Mechanisms of PEDF-mediated protection against reactive oxygen species damage in diabetic retinopathy and neuropathy

Mina Elahy, Swati Baindur-Hudson, Vinicius F Cruzat[1,2], Philip Newsholme[1,2] and Crispin R Dass[2,3]

College of Health and Biomedicine, Victoria University, St Albans, Victoria 3021, Australia
[1]School of Biomedical Sciences, [2]Biosciences Research Precinct, and [3]School of Pharmacy, Curtin University, Bentley, Perth, Western Australia 6102, Australia

Correspondence should be addressed to C R Dass
Email
Crispin.Dass@curtin.edu.au

Abstract

Pigment epithelium-derived factor (PEDF) is a pluripotent glycoprotein belonging to the serpin family. PEDF can stimulate several physiological processes such as angiogenesis, cell proliferation, and survival. Oxidative stress plays an important role in the occurrence of diabetic retinopathy (DR), which is the major cause of blindness in young diabetic adults. PEDF plays a protective role in DR and there is accumulating evidence of the neuroprotective effect of PEDF. In this paper, we review the role of PEDF and the mechanisms involved in its antioxidative, anti-inflammatory, and neuroprotective properties.

Key Words
- PEDF
- diabetes
- oxidative stress
- pericyte
- signal transduction
- redox balance

Journal of Endocrinology (2014) **222**, R129–R139

Introduction

Nontransmissible chronic diseases are increasing all over the world, resulting in financial and logistical challenges for all health care systems in the 21st century. Contributing to this scenario, diabetic retinopathy (DR), one of the most devastating acquired vascular complications of diabetes mellitus, is responsible for affecting overall life quality worldwide. It has been estimated that the number of Americans suffering from DR will be 16 million by 2050 (Milne & Brownstein 2013).

In DR disease, premature death of pericytes occurs via apoptosis, and may result in a dramatic reduction in retinal function, due to the formation of pericyte ghosts in the basement membrane, subsequently leading to nonproliferative DR (Amano *et al.* 2005, Hammes 2005, Ejaz 2008). Pericytes are one of the main cell types of retinal microvessels, playing an important role in retinal capillary homeostasis via control of proliferation of endothelial cells (ECs). Furthermore, experimental evidence shows that pericytes are responsible for protection of ECs against lipid peroxide-induced injuries, preserving their capacity to produce prostacyclins (Yamagishi *et al.* 1993*a*,*b*). Therefore, in DR, major structural change occurs, including thickening of the basement membrane, hyperpermeability, and the formation of microaneurysms. These changes ultimately predispose the capillaries to neovascularization, angiogenesis, ECs injuries, and the proliferative form of DR, which mostly results in vision loss due to macular edema (Yamagishi & Matsui 2011).

Metabolic and signaling disturbances in diabetes can initiate apoptosis in retinal capillaries and may culminate

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

in pericyte apoptosis and depletion (Ejaz 2008, Yamagishi & Matsui 2011). These disturbances include formation of advanced glycation end products (AGEs), upregulation of protein kinase C, the polyol pathway, focal leukostasis, and oxidative stress. In DR, promoted by an islet-based inability to secrete or failure of target tissues to optimally respond to insulin, hyperglycemic events are common and these, *per se*, promote the aberrant production of reactive oxygen species (ROS) and an overwhelmed detoxification system in insulin-responsive cells, which leads to oxidative stress (Yamagishi *et al*. 2002*a*, Newsholme *et al*. 2012). In this context, pigment epithelium-derived factor (PEDF, a glycoprotein (50 kDa, 418 amino acids) widely expressed in most body tissues) exerts anti-inflammatory functions, attenuating the expression of chemical mediators, such as vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNFα), and intercellular adhesion molecule 1 (ICAM1) in retinal vascular ECs (Zhang *et al*. 2008, 2011).

Recent advances in molecular and cell biology have provided the basis for the discovery of inhibitory activity of PEDF against cancers, such as osteosarcoma (Dass *et al*. 2007, Ek *et al*. 2007*a*,*b*,*c*, Ta *et al*. 2009), breast and prostate cancers (Filiz & Dass 2012), and chondrosarcoma (Tan *et al*. 2010). It also protects against oxidative stress, which includes diabetic damage in the eye and angiogenic-related disease (Yamagishi *et al*. 2003), vascular injuries (Yoshida *et al*. 2006, Nakamura *et al*. 2007), and neurotoxicity (Araki *et al*. 1998, Yabe *et al*. 2005*a*). However, recent work has provided evidence that in uncontrolled diabetes, PEDF levels in the retina and vitreous fluids are low, which may contribute to proliferative DR (Boehm *et al*. 2003, Yokoi *et al*. 2007). Considering the epidemic challenge of diabetes and its complications, a better understanding of DR, its mechanisms, and targets will be essential to future new strategies and treatments. In the following sections, first the mechanisms and pathways that are involved in the development of DR and pericyte loss is discussed and next the inhibitory and protective role of PEDF will be presented.

