Review

Study of Pigment Epithelium-derived Factor in Pathogenesis of Diabetic Retinopathy

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Abstract

Diabetic retinopathy (DR), a major micro-vascular complication of diabetes, has emerged as a leading cause of visual impairment and blindness among adults worldwide. However, aside from pathological damage, the traditional laser and multi-needle operation treatments required for more advanced disease can cause further damage to the visual field and increase the operation risk. Therefore, the development of new therapeutic strategies for the prevention and treatment of DR is essential. Some emerging evidence now indicates that pigment epithelium-derived factor (PEDF), a multifunctional protein, can target multiple pathways to exert neurotropic, neuroprotective, anti-angiogenic, anti-vasopermeability, anti-inflammation, anti-thrombogenic, and anti-oxidative effects against DR. This review addresses the functions of PEDF in different pathways that could lead to potential therapeutics for the treatment of DR. (***Eye Science 2015; 30:81-88***)

Keywords: diabetic retinopathy; pigment epithelium-derived factor; pleiotropic functions

Diabetic retinopathy (DR), the most common microvascular complication in diabetic patients, has become the primary cause of visual impairment and blindness in developed countries[1]. A recent study reported that diabetes is a major public health issue in Chinese adults[2], and the present incidence of DR in diabetic patients of 37% is likely to increase to 54% in 10 to 20 years[3]. Currently, common therapies for treatment of DR consist of systemic control of blood pressure, glucose, and lipid levels and topical treatments, such as retinal photocoagulation, vitrectomy, and anti-vascular endothelial growth factor (VEGF) therapy, etc[4-6]. Conventional laser and surgical approaches are mainly applied to patients with advanced DR, as these options may cause visual field injury, increase surgical risk, and fail to unravel the pathogenesis of DR. Therefore, exploring novel approaches is extremely important for preventing and treating DR.

DR is mainly pathologically characterized as neovascularization. The accumulated evidence has demonstrated that neovascularization results from the synergistic effect of a variety of cytokines. Among these, pigment epithelium-derived factor (PEDF), a protein discovered in the past 20 years, shows a wide array of biological activities. It is a natural inhibitor of neovascularization, so PEDF is capturing increasing attention in DR studies.

About PEDF

PEDF is a secreted glycoprotein around 50 kDa in size. It was originally discovered and isolated in 1989 from neonatal retinal pigment epithelium (RPE) by Joyce Tombran-Tink and Lincoln Johnson[7]. It is 418 amino acids in length and the N-terminus contains a leader sequence at residues 44-121 that is responsible for PEDF neurotrophic properties. A reactive center loop (RCL) lies near the C-terminus, at residue 385; this is normally involved in serine protease inhibitor activity. However, in contrast to similar proteins, PEDF has no ability to inhibit proteinases. The gene encoding human PEDF is localized to the 17th chromosome, at position 17p13.1 and consists of 8 exons and 7 introns.

PEDF can induce the outgrowth of human Y-79

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retinoblastoma cells and can even lead to widespread nerve cell differentiation[8,9]. It was initially isolated from human RPE cell solutions, and the PEDF protein is mainly secreted by RPE cells. A substantial quantity of PEDF accumulates in the stromal layer of inner photoreceptors, where it performs vital functions[10]. Other research has indicated that PEDF can be secreted and expressed in multiple tissues and cells other than the RPE, including the aqueous humor, vitreous body, choroid, corneal epithelium, and photoreceptors[11]. Dawson et al[12] confirmed that PEDF has strong anti-angiogenic function. It can be detected in the vitreous body and aqueous humor, where it probably functions to prevent neovascularization in the cornea, vitreous body, and aqueous humor.

PEDF has an asymmetrical charge distribution across the whole protein, which is possibly correlated with its anti-angiogenic function[16]. The expression levels of PEDF in the aqueous humors and vitreous bodies of DR patients have been positively correlated with the severity of DR[13-15]; thus, severe DR most likely results from a low level of PEDF. Previous research demonstrated that supplying PEDF at 50 ng/mL can completely suppress the endothelial cell migration induced by VEGF[17]. An imbalance between PEDF and VEGF is associated with neovascularization, such that a high concentration of VEGF and low level of PEDF can promote angiogenesis. Therefore, higher levels of PEDF, a natural potent inhibitor of neovascularization, should effectively suppress the neovascularization commonly seen in DR patients.

