Review Article

PEDF in Diabetic Retinopathy: A Protective Effect of Oxidative Stress

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Diabetic retinopathy (DR) is a major cause of blindness in working age adults, and oxidative stress plays a vital role in the pathogenesis of DR. Pigment-epithelium-derived factor (PEDF), a multifunctional protein, has shown to inhibit the development of DR by accumulating evidence. This paper highlights the current understanding of probable mechanism about how PEDF blocks the deterioration of DR through its antioxidative properties and application prospects of PEDF as a novel therapeutic target in DR. Gene therapy of PEDF is becoming more and more acceptable and will widely be applied to the actual treatment in the near future.

1. Introduction

Diabetic retinopathy (DR) is the leading cause of acquired vision loss among adults of working age in developed countries worldwide and has been perceived as the most common microvascular complications of diabetes [1–3]. Therefore, within three to five years after the onset of type 1 diabetes and shortly after the diagnosis of type 2 diabetes, the diabetic patients are recommended to be screened for retinopathy with an initial dilated and comprehensive eye examination by an ophthalmologist regularly [4]. Chronic hyperglycemia which is well documented is a primary initiator of DR [5]. In principle, intensive glycemic control can delay the development of DR [5]. However, it is noteworthy that some patients may still develop DR even with good glycemic control [6, 7]. This remaining effect, prior to glycemic control, suggests a "metabolic memory" phenomenon. Furthermore, a growing number of studies have shown that the retina experience increased oxidative damage continuously, even in tight glycemic control, and oxidative stress plays a vital role in the pathogenesis of DR [6, 8].

The molecular mechanisms of hyperglycemia-induced DR are not fully clear, and the majority of publications focus on multiple biochemical pathways, including the augmentation of polyol pathway [9], protein kinase C (PKC) activation

[10], increased advanced glycation endproducts (AGEs) formation, the receptor for AGEs and its activating ligands [11], and overactivity of the hexosamine pathway [12]. However, all the mechanisms are activated by a single event: aberrant production of the mitochondria-derived reactive oxygen species (ROS) to increase the level of oxidative stress [13]. Therefore, antioxidant therapy is being studied to prevent induction of the various pathogenic mechanisms of DR. With the failure of demonstrating that dietary supplementations of multiantioxidants have beneficial effects clinically [14], an antioxidant which could specifically target pathogenesis of DR is no time to delay. Pigment-epithelium-derived factor (PEDF), a 50-kDa secreted glycoprotein, recently is shown to inhibit the development of DR through its antioxidative properties. This paper summarizes the probable protective mechanism of PEDF in high-glucose-induced oxidative stress and application prospects of PEDF as a novel therapeutic target in DR.

2. PEDF and Its Potential Protective Role of Oxidative Stress in Diabetic Retinopathy

2.1. PEDF and Its Biological Function. PEDF is a 418-aminoacid, 50-KDa protein which was first purified from

conditioned medium from both fetal and adult retinal pigment epithelial (RPE) cells [15–18] and it is a noninhibitory member of the serine protease inhibitor (serpin) family. PEDF is widely expressing throughout the body, especially in the nervous system and the retina. According to current research, PEDF has shown that it is a multifunctional protein with demonstrable neurotrophic [19, 20], antiangiogenic [16], antivasopermeability [21], antiinflammatory [22], antifibrosis [23], and antitumorigenic [24] properties and inhibited the development of DR through its antioxidative properties by accumulating evidence [25–28].

Earlier clinical studies demonstrated an inverse correlation of the levels of intraocular PEDF and the development of abnormal angiogenesis in some ocular diseases, such as proliferative diabetic retinopathy (PDR) [16, 29–32]. Similarly, in mouse model of type 2 diabetes mellitus, lower vitreous or aqueous humour levels of PEDF are associated with early phase of experimental DR [16, 31, 33], and decreased protein levels of PEDF in the retina are associated with ischemia-induced retinal neovascularization in the oxygen-induced retinopathy (OIR) model as well [34].

Therefore, existing research data demonstrated that upregulation or substitution of PEDF may be a promising therapeutic target for DR [16, 25, 35–37], especially oxidative stress-involved retinal tissue damage [38]. Then, experimental interventions to increase locally PEDF concentrations, either by an adenovirus expressing human PEDF or a purified recombinant PEDF protein, have shown to attenuate retinal tissue damage in different animal models [22, 39]. Thus, finding a good way for controlling PEDF expression and action in the retina has become a research focus.