Role of oxidative stress and inflammation in the development of DR

The Maillard process, a nonenzymatic reaction between a reducing sugar and free amino groups in proteins (the carbonyl group of the sugar reacts with the amino group producing *N*-substituted glycosylamine and water), is important for the development of DR. The glycosylamine undergoes Amadori rearrangement to form various ketosamines that undergo further rearrangement, important for the creation of glycation products which can undergo further complex reactions such as dehydration, condensation, and rearrangement, and become permanently cross-linked to form AGEs (Sato *et al*. 2006, Yamagishi *et al*. 2007*a*, Yamagishi & Matsui 2011). During the progression of diabetes, the formation and accumulation of AGEs increase. Retinal pericytes are associated with higher levels of AGEs, which then contribute to retinal vascular hyperpermeability and DR (Yamagishi *et al*. 2002*a*, 2007*a*, Sato *et al*. 2006). AGEs, and signaling stimulated by their receptors (RAGEs), can induce the generation of intracellular ROS and provoke oxidative stress, initiating vascular inflammation and complications in diabetes (Fukami *et al*. 2004). Furthermore, AGE–RAGE interaction can also cause apoptosis in retinal pericytes and become embroiled in the early phase of DR (Hammes *et al*. 1999, Yamagishi *et al*. 2002*a*,*b*).

Free radical (containing an unpaired electron) and nonradical ROS can be produced through different mechanisms including the plasma or organelle membrane-bound NADPH oxidase (NOX) family of enzymes, ischemia/reperfusion, inflammatory response, transition metal ions, and inefficient electron transport chain reactivity in organelles such as mitochondria. Some ROS such as superoxide anion ($O_2{\cdot}^-$) or hydroxyl radicals ($OH{\cdot}$) are extremely unstable, whereas others such as hydrogen peroxide (H_2O_2) are freely diffusible and relatively long-lived, from nanoseconds to milliseconds (Newsholme *et al*. 2012). In general, ROS are considered highly reactive molecules as they tend to capture electrons from other molecules (oxidation) and produce other ROS, such as peroxynitrite ($ONOO^-$), thiol-based radicals ($RS{\cdot}$), and others (Brownlee 2001). Moreover, these unstable molecules can promote DNA damage by reacting with nucleotides, proteins, and especially structural components in the cell such as neutral lipids and phospholipids of the membranes via a process known as lipid peroxidation (propagation step of ROS; Finkel & Holbrook 2000). Lipid peroxidation changes the fluidity of cell membranes, reduces the capacity to maintain defined ion gradients (e.g. Na^+ and K^+), and also increases membrane permeability. Consequently, lipid peroxidation leads to a loss of intracellular proteins, reduces Ca^{2+} transport across the cell and endoplasmic reticulum membranes, altering mitochondrial voltage channels, and cell function (Dias & Griffiths 2014).

It has been well-documented that high glucose, fatty acids, and AGEs can increase intracellular ROS generation

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

and induce apoptosis in retinal pericytes (Amano *et al.* 2002, Yamagishi *et al.* 2002*a*,*b*,*c*). High glucose and fatty acid levels may overstimulate electron transport activity in the mitochondria, leading to excessive generation of superoxide (Newsholme *et al.* 2007).

Characterized by increased levels of ROS due to excessive production and slow removal by the anti-oxidant systems, the phenomenon of oxidative stress has attracted attention in the last decades. The rationale for this scientific interest arises from the fact that oxidative stress, and consequently the change in the intracellular redox state, occurs in several disease mechanisms (Krause & de Bittencourt 2008, Cruzat *et al.* 2014), including the complications of diabetes (Newsholme *et al.* 2007) and aging (Ristow *et al.* 2009). The detrimental effects of AGEs on pericyte survival and function are mediated via increased ROS generation, which then leads to apoptosis. It has been shown that AGE-modified BSA (AGE–BSA) has the potential to stimulate glucose transport into retinal pericytes followed by an elevation in ROS production therefore provoking cell death. The activation of AGE-sensitive cell surface receptors, such as RAGE, or nonreceptor-dependent pathways may be involved in increasing ROS generation (Schmidt *et al.* 2001; Fig. 1).

The BCL2 family of proteins are key players in the regulation of apoptosis. The anti-apoptotic members of this family, such as BCL2, inhibit apoptosis by blocking the release of cytochrome *c* from mitochondria. However, the pro-apoptotic members of this family, including BAX, enhance the release of cytochrome *c*, which subsequently