PEDF expression in DR patients

Ogata et al[18] used ELISA to measure the concentration of PEDF in the vitreous chambers of 34 patients with either DR, rhegmatogenous retinal detachment, or idiopathic macular hole and found that PEDF levels were the lowest in DR patients, and especially those with proliferative diabetic retinopathy (PDR). The concentration of PEDF was significantly lower in patients with active DR than in their inactive counterparts, hinting that low levels of PEDF might be correlated with neovascularization in DR and with the incidence of active PDR. Clinical trials demonstrated that the VEGF level in the vitreous chamber of DR patients was considerably elevated. The VEGF level in PDR patients was higher than in their non-proliferative counterparts and the same findings were observed in active and inactive DR individuals. Ogata et al. found that the vitreous level of PEDF was significantly lower in DR patients than in healthy controls, especially in PDR patients, whereas the opposite tendency was documented in terms of VEGF[19], which suggested that an imbalance between the inhibitor and promoter of angiogenesis might give rise to the observed incidence and progression of DR.

Spranger et al. utilized western blotting and immunohistochemical analysis to detect the PEDF expressed in intraocular fluid and retinal specimens in DR patients and found an associated decrease in the concentration of PEDF. The PDR patients receiving panretinal photocoagulation had significantly enhanced levels of PEDF when compared with their counterparts who did not undergo panretinal photocoagulation[20]. Ogata et al[21]. reported that the in vitro expression level of PEDF peaked at 6 h after photocoagulation and then gradually declined. They also found that the *in vivo* PEDF level began to increase at 6 h following photocoagulation and continued to rise until 14 d, hinting that laser photocoagulation can up-regulate the expression of PEDF by reducing the retinal ischemic area, thereby suppressing neovascularization. The PDEF levels were equally up-regulated, but remained below the normal range, probably due to the recurrence of neovascularization after photocoagulation.

Previous studies on patients with diabetes complicated with cataract indicated that the PEDF levels in the aqueous humor were lower than those in cataract patients. During a mean follow-up of 69 months, approximately 30% of the diabetic patients developed retinopathy, which was not associated with age, course of diabetes, hypertension, or HbAlc. The concentration of PEDF was lower in patients with retinopathy than without retinopathy. These results demonstrated that PEDF is a pivotal negative regulator of neovascularization of the aqueous humor. A low level of PEDF in the aqueous humor strongly predicts the risk of retinopathy in diabetic

patients, hinting that PEDF level in aqueous humor can serve as an index to predict the incidence and development of DR[22].

Previous research indicated that low levels of PEDF (0.5-5.0 μg/ml) could inhibit the migration of endothelial cells and the induction of angiogenesis, whereas high concentrations of PEDF (25-50 μg/ml) stimulated the migration of endothelial cells and secretion of VEGF, indicating that PEDF can exert a concentration-dependent double effect on neovascularization[23]. The levels of PEDF were significantly higher in type I diabetic patients with capillary complications than in healthy controls[24]. Type II diabetic patients, regardless of alternative complications, had significantly elevated serum levels of PEDF when compared with healthy controls. Ogata et al[25]. reported significantly elevated PEDF levels in two diabetic patients compared to a control group and an even higher serum PEDF concentration in PDR patients. The PEDF level was significantly higher in type I diabetic patients with DR than in those without DR. The PEDF level in the aqueous humor was also higher in diabetic patients with DR than in the control group, although this difference was not statistically significant.

