸ PU or above is able to significantly improve the symptoms of advanced neovascular AMD patients [60].

Table 1 Basic research for the structure-function relationship of PEDF in ocular compartment

Derived peptides	Amino acids	Functions	Experimental models	References
34 mer	44-77	Anti-angiogenic activity Proapoptosis activity	OIR BRCEC	18
34 mer	44-77	Anti-angiogenic activity	Laser-induced CNV	62
25 mer	46-70	Anti-angiogenic activity Proapoptosis activity	Corneal angiogenesis	63
14 mer	43-57	Blocking endothelial cell chemotaxis	HUVECs and HMVECs	64
18 mer	39-57	Blocking endothelial cell chemotaxis Proapoptosis activity Anti-angiogenic activity	HUVECs and HMVECs HMVECs Corneal angiogenesis	64
23 mer	34-57	Proapoptosis activity	HMVECs	65
44 mer	78-121	Anti-vasopermeability	VEGF-induced retinal vascular permeability in mouse	40
34 mer	24-57	Proapoptosis activity Anti-angiogenic activity	HUVEC and corneal angiogenesis	65
44 mer	58-101	Neurotrophic function	Y-79 retinoblastoma cells	65
TGA	16-26	Proapoptosis activity Anti-angiogenic activity	HUVEC and corneal angiogenesis	65
ERT	78-94	Proapoptosis activity Anti-angiogenic activity Neurotrophic function	HUVEC and corneal angiogenesis Y-79 retinoblastoma cells	65
PEDF82-1 21	82-121	Neuroprotective function	Retinal transient ischemic injury	66
44 mer	78-121	PEDF receptor binding site	Y-79 retinoblastoma cells	67

OIR: Oxygen-induced retinopathy; BRCEC: Bovine retinal capillary endothelial cell; CNV: Choroidal neovascularization; HUVECs: Human umbilical vein endothelial cells; HMVECs: Human microvascular endothelial cells; VEGF: Vascular endothelial growth factor; PEDF: Pigment epithelium-derived factor.

PEDF can be applied to treat diabetic retinopathy in clinic in the future. Intravitreal injection of full-length soluble PEDF protein into research animals has been shown to prevent neuronal derangements, and significantly reduce the vascular hyperpermeability as well as retinal leukostasis in STZ-induced diabetic rats [21, 26, 52]. Furthermore, studies on OIR rat model, a common model for PDR, showed that intravitreal injections of full-length soluble PEDF protein significantly reduced vascular hyperpermeability, retinal ischemia and neovascularization [58, 61]. Therefore, the administration of PEDF may be a promising strategy to prevent the development of DR.

However, the size of PEDF may limit its utility as a therapeutic agent; some synthetic PEDF-derived peptides with certain biological active fragment may be needed. Therefore, it is important to clarify the structure-function relationship of PEDF. The residues of four amino acids (glutamate-101, isoleucine-103, leucine-12 and serine-115) in PEDF peptide contribute to the inhibition of VEGF-induced vascular permeability [40]. Furthermore, studies on OIR, a common model for PDR, showed that a 34 mer-derived peptide of PEDF is able to inhibit the pathological retinal neovascularization [18]. Recently, a variety of the functional sites of PEDF in ocular disease have been identified in preclinical study (Table 1), which indicate that PEDF could be clinically applied for treatment of DR in the future.

However, the application of PEDF protein in clinic might be restricted because of its instability and short half-life. Therefore, exploring a new method to delivery PEDF with long-term effect is necessary before clinical application of PEDF being available. Delivery of PEDF via virus mediated gene transfer to the retina has been shown to attenuate the pathological ocular neovascularization and vascular leakage in OIR, with no affection normal vascular formation [68]. Nevertheless, gene therapy might cause a few problems, such potential oncology-inducing property, immunogenicity, uncertain quantitative expression and safety, etc. A recent study shows that the polyethylene glycol (PEG)-modified PEDF can effectively inhibit the development of neovascularization in OIR rat model and has a long-lasting effect in both plasma concentration and retinal concentration, which could be a promising long-term approach for the treatment of DR [69].

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