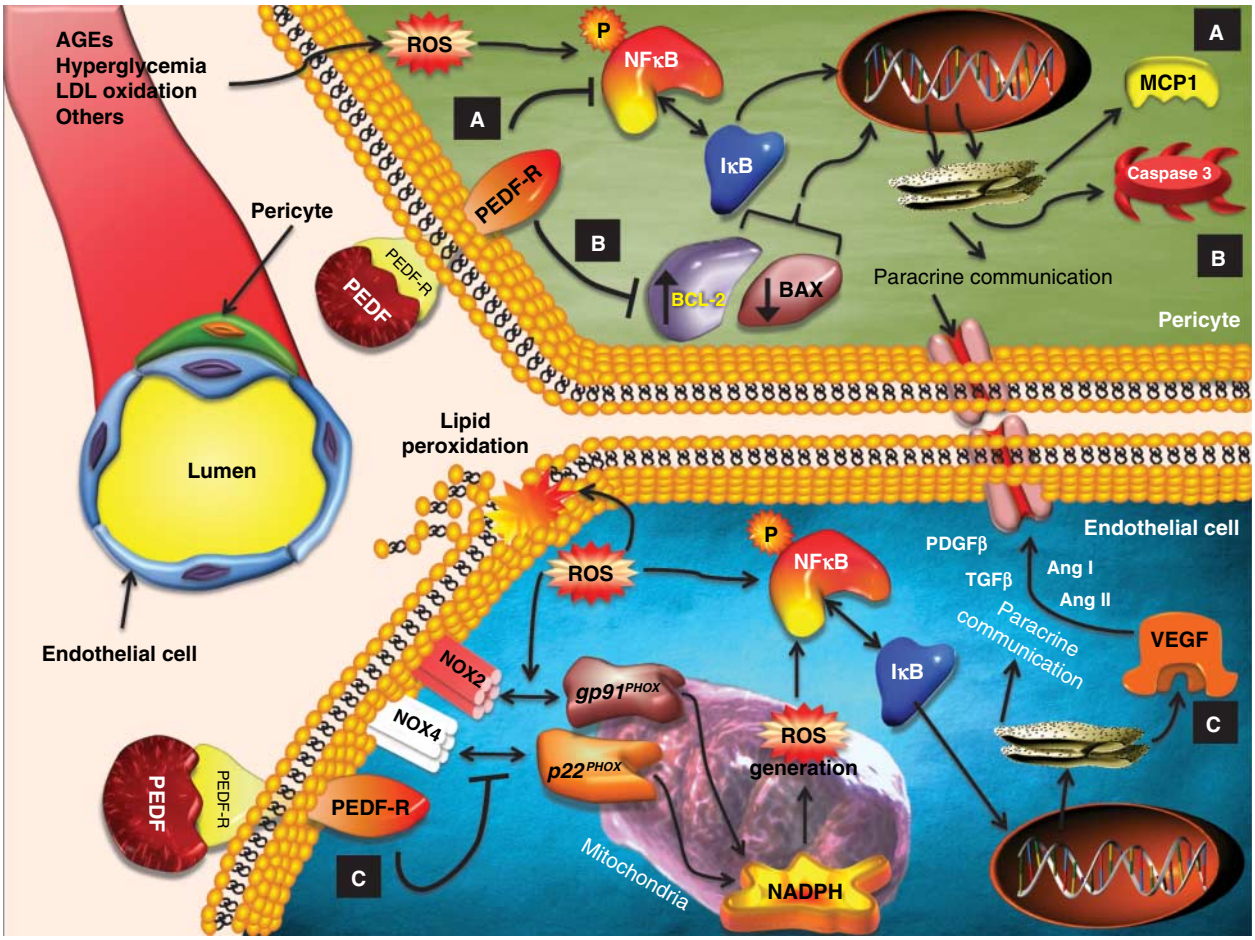

Figure 1
A brief overview of the protective mechanisms mediated by PEDF in conditions of oxidative stress caused by advanced glycation end products (AGEs), NADPH oxidase activation and glycated LDL in pericytes and endothelial cells (EC). (A) In LDL-exposed pericytes, PEDF can suppress the binding of nuclear factor kappa B (NFκB) to DNA and, as a result, inhibit the monocyte chemoattractant protein 1 (MCP1) (Zhang *et al.* 2008). (B) In AGE-exposed pericytes, PEDF is able to attenuate caspase 3 activity by improving the ratio of BCL2/BAX (Yamagishi *et al.* 2002*a*,*c*). (C) In AGE-exposed EC, PEDF can reduce reactive oxygen species (ROS) generation by downregulating p^{22PHOX} and $gp91^{PHOX}$ thus suppressing NADPH oxidase activity (Yoshida *et al.* 2009, Yamagishi *et al.* 2006*a*). A full colour version of this figure is available at http://dx.doi.org/10.1530/JOE-14-0065.

Published by Bioscientifica Ltd.

Journal of Endocrinology

leads to activation of different caspase molecules (such as caspases 3 and 9) that cleave various downstream pro-caspases within the cell to induce full-blown apoptosis (Cory & Adams 2002, Broadhead *et al*. 2009). Furthermore, inflammatory reactions and apoptosis are initiated as a result of activation of MAP kinase/RAS, nuclear factor kappa B (NFκB), AKT, and p38 in addition to key molecules in apoptotic pathways, for example forkhead transcription factors (FOXO) and c-JUN (Min *et al*. 1999, Alikhani *et al*. 2007). During pericyte apoptosis, caspase 3, a key enzyme required for the execution of apoptosis, increases in concentration due to a decreased ratio of BCL2/BAX.