In addition, PEDF suppresses vascular endothelial growth-factor-induced (VEGF-induced) retinal microvascular endothelial cell proliferation and migration [40] and inhibits VEGF activation in human retinal endothelial cells in vitro condition [41]. In contrast, PEDF has a synergistic action with VEGF on cell proliferation in endothelial cells cultured in the presence of VEGF [42]. Moreover, the VEGF and PEDF expression in Müller cells is unbalanced under high-glucose concentration, which contributes to retinal neovascularization in DR [43]. Since VEGF plays a pivotal role in the formation of ROS, to defining the specific effects of PEDF, high-glucose-induced oxidative stress will also be important.

2.2. Antioxidative Properties of PEDF in Diabetic Retinopathy

2.2.1. Inhibit AGEs-Induced Injury.
AGEs, senescent macroproteins formed at an accelerated rate in diabetes, cause apoptotic cell death in retinal pericytes [44, 45]. Studies have found AGEs significantly decreased endothelial mRNA levels of PEDF in endothelial cells [46], and PEDF proteins protect cultured retinal pericytes from AGEs-induced injury probably via oxidative stress generation [25]. Thus, we will introduce the different possible mechanisms of PEDF to inhibit AGEs-induced injury in DR.

Retinal Pericytes. Earlier studies have found that loss of pericytes and increased vascular permeability, followed by

microvascular occlusion in the retinas, ultimately leaded to the development of DR [47, 48]. Experimental analysis revealed that pericytes possessed of a membrane protein with binding affinity for PEDF which significantly inhibited AGEs-induced ROS generation and the subsequent decrease in DNA synthesis and apoptotic cell death in pericytes. Furthermore, PEDF proteins completely restored the downregulation of bcl-2 (an antiapoptotic molecule) gene expressing in AGEs-exposed pericytes [25]. Similarly, the studies have demonstrated that PEDF completely blocked high-glucose- or H_2O_2-induced intracellular ROS generation and an increased ratio of bax to bcl-2 mRNA level with subsequent activation of caspase-3 in pericytes [49]. In addition, PEDF protected high glucose- (30 mM) or H_2O_2-induced pericyte apoptosis and dysfunction through its antioxidative properties via glutathione peroxidase (GPx) induction. Simultaneously, the study also found that PEDF's mRNA levels themselves were downregulated in high-glucose (HG-) or H_2O_2-exposed pericytes.

These results all demonstrated that PEDF proteins protected cultured pericytes from AGEs-induced cytotoxicity through its antioxidative properties, and substitution of PEDF proteins may be a promising strategy in treatment of patients with DR.

Monocyte Chemoattractant Protein-1 (MCP-1). Further work have shown that PEDF prevented the AGEs-induced upregulation of monocyte chemoattractant protein-1 (MCP-1) mRNA contents as well as protein production in microvascular endothelial cells (ECs) [50]. Moreover, levels of MCP-1 in vitreous fluids have been correlated with the severity of PDR [51].

Rage. There is a growing body of evidence that RAGE is a signal-transducing receptor for AGEs, and that engagement of RAGE by AGEs elicits vascular inflammation and alters gene expression in retinal vascular wall cells, thereby it is involved in the development and progression of DR [45, 50, 52–54]. Recent studies have shown that PEDF could inhibit diabetes- (in the eye of diabetic rats) or AGEs-induced (in vitro) RAGE gene expression by blocking the superoxide-mediated NF-kappaB activation [36].

Endothelial NO Synthase (eNOS). Studies have shown that PEDF prevented the AGEs-elicited endothelial NO synthase (eNOS) reduction through its antioxidative properties in AGEs-exposed human umbilical vein ECs (HUVECs) [55]. And endothelial dysfunction due to reduced synthesis and/or bioavailability of nitric oxide (NO) is an initial step of atherosclerotic vascular disease in diabetes [56–58].

Platelet Activation and Aggregation. There is accumulating evidence that the oxidative stress generation is involved in platelet activation and aggregation [59, 60]. These observations suggest that the inhibition of platelet activation and aggregation may be a novel therapeutic target for preventing the development and progression of vascular complications in patients with diabetes. Further, the researchers have found

that PEDF prevented platelet activation and aggregation in diabetic rats or AGEs-injected rats through its antioxidative properties by suppressing NADPH oxidase-driven superoxide generation, deleterious effects of AGEs [61].

The Src Pathway. PEDF inhibited AGEs-induced ROS generation by increasing levels of SOD and GSH and also blocked the activation of caspase-3. Furthermore, PEDF induced cell survival via the Src pathway by Src phosphorylation at Y419, as evidenced by a pharmacological inhibitor and Src mutants [62].