Role of PEDF in DR

Anti-angiogenic function of PEDF

PDR is characterized by neovascularization and PDR is the primary cause of visual loss in DR patients. A majority of angiogenesis-inducing factors, such as VEGF, basic fibroblast growth factor (bFGF), insulin-like growth factor I (IGF-I) and interleukin-8 (IL-8), are up-regulated in PDR patients. PEDF is able to suppress the angiogenesis induced by a wide range of stimuli and it inhibits VEGF infiltration caused by multiple angiogenesis inducers, thereby exerting an anti-neovascularization function[26]. Previous research indicated that PEDF inhibits neovascularization in vivo mainly through acceleration of apoptosis in active endothelial cells. The increasing concentration of PEDF is accompanied by a corresponding enhancement in the number of apoptotic cells. This proapoptotic function is realized mainly via the Fas and FasL signaling pathway. The underlying mechanism is to involve angiogenesis inducers that up-regulate the expression of Fas on the VEGF membrane and down-regulate the expression of anti-apoptosis proteins. Nevertheless, PEDF is capable of up-regulating the expression of FasL, activating Fas/FasL, promoting vascular endothelial cell apoptosis and inhibiting neovascularization[27,28].

The imbalance between angiogenesis inducers and inhibitors is now accepted as the primary cause of pathological neovascularization. The blocking of the VEGF and VEGF-mediated signaling pathway identifies PEDF as a probable inhibitor of ocular angiogenesis in mammals[29]. VEGF-induced MAPK increases vascular permeability and raises the glucose concentration in the retina in diabetic rats[30,31]. Previous research demonstrated that PEDF could partially inhibit the activation of MAPK and hypoxia inducible factor-1, and then down-regulate the expression level of VEGF. PEDF can also suppress the VEGF-induced phosphorylation of the VEGF-1 receptor, which plays a pivotal role in regulating VEGF receptor-induced angiogenesis[32,33]. In the retinas of DR patients and rats with STZ-induced diabetes, the activation of Wnt signaling pathway participates in regulating pro-angiogenic factors, such as VEGF[34]. PEDF can also bind to the receptor and block the activation of the Wnt/β-catenin signaling pathway, thereby down-regulating the expression of VEGF and promoting anti-angiogenesis[35].

PEDF exerts its anti-angiogenic function by inhibiting the expression of VEGF via transcription and VEGF-intervention pathways. Stellmach et al[36]. found that PEDF inhibits abnormal neovascularization while causing no evident retinal vessel injury. Even at high doses, PEDF has no effect on the formation and development of vascular epithelial cells. Moreover, the numbers of endothelial cells are similar in PEDF-treated animal retinas and in animals treated with vehicle alone, suggesting that PEDF causes vascular injury only during pathological neovascularization.

PEDF not only inhibits the incidence of neovascularization, but also reverses the process of neovascularization. For example, Mori et al[37]. successfully constructed laser-induced CNV mouse models transfected with VEGF and divided the animals into PEDF (AdPEDF) and control groups (AdNull).

They detected an apparent recession of neovascularization and significant endothelial cell apoptosis. More importantly, PEDF shows a selective anti-angiogenic function. It can suppress pathological neovascularization but has no effect upon normal physiological neovascularization. In transgenic mouse models, endogenous PEDF at a dose > 3.5 times the physiological concentration is unlikely to exert any apparent or persistent effect on retinal neovascularization and differentiation in newborn mice[30]. Therefore, PEDF is a promising approach for treating retinal neovascular diseases.

Antioxidant property of PEDF

The pathogenesis of DR is correlated with oxidative stress. Oxidative stress responses tend to be strengthened under high glucose conditions, which probably accelerates cellular apoptosis, leads to microvessel injury, and destroys the blood-retina barrier. An initial pathological characteristic of DR is the exfoliation of retinal capillary pericytes. The loss of these pericytes causes hyperglycemia-induced endothelial cell dysfunction and death, thereby leading to the formation of acellular retinal capillaries in DR patients. This observation suggests that pericytes play a crucial role in maintaining a balance in the mechanisms that alleviate oxidative stress responses[38]. Yamagishi et al[39]. concluded that PEDF could significantly inhibit the production of active oxygen free radicals induced by advanced glycation end products (AGEs) and subsequently decrease the suppression of DNA synthesis and induction of apoptosis in pericytes. They proposed that PEDF functions by intervening in the nonenzymatic glycation pathway.