Oxidative stress can result in direct, free radical-based DNA damage, but can also trigger redox pathways required for transcriptional activation. NFκB in retinal pericytes is extremely sensitive to the redox status of the cells, and normally remains in an inactive form, as it is bound with an inhibitory IκB protein. Several inflammatory stimuli, such as TNFα, and also elevated levels oxidative stress can promote specific signal transducing pathways to enable phosphorylation of the IκB and subsequent degradation by the 26S proteosome (Sethi *et al*. 2008). The phosphorylation of IκB releases NFκB from IκB protein and permits NFκB to translocate to the nucleus (Heck *et al*. 2011). The subunit composition of NFκB can vary, although NFκB p65 (Rel A) and NFκB p50 (NFκB1) are the classical NFκB pathway components studied in inflammation (Sethi *et al*. 2008). Many target genes related to pro-inflammatory response (e.g. NFκBIA, NFκB1, COX2, MYD88, and IRAK1) are cyclically activated by NFκB. The imbalance between NFκB and IκB has several consequences, such as hyper-inflammation and loss of cell repair and function, which lead to apoptosis and DR disease evolution (Duarte *et al*. 2013).

Summary of the key roles of PEDF and potential mechanisms of protection in oxidative stress conditions

Apoptosis and PEDF: balance of BCL2 and BAX PEDF has neurotrophic and neuroprotective effects on dopaminergic neurons (Falk *et al*. 2009), as well as protective effect on pericytes. A dose-dependent effect of PEDF on BCL2 was observed in cultured cortical neurons where PEDF upregulated the expression of BCL2 and promoted neuronal survival against oxidative stress (Sanchez *et al*. 2012). In photoreceptor cells, the nuclear translocation of apoptosis-inducing factor (AIF) from mitochondria intermembrane space during apoptosis results in chromatin condensation and DNA fragmentation. The upregulation of BCL2 by PEDF leads to inhibition of the nuclear translocation of AIF, resulting in prevention of the apoptosis in both *in vitro* and *in vivo* (Murakami *et al*. 2008). PEDF significantly prevents the arrest of DNA synthesis in cultured AGE-exposed pericytes by reversing the reduction in expression of BCL2, as well as inactivating BAX expression in retinal pericytes and thus aids pericyte survival (Fig. 1; Yamagishi *et al*. 2002*a*,*c*).

PEDF and inflammatory signal transduction The protective effect of PEDF on retinal pericytes exposed to high-glucose or H_2O_2 is via stimulation of antioxidative mechanisms, such as inhibition of ROS production, and normalizing or enhancing the level of antioxidant enzymes such as phospholipid hydroperoxide/glutathione peroxidase (GSH-Px). PEDF is able to induce and increase the mRNA expression level of *GSH-Px* (Yamagishi *et al*. 2002*a*,*c*, Amano *et al*. 2005). However, the role of PEDF in regulating the levels and activity of the other major antioxidant enzymes – catalase and Cu/Zn superoxide dismutase (SOD) – has yet to be elucidated. Similarly, JAK2/STAT3 and ERKs (ERK1/2) are activated in bovine retinal capillary ECs (BRECs) (Zheng *et al*. 2010) and human retinal pigment epithelial cells (ARPE-19) respectively (Tsao *et al*. 2006). PEDF decreased the level of mitochondria-generated ROS, suppressed JAK2/STAT3 activation, leading to lower VEGF mRNA expression (Zheng *et al*. 2010). On the contrary, PEDF can induce ERK1/2 phosphorylation and activation and protect ARPE-19 cells against H_2O_2-mediated oxidative stress (Tsao *et al*. 2006). Similar pathways are involved in PEDF-mediated protection in cerebellum granule cells (Taniwaki *et al*. 1995, 1997), hippocampal neurons (DeCoster *et al*. 1999), and spinal motor neurons (Bilak *et al*. 1999) against glutamate toxicity. PEDF can induce ERK1/2 phosphorylation followed by phosphorylation and activation of cAMP-responsive element-binding protein (CREB) – the two key molecules in the cell survival signal transduction pathway – therefore providing protective properties in cultured rat cerebellar granule cells (CGCs) (Yabe *et al*. 2005*b*). Interestingly, the protective effect of PEDF has been observed in immature CGCs rather than mature cells (Taniwaki *et al*. 1995, Araki *et al*. 1998). The suggested mechanism underlying glutamate neurotoxicity is the elevation of intracellular Ca^{2+} as a result of opening *N*-methyl-D-aspartate (NMDA) channels. The high free intracellular Ca^{2+} leads to activation of Ca^{2+}-dependent enzymes – nucleases, proteases, protein kinases, and protein phosphatases – and may also lead to the generation of free radicals. It has been postulated that PEDF can block the initial signal transduction, which leads

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

to the opening of NMDA channels as well as maintain Ca^{2+} homeostasis through removal of excess Ca^{2+}, thus helping cell survival (Taniwaki *et al.* 1997).