Phosphatidylinositol 3-Kinase (PI3K)/Akt Pathway. Recent studies demonstrated that PEDF could inhibit the AGEs-BSA-induced permeability via phosphatidylinositol 3-kinase (PI3K)/Akt pathway. AGEs-BSA increased the ECs permeability by stimulating ROS generation via NADPH oxidase activity and Akt phosphorylation at Ser473. PEDF decreased ROS generation in AGEs-BSA-exposed endothelial cells by suppressing the NADPH oxidase activity via downregulating the phosphorylation of p22^{PHOX} at Thr147. This led to blockade of AGEs induction of PI3K/Akt activation in permeability. Furthermore, PEDF inhibited the AGEs-BSA-induced permeability by increased expression of tight junction protein zona occludens-1 (ZO-1), coincident with an increase in barrier properties of endothelial monolayer [63].

2.2.2. Inhibit Leptin-Induced Injury. PEDF was found to inhibit the leptin-induced ROS generation and upregulation of VEGF mRNA levels including any increase in DNA synthesis in microvascular ECs [64]. Indeed, leptin levels in vitreous were correlated with PDR [65].

2.2.3. Inhibit Tumor Necrosis Factor-α (TNF-α-) Induced Injury. The studies demonstrated that PEDF inhibited tumor necrosis factor-α (TNF-α-) induced redox-sensitive transcriptional factor NF-kappaB activation and subsequent interleukin IL-6 overexpression at both mRNA and protein levels in human umbilical vein endothelial cell (HUVEC) by suppressing NADPH oxidase-mediated ROS generation [66]. In addition, TNF-alpha which is initially involved in the pathogenesis of atherosclerosis [67, 68] and the classic proinflammatory cytokines, downregulated PEDF mRNA levels [66].

2.2.4. Inhibit Angiopoietin-II (Ang II-) Induced Injury. The researchers have found that Angiopoietin-II (Ang II) significantly induced NF-kappaB activation and subsequent MCP-1 expression in HUVEC, both of which were completely inhibited by PEDF. Subsequently, PEDF inhibited Ang-II-induced upregulation of mRNA levels of p22^{PHOX}, Nox4, and gp91^{PHOX}/Nox2, which are membrane components of NADPH oxidase and its enzymatic activity in HUVEC [69]. Another study found that Ang II also significantly decreased PEDF mRNA levels in ECs, which was completely reversed

by an Ang II type 1 receptor blocker, telmisartan [70]. Furthermore, anti-PEDF Ab significantly inhibited the growth-stimulating effects of cocultured ECs on pericytes. These results demonstrated that PEDF, an EC-derived mitogen or survival factor for retinal pericytes, inhibited Ang-II-induced ECs activation by suppressing NADPH-oxidase-mediated ROS generation, and suppression by Ang II of the EC-derived PEDF may be involved in exacerbation of DR in patients with hypertension. In addition, PEDF was found to completely inhibit high-glucose- or H_2O_2-induced increase in a mRNA ratio of Ang II to Angiopoietin-I (Ang I) and upregulation of VEGF mRNA levels in pericytes. VEGF and angiopoietin (Ang) have been known that they were the major regulators of vascular integrity and involved in DR as well [71].

2.2.5. Inhibit HOL-LDL-Induced Injury. PEDF ameliorated HOL-LDL-induced MCP-1 and the subsequent NF-kappaB activation effectively. Moreover, PEDF significantly ameliorated HOG-LDL-induced ROS generation through upregulation of superoxide dismutase 1 expression [72]. This study represented a new mechanism for the salutary effect of PEDF in DR.

2.2.6. Inhibit High-Glucose (HG)-Induced JAK2/STAT3 Activation. A recent report suggested that ACEI exerted a protective effect on DR, and this protective effect could be reflected by a decreased VEGF-to-PEDF ratio, which is a result of reduced mitochondrial ROS production itselfs caused by ACEI-induced increase of proliferator-activated receptor gamma (PPARγ) and subsequent upregulation of uncoupling protein-2 (UCP-2) expression [73]. Further work in vitro has demonstrated that PEDF could decrease mitochondria-derived ROS generation and subsequently downregulate VEGF expression, possibly through inhibiting HG-induced JAK2/STAT3 activation [74]. These studies pave a new way for future in treatment of DR.

2.2.7. Regulation of PEDF Expression In Vivo. PEDF levels in aqueous humor or vitreous were associated with total antioxidant capacity in humans [38, 75] and suggested that PEDF may act as an endogenous antioxidant in the eye and upregulation or substitution of PEDF may be a therapeutic target for oxidative stress-involved eye diseases, especially PDR.

Retinal PEDF levels were reduced in diabetic rat, which were restored by PEDF injections. Decreased amplitudes of a- and b-wave in the ERG in diabetic rats, which were in parallel with GFAP overexpression in the Müller cells, also could be blocked by PEDF injections. Further, retinal 8-OHdG, p22^{PHOX}, VEGF levels, and NADPH oxidase activity were increased, and BRB was broken in diabetic rats, both of which were ameliorated by the treatment of PEDF [26].