PEDF also exerts its inhibitory effects through the phosphorylation or mutation of Src[40]. The P13K/Akt signaling pathway is an essential pathway for cell survival and influences the effects of PEDF on the protection and survival of pericytes[41]. Recent in vitro studies demonstrated that PEDF showed a dose-dependent inhibition of hydrogen peroxide-induced apoptosis of retinal pigment epithelium. PEDF can also protect cells by stimulating the phosphorylation of extracellular signal-regulated kinases[42]. Furthermore, it can counteract the increases in reactive oxygen species (ROS) in the outer vascular membrane that occur in response to high blood glucose levels, thereby preventing growth retardation and apoptosis[43]. In vitro experiments have indicated that PEDF causes a dose-dependent suppression of the production of reactive oxygen induced by AGE and completely inhibits the expression of monocyte chemoattractant protein (MCP) at both the RNA and protein levels, hinting that PEDF probably has a therapeutic effect on PDR[44]. PEDF can also prevent the elevation in levels of angiopoietin-1 and -2 mRNA and possibly interfere with the interaction between pericytes and endothelial cells[45]. Angiotensin II can significantly induce the activation of nuclear factor-κB (NF-κB) and subsequently influence the expression of MCP-1[46]. Intravitreal expression of MCP-1 is probably correlated with the severity of PDR in diabetic patients.

Another angiogenic factor, leptin, is closely associated with the incidence of PDR. It can increase the production of reactive oxygen products in microvascular endothelial cells and up-regulate the expression level of VEGF mRNA, thereby accelerating the incidence of DR. PEDF can counteract these events by virtue of its antioxidant characteristics[47]. NF-κB probably acts as a proinflammatory and and proapoptotic factor in the pathogenesis of DR. It can activate the secretion of PEDF by retinal glial cells for protection of retinal ganglion cells from ischemia or hypoxia[70]. PEDF can fully suppress the increase in NADPH oxidation induced by tumor necrosis factor-α, thereby evidently inhibiting the activation of NF-κB and expression of IL-6[48]. Recent research has demonstrated that the activation of NF-κB is blocked after administration of PEDF in diabetic or AGE-treated rats[49]. Up-regulation of AGE can inhibit the expression of PEDF mRNA in retinal pericytes (through acceleration of intracellular ROS production), down-regulate the PEDF level, aggravate the apoptosis and dysfunction of pericytes induced by oxidative stress, and eventually promote the development of DR[50].

Anti-vascular permeability function of PEDF

PEDF plays a critical role in maintaining retinal vessel permeability and vascular balance, while changes in vascular permeability probably promote the development of DR[51]. A low level of PEDF in the vitreous body is correlated with an increase in retinal vessel permeability and aggravation of cystoid macu-

lar edema[52,53]. Some scholars have indicated that down-regulation of PEDF expression probably leads to severe cystoid macular edema[52]. Both *in vivo* and *in vitro* research demonstrated that PEDF exerts its effects on vascular permeability through an anti-VEGF action[54]. Liu et al[55]. successfully established non-PDR mouse models using VEGF and found that PEDF could significantly suppress the VEGF-induced vascular permeability with an inhibition rate of 95.6%. The N-terminal domain, consisting of 44 amino acid residues, especially the amino acids glutamic acid (101), isoleucine (103), leucine (112), and serine (115), is associated with the function of anti-vascular permeability.

PEDF and its derivatives can be used to treat diabetic macular edema and restore visual acuity via its anti-vascular permeability function. Intravitreal injection of PEDF in streptozotocin (STZ)-induced diabetic rats can significantly reduce vascular permeability by inhibiting the production of retinal VEGF and the function of VEGF receptor-2 (VEGFR-2) and by down-regulating the expression of inflammatory factors, such as MCP-1, TNF-α, and intercellular adhesion molecule-1 (ICAM-1). The decreasing vascular permeability is probably mediated by the anti-inflammatory activity of PEDF[56]. Recent study demonstrated that PEDF inhibition of VEGF-induced permeability, both in cultured microvascular endothelial cell monolayers and *in vivo* in the mouse retinal vasculature, is mediated by γ-secretase and prevention of the dissociation of endothelial adhesion[57].