As mentioned before NFκB is one of the transcription factors activated during oxidative stress. PEDF inhibition of this particular pathway results in protection for AGE-exposed mesangial cells. In this situation, the coupling of RAGE and AGE can initiate downstream signaling and stimulate ROS-generated inflammatory and thrombogenic reactions via redox-sensitive transcriptional factor NFκB. PEDF can inhibit ROS generation, attenuating NFκB activation and subsequently inhibiting the expression of inflammatory and thrombogenic genes such as monocyte chemoattractant protein 1 (*MCP1*), vascular cell adhesion molecule 1 (*VCAM1*), and plasminogen activator inhibitor 1 (*PAI1*) (Ide *et al.* 2010). Furthermore, there is a correlation between MCP1 protein abundance in vitreous fluids and progression of proliferative DR (Mitamura *et al.* 2001). However, PEDF can inhibit AGE-induced overexpression of MCP1 in ECs by suppressing the generation of intracellular ROSs (Inagaki *et al.* 2003). This may be similar to the situation in retinal pericytes when exposed to glycated LDL. This oxidizing factor could activate the NFκB pathway and lead to overexpression of MCP1. PEDF has an inhibitory effect on MCP1 expression, which consequently results in decreased cell permeability and leakage and ultimately neovascularization in DR (Fig. 1). It also has been shown that PEDF can suppress the binding of NFκB to DNA and its transcription activation in a cell-type-specific manner (Yabe *et al.* 2001, Zhang *et al.* 2008). Production of pro-inflammatory cytokines can be inhibited by the activation of NFκB or CREB in cultured microglia (Sanagi *et al.* 2005), neonatal astrocytes (Yabe *et al.* 2005*a*), and rat culture CGCs (Yabe *et al.* 2005*b*). PEDF regulates the level of these transcription factors and therefore acts as a neuroimmune modulator in the CNS (Sanagi *et al.* 2005).

In relation to AGE-induced apoptosis in podocytes (epithelial cells around glomerular capillaries), restoration of transcriptional activity of peroxisome proliferator-activated receptor gamma (PPARγ) is the proposed pathway for PEDF protection, although it did not affect the AGE-induced reduction in PPARγ protein expression (Ishibashi *et al.* 2013). The antagonist effect of PEDF/RAGE also contributes to activation of PPARγ therefore inhibiting generation of ROS. PPARγ activation by PEDF can inhibit platelet-derived growth factor (PDGF)-induced migration and proliferation of smooth muscle cell (SMC) as well as suppress macrophage-mediated inflammatory reactions (Yang *et al.* 2010, Wang *et al.* 2012) which ultimately would lead to atherosclerosis as a result of ROS-induced signal transduction involving angiotensin II mediated EC activation and SMC proliferation (Nishikawa *et al.* 2000, Sorescu *et al.* 2002).

PEDF and NADPH NOX-mediated ROS production and initiation of the redox-dependent signaling cascade as a result of Ang II expression and stimulation is an important event in vascular injury and inflammation (Yamagishi *et al.* 2005). PEDF can inhibit NOX ROS generation and in the case of MOLT-3 T cells, an immortalized T cell line, it leads to blocking and suppressing Ang II-induced VEGF expression (Yamagishi *et al.* 2006*a*). The protective effect of PEDF via its antioxidative effect has also been observed in Ang II-exposed human umbilical vein ECs (HUVECs). The activation of redox-sensitive transcription factor NFκB, and as a result, overexpression of MCP1 in HUVECs, is induced by activation of Ang II. PEDF can protect HUVECs via downregulation of the mRNA level of $p22^{PHOX}$ associated with NOX4 and $gp91^{PHOX}$ associated with NOX2. These subunits are membrane-bound components of NOX. A reduction in the level of these proteins can inhibit Ang II-induced ROS production (Yamagishi *et al.* 2005).

Studies on vascular hyperpermeability and oxidative stress in retinal ECs also include examination of the role of NOX and its various membrane components. NOX will be critical to superoxide and subsequently H_2O_2 generation (via SOD) in PEDF-stimulated ECs (Fig. 1). Some findings suggest that PEDF has an inhibitory effect on AGE-mediated VEGF-induced vascular hyperpermeability via suppression of VEGF expression (Yamagishi *et al.* 2006*b*, Yoshida *et al.* 2009). The latter authors have also shown that NOX activity has an important role in elevating ROS generation and ultimately in apoptosis and increased cell permeability. PEDF can downregulate p^{22PHOX} and $gp91^{PHOX}$ mRNA levels and subsequently suppress NOX protein levels and activity (Yamagishi *et al.* 2006*b*, 2007*b*, Yoshida *et al.* 2009). Reduced NOX activity inhibits NFκB-dependent VEGF expression in ECs, affecting EC's vascular lining permeability and inhibiting ROS generation (Fig. 1). Furthermore, PEDF also has a protective effect in H_2O_2-induced retinal pigment epithelium (RPE) permeability. It has been shown that in H_2O_2-induced oxidative stress, PEDF is able to suppress the stress-activated p38/MAPK signaling pathway by inhibiting the phosphorylation and activation of a key substrate (HSP27; Ho *et al.* 2006). In a leptin-induced ROS generation model, PEDF inhibited VEGF expression, thus potentially eliminating the