In addition, studies with rats, intravenous administration of AGEs, and simultaneous treatments with PEDF demonstrated that PEDF decreased ROS generation in AGEs-exposed endothelial cells by suppressing NADPH oxidase activity via downregulation of mRNA levels of p22^{PHOX} and gp91^{PHOX} and inhibited the AGEs-induced vascular

hyperpermeability, the characteristic feature of early DR, by suppressing VEGF expression [76].

Furthermore, the action of PEDF not only varied with the cell type but also depended on its concentration and environmental conditions [77].

3. Application Prospects of PEDF in Diabetic Retinopathy

Large prospective clinical studies have shown that intensive blood glucose control reduced the incidence and progression of DR [5, 48]. However, strict control of hyperglycemia is often difficult to maintain and may increase the risk of severe hypoglycemia in diabetic patients. In addition, photocoagulation and vitrectomy, current conventional therapeutic options for the treatment of PDR, are limited by considerable side effects. Therefore, developing novel therapeutic strategies that specifically target pathogenesis of DR is desired for patients with diabetes. Based on the above role and regulation mechanism of PEDF in DR, the research on the treatment of PDR has turned to the regulation of angiogenesis inhibitors and growth factors.

3.1. Current and Potential Molecular Therapie of PEDF

3.1.1. PEDF and PEDF-Derived Peptide. The multifunctional PEDF, more effective than other antiangiogenic factors, is a good candidate for treatment of DR. Because a large part of endogenous PEDF-binding affinity to extracellular matrix components and cell-surface receptors, it would not achieve a protective effect. However, injection of exogenous PEDF, competitive binding of PEDF in the extracellular binding sites, will release PEDF from extracellular matrix and subsequently achieve therapeutic concentrations. The problems of current stage are as follow: (1) PEDF is a 50-kDa protein, and its structural features limit practical application as pharmaceuticals; (2) underestimated the carrier itself could induce the inflammatory response; (3) currently there is no good control methods for PEDF which has secreted into the extracellular. However, many biologically active fragment of PEDF has been known, and if we can find the active fragment, equivalent or near equivalent with PEDF, which is replaced with small peptides, we will avoid or reduce the inflammatory reaction by virus or other carriers through slow release of directly permeating sclera or intravitreal injection [78]. A recent study demonstrated that an antiangiogenic peptide, PEDF-34, reduced circulating endothelial cells during ischemia-induced neovascularization [79]. Thus, the PEDF-34 peptide could be a superior biological therapeutic for the treatment of PDR and has great potential for large-scale pharmaceutical development. A disadvantage, however, of using small peptide derivatives is that they tend to be cleared rapidly from tissues and thus may be less effective therapeutically unless they are protected from rapid enzymatic degradation and tissue clearance.

3.1.2. Biodegradable Nanospheres. Nanospheres, biocompatible, biodegradable, and producer of fewer side effects, is one way to avoid disadvantage of peptide. Moreover, there is abundant experimental evidence that intravitreal injections of drugs encapsulated in PLGA-poly (lactide-co-glycolide) nanospheres are both useful and effective [80, 81]. A recent study has found that delivering $PEDF_{82-121}$, a free peptide, in PLGA nanospheres is an effective way of achieving controlled release of therapeutically active levels of the peptide [82]. Such delivery systems can be manipulated to provide controlled release of physiological levels of bioactive products for both short- and long-term needs [83].

3.1.3. Gene Therapy. Gene therapy involves the replacement of a faulty gene or the insertion of a new gene. Treatment of angiogenic disease can be achieved by the insertion of genes that encode antiangiogenic proteins, including the vitreous body and subretinal injection. Adenovirus and adeno-associated virus (AAV) has shown particular promise in the delivery of antiangiogenic DNA. The first human trial of recombinant PEDF introduced via the adenoviral vector AdPEDF.11 suggested that intravitreal delivery is relatively well tolerated with the antiangiogenic effect of PEDF persisting for several months [84]. PEDF transgenic (PEDF-Tg) mice that ubiquitously express human PEDF driven by the β-actin promoter inhibited neovascular disorders such as DR [85]. These results have provided valuable information with regards to gene therapy for the treatment of DR.

4. Conclusion

PEDF, a multifunctional factor, has shown to inhibit the development of DR by accumulating evidence. This paper highlights the current understanding of probable mechanism about how PEDF blocks the deterioration of DR through its antioxidative properties and application prospects of PEDF as a novel therapeutic target in DR. Gene therapy is becoming more and more acceptable and widely applied to the actual treatment. PEDF and its fragments, transferred into virus vector or made of biodegradable implant for local injection, have broad application prospects in treatment of DR. However, the drug screening and selection of the best way to enter the human body need large number of researches.

Acknowledgments

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