Inhibition of inflammatory responses by PEDF

Chronic inflammatory responses play a clear role in the incidence and development of diabetic microvascular complications. Previous research showed that PEDF levels were significantly decreased in the retina and plasma of rats with endotoxin-induced uveitis. Intravitreal injection of PEDF considerably reduced vascular hyper-permeability and oxygen-induced retinopathy in rat models of diabetes and these responses were correlated with decreased levels of retinal inflammatory factors, including VEGF, VEGFR-2, and MCP-1. In cell cultures, PEDF levels were inversely correlated with the changes in these factors[58], suggesting that PEDF probably serves as an endogenous anti-inflammatory factor in the eye, and that decreased levels of PEDF increases vascular permeability and enhances the concentration of inflammatory factors. Previous studies indicated increased concentrations of IL-6 and IL-8 in the vitreous fluid of PDR patients and in the retinas of DR mice[59,60]. PEDF has a confirmed anti-inflammatory function[61]; for example, decreased expression level of PEDF in retinal Muller cells enhanced the secretion of VEGF and TNF, while intravitreal injection of PEDF reduced the levels of retinal pro-inflammatory factors, such as VEGF, MCP-1, TNF, and I-CAM. A recent role for PEDF was postulated in the adhesion of white blood cells to vascular endothelial cells[62,63]. Spontaneously diabetic Torii (SDT) and streptozotocin-induced diabetic (STZ) rats showed a significant increase in ICAM-1 levels and PEDF expression when compared to control SD rats, but SDT rats had notably lower levels of retinal leukostasis when compared to STZ rats[64]. PEDF promotes leukostasis in diabetic patients and in AGE-induced rats by blocking oxidative stress and inhibiting ICAM-1 expression[52]. Therefore, the regulation of I-CAM-1 by PEDF is probably the mechanism of suppressing white blood cell adhesion in the central nervous system in DR patients.

Prospects for application

PEDF is a potent angiogenic inhibitor, showing anti-angiogenic, anti-tumorigenic, and neurotrophic functions. As an antiangiogenic protein, PEDF helps to maintain the avascular status of transparent ocular tissues and suppresses unwanted neovascularization of the eye. In addition, PEDF can selectively inhibit pathological angiogenesis by various mechanisms without impairing vision. Hence, it has widespread prospects for clinical application. The exact mechanism underlying its inhibition of angiogenesis remains largely unknown. The association between the PEDF levels in the vitreous body/subretinal fluid and eye diseases, especially PDR, remains to be fully investigated. The possibility that PEDF could inhibit neovascularization needs to be validated by clinical trials. Animal experiments have confirmed that topical intraocular injection of PEDF may ameliorate ischemia, as well as VEGF-induced retinal neovascularization[65] and laser-induced choroidal neo-

vascularization[66]. PEDF shows efficacious, safe, and specific characteristics, and it can theoretically be used for regulation of systemic neovascularization. The PEDF protein can be directly injected into the vitreous chamber, although some scholars found that exogenously applied PEDF could be rapidly eliminated from vitreous body, thereby necessitating repeated injections[67] and risking ocular pain and other complications. The development of gene therapy, by contrast, offers novel options. Transferring of adenovirus or adeno-associated virus vectors encoding the PEDF gene would allow persistent expression of PEDF, thereby avoiding long-term repeated procedures and minimizing systemic adverse events as much as possible. Takita et al[68]. performed intravitreal injection of an adenovirus vector expressing PEDF in a retinal ischemia rat model and documented a clear increase in the retinal ganglion cell layer and in the inner and outer nuclear layer cells and amelioration of the ischemic injury. Polyethylene glycol (PEG)-modified PEDF has effectively inhibited neovascularization in OIR rat models and, indicating a potential therapeutic role for PEDF in DR[69]. Options with greater effectiveness and safety remain to be explored for the treatment of human eye diseases, but all indications suggest that PEDF can be widely applied in treating multiple diseases.

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