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

Table 1 Summary of PEDF protective mechanisms in oxidative stress condition

Regulatory molecules/ pathways	Condition	Cell line	Effects	References
BCL2/BAX	AGE exposure Oxidative stress	Pericytes Cultured cortical neurons	Inhibition of apoptosis	Yamagishi *et al.* (2002*a*), Murakami *et al.* (2008), Falk *et al.* (2009) and Sanchez *et al.* (2012)
Antioxidative molecule (GSH-Px)	High glucose H_2O_2 exposure	Pericytes	Inhibition of ROS generation Normalization of the level of anti-oxidant enzymes	Yamagishi *et al.* (2002*a*,*c*) and Amano *et al.* (2005)
PPARγ	AGE exposure	Podocytes	Blocking of RAGE expression and ROS generation	Nishikawa *et al.* (2000), Sorescu *et al.* (2002), Yang *et al.* (2010) and Ishibashi *et al.* (2013)
RAGE	AGE exposure	Proximal tubular cells	Inhibition of ROS generation Blocking of the inflammatory and fibrogenic gene expression	Nishikawa *et al.* (2000), Sorescu *et al.* (2002) and Yang *et al.* (2010)
ERK1/2	H_2O_2 exposure	ARPE-19 Cerebellar granule cells	Activation of CREB	Taniwaki *et al.* (1995, 1997), Araki *et al.* (1998), Bilak *et al.* (1999), DeCoster *et al.* (1999), Tsao *et al.* (2006) and Zheng *et al.* (2010)
JAK2/STAT3	High glucose	BRECs	Inhibition of ROS generation Downregulation of VEGF	Taniwaki *et al.* (1995, 1997), Araki *et al.* (1998), Bilak *et al.* (1999), DeCoster *et al.* (1999), Tsao *et al.* (2006) and Zheng *et al.* (2010)
NFκB	AGE exposure LDL exposure	Mesangial cells and ECs Pericytes	Inhibition of ROS generation Blocking of the expression of inflammatory and fibrogenic genes such as *MCP1*, *VCAM1*, and *PAI1*	Yabe *et al.* (2001, 2005*a*,*b*), Sanagi *et al.* (2005), Yamagishi *et al.* (2005) and Zhang *et al.* (2008)
		HUVEC	Inhibition of cell permeability and neovascularization	
	Ang II exposure	Cultured microglia	Inhibition of NADPH-mediated and Ang II-induced ROS generation	
	Glutamate toxicity	Cultured rat cerebellar cells Neonatal astrocytes	Downregulation of membrane component of NADPH oxidase	
ICAM1	AGE exposure	ECs	Suppression of ICAM1 overexpression	Yamagishi *et al.* (2006*c*)
P38/MAPK signaling	H_2O_2 exposure	RPE	Inhibition of substrate activation via suppression of phosphorylation	Yamagishi *et al.* (2003) and Ho *et al.* (2006)
VEGF	H_2O_2 exposure	MOLT-3 T cells	Suppression of the Ang II-induced VEGF expression	Yamagishi *et al.* (2002*b*, 2006*a*,*b*) and Yoshida *et al.* (2009)
	Leptin exposure	ECs	Blocking of receptor–Ang II interaction Inhibition of *VEGF* mRNA overexpression	

angiogenic effect of leptin and protecting ECs through its antioxidant properties (Yamagishi *et al*. 2003).

Adhesion of leukocytes and inflammatory cells to the capillary endothelium (leukostasis) is one of the possible mechanisms of DR, which can be related to ICAM1 levels. In AGE-induced oxidative stress conditions, ICAM1 overexpression may result in retinal leukostasis. PEDF can inhibit the overexpression of ICAM1 in ECs via its antioxidative properties (Yamagishi *et al*. 2006*c*).

Table 1 summarizes the suggested mechanisms of PEDF action in various cell types and PEDF-mediated survival in oxidative stress conditions.

Pericyte–EC communication and PEDF

The communication system between pericytes and ECs during angiogenesis and maturation of the vasculature system consists of several complex paracrine signaling pathways such as PDGFβ, activated transforming growth factor beta, VEGF, angiopoietin 1 (Ang I), and its antagonist angiopoietin 2 (Ang II) (Milne & Brownstein 2013). The regulatory effect of PEDF on paracrine signaling and its role in the maintenance of homeostasis between pericytes and ECs are also dependent on the antioxidative function of PEDF. Platelet activation and aggregation is a common cause of vascular complications in diabetic patients and oxidative stress via the action of AGEs (Yamagishi *et al*. 2001). The antioxidative activity of PEDF can reduce the production of NOX-driven superoxide, and can inhibit platelet activation and aggregation as well as have a detrimental effect on AGEs in diabetic rat models (Yamagishi *et al*. 2009).

Moreover, both *in vivo* and *in vitro* hyperglycemic conditions can result in activation of NFκB in retinal pericytes which can upregulate BAX and TNFα (Romeo *et al*. 2002). Ang I has a protective effect on pericytes in such conditions; however, Ang II accelerates TNFα- and hyperglycemia-induced apoptosis as well as pericyte migration from retinal capillaries, which lead to pericyte loss and EC proliferation (Cai *et al*. 2008, Pfister *et al*. 2008). In a high-glucose ROS-induced condition, the mRNA ratio of Ang II to Ang I increases and consequently elevates the *VEGF* mRNA level in pericytes (Amano *et al*. 2005). This may disrupt pericyte–EC interactions and induce angiogenesis-related gene expression. Through its antioxidative properties, PEDF can inhibit pericyte apoptosis, modifying VEGF-mediated gene expression and ultimately delaying or even halting the progression of DR (Amano *et al*. 2005).

Clinical implications and possible future studies

In the normal adult eye (especially in macular region), the concentration of PEDF is tenfold higher than VEGF, which may suggest that PEDF is the main factor responsible for the low number of blood vessels associated with macular avascularity (Kociok & Joussen 2007, Kozulin *et al*. 2010). However, the level of PEDF in the vitreous of the eye in patients suffering from proliferative diseases such as PDR is significantly lower than in the normal eye, which may be a biomarker of oxidative stress in the eye, and the pharmacological upregulation or administration of PEDF may be a therapeutic strategy to address PDR (Yokoi *et al*. 2007). The imbalanced ratio of VEGF and PEDF bring the concept of anti-angiogenic therapy into perspective. Anti-VEGF antibodies have been used clinically and showed significant positive results, but the efficacy is limited by short half-life (10 days). Therefore, to maintain the therapeutic effect, regular dosing is required, although repetitive injections carry substantial risks for the patient, such as retinal detachment, endophthalmitis, cataract formation, ocular hypertension, and submacular hemorrhage. In this approach, using PEDF (a potent anti-angiogenic molecule) seems a promising strategy. However, the short half-life is still an issue. In this regard, some new delivery systems have been tested in order to increase the efficiency of delivery and half-life of the *PEDF* gene therapy method. The examples include adeno-associated virus vector-mediated PEDF delivery, which has been recently described (Streck *et al*. 2005, Park *et al*. 2011, He *et al*. 2012). However, because of its potential carcinogenic properties, immunogenicity, uncertain quantitative expression, and lower production rate, this application is limited for therapy. Other studies have also used poly(D,L-lactide-coglycolide acid) nanoparticles for efficient *PEDF* gene delivery, but processing and formulation lead to loss of activity of *PEDF* (Pai *et al*. 2009). Polyethylene glycol is a polyether with many applications in medicine and has recently been used to improve the pharmacokinetic and pharmacodynamic properties of administered PEDF. This strategy provided promising results for long-term therapy of PDR as well as other retinal angiogenic diseases (Bai *et al*. 2012).

Conclusion

Pericytes and ECs respond in different ways to oxidative stress. AGE-induced ROS inhibits the growth of pericytes. Oxidative stress commonly occurs in chronic diseases such as diabetes mellitus, thus PEDF could protect retinal

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

pericytes exposed to such stress through its antioxidative properties as well as through inhibition of EC activation. PEDF may act directly on ECs to prevent inflammation-mediated pro-proliferative responses, therefore playing a protective role against angiogenesis. Furthermore, PEDF could affect or upregulate anti-apoptotic gene expression in neural cells that can improve neuronal survival. Taken together, PEDF is emerging as a novel and suitable candidate for new therapeutic approaches in neuro-degenerative disorders and vascular complications in diseases such as diabetes mellitus.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

M E is supported by an IPR Scholarship from Victoria University and CRD by a Curtin Academic50 scheme. V F C is supported by Brazilian National Council for Scientific and Technological Development (CNPq)/Science without borders Programme (process 245562/2012-5). P N is supported by the School of Biomedical Sciences and Faculty of Health Sciences with respect to diabetes and metabolism research at Curtin University.

Author contribution statement

M E and C D designed the outline of the review, and M E was responsible for writing the first draft of this review. S B-H, V F C, P N, and C D were responsible for subsequent revisions to the manuscript. All authors approved the final version of the manuscript.

References

Alikhani M, MacLellan CM, Raptis M, Vora S, Trackman PC & Graves DT 2007 Advanced glycation end products induce apoptosis in fibroblasts through activation of ROS, MAP kinases, and the FOXO1 transcription factor. *American Journal of Physiology. Cell Physiology* **292** C850–C856. (doi:10.1152/ajpcell.00356.2006)

Amano S, Yamagishi SI, Kato N, Inagaki Y, Okamoto T, Makino M, Taniko K, Hirooka H, Jomori T & Takeuchi M 2002 Sorbitol dehydrogenase overexpression potentiates glucose toxicity to cultured retinal pericytes. *Biochemical and Biophysical Research Communications* **299** 183–188. (doi:10.1016/S0006-291X(02)02584-6)

Amano S, Yamagishi SI, Inagaki Y, Nakamura K, Takeuchi M, Inoue H & Imaizumi T 2005 Pigment epithelium-derived factor inhibits oxidative stress-induced apoptosis and dysfunction of cultured retinal pericytes. *Microvascular Research* **69** 45–55. (doi:10.1016/j.mvr.2004.11.001)

Araki T, Taniwaki T, Becerra SP, Chader GJ & Schwartz JP 1998 Pigment epithelium-derived factor (PEDF) differentially protects immature but not mature cerebellar granule cells against apoptotic cell death. *Journal of Neuroscience Research* **53** 7–15. (doi:10.1002/(SICI)1097-4547(19980701)53:1%3C;7::AID-JNR2%3E;3.0.CO;2-F)

Bai Y-J, Huang L-Z, Xu X-L, Du W, Zhou A-Y, Yu W-Z & Li X-X 2012 Polyethylene glycol-modified pigment epithelial-derived factor: new prospects for treatment of retinal neovascularization. *Journal of Pharmacology and Experimental Therapeutics* **342** 131–139. (doi:10.1124/jpet.112.192575)

Bilak MM, Corse AM, Bilak SR, Lehar M, Tombran-Tink J & Kuncl RW 1999 Pigment epithelium-derived factor (PEDF) protects motor neurons from chronic glutamate-mediated neurodegeneration. *Journal of Neuropathology and Experimental Neurology* **58** 719–728. (doi:10.1097/00005072-199907000-00006)

Boehm BO, Lang G, Volpert O, Jehle PM, Kurkhaus A, Rosinger S, Lang GK & Bouck N 2003 Low content of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor predicts progression of diabetic retinopathy. *Diabetologia* **46** 394–400.

Broadhead ML, Dass CR & Choong PFM 2009 Cancer cell apoptotic pathways mediated by PEDF: prospects for therapy. *Trends in Molecular Medicine* **15** 461–467. (doi:10.1016/j.molmed.2009.08.003)

Brownlee M 2001 Biochemistry and molecular cell biology of diabetic complications. *Nature* **414** 813–820. (doi:10.1038/414813a)

Cai J, Kehoe O, Smith GM, Hykin P & Boulton ME 2008 The angiopoietin/Tie-2 system regulates pericyte survival and recruitment in diabetic retinopathy. *Investigative Ophthalmology & Visual Science* **49** 2163–2171. (doi:10.1167/iovs.07-1206)

Cory S & Adams JM 2002 The BCL2 family: regulators of the cellular life-or-death switch. *Nature Reviews. Cancer* **2** 647–656. (doi:10.1038/nrc883)

Cruzat VF, Pantaleao LC, Donato J Jr, de Bittencourt PI Jr & Tirapegui J 2014 Oral supplementations with free and dipeptide forms of L-glutamine in endotoxemic mice: effects on muscle glutamine–glutathione axis and heat shock proteins. *Journal of Nutritional Biochemistry* **25** 345–352. (doi:10.1016/j.jnutbio.2013.11.009)

Dass CR, Contreras KG, Dunstan DE & Choong PF 2007 Chitosan microparticles encapsulating PEDF plasmid demonstrate efficacy in an orthotopic metastatic model of osteosarcoma. *Biomaterials* **28** 3026–3033. (doi:10.1016/j.biomaterials.2007.03.016)

DeCoster MA, Schabelman E, Tombran-Tink J & Bazan NG 1999 Neuroprotection by pigment epithelial-derived factor against glutamate toxicity in developing primary hippocampal neurons. *Journal of Neuroscience Research* **56** 604–610. (doi:10.1002/(SICI)1097-4547(19990615)56:6%3C;604::AID-JNR6%3E;3.0.CO;2-B)

Dias IH & Griffiths HR 2014 Oxidative stress in diabetes – circulating advanced glycation end products, lipid oxidation and vascular disease. *Annals of Clinical Biochemistry* **51** 125–127. (doi:10.1177/0004563213508747)

Duarte DA, Silva KC, Rosales MA, Lopes de Faria JB & Lopes de Faria JM 2013 The concomitance of hypertension and diabetes exacerbating retinopathy: the role of inflammation and oxidative stress. *Current Clinical Pharmacology* **8** 266–277. (doi:10.2174/1574884711308040002)

Ejaz S 2008 Importance of pericytes and mechanisms of pericyte loss during diabetes retinopathy. *Diabetes, Obesity & Metabolism* **10** 53–63.

Ek ET, Dass CR, Contreras KG & Choong PF 2007*a* Inhibition of orthotopic osteosarcoma growth and metastasis by multitargeted antitumor activities of pigment epithelium-derived factor. *Clinical & Experimental Metastasis* **24** 93–106. (doi:10.1007/s10585-007-9062-1)

Ek ET, Dass CR, Contreras KG & Choong PF 2007*b* Pigment epithelium-derived factor overexpression inhibits orthotopic osteosarcoma growth, angiogenesis and metastasis. *Cancer Gene Therapy* **14** 616–626. (doi:10.1038/sj.cgt.7701044)

Ek ET, Dass CR, Contreras KG & Choong PF 2007*c* PEDF-derived synthetic peptides exhibit antitumor activity in an orthotopic model of human osteosarcoma. *Journal of Orthopaedic Research* **25** 1671–1680. (doi:10.1002/jor.20434)

Falk T, Zhang S & Sherman SJ 2009 Pigment epithelium derived factor (PEDF) is neuroprotective in two *in vitro* models of Parkinson's disease. *Neuroscience Letters* **458** 49–52. (doi:10.1016/j.neulet.2009.04.018)

Filiz G & Dass CR 2012 Reduction in tumour cell invasion by pigment epithelium-derived factor is mediated by membrane type-1 matrix metalloproteinase downregulation. *Die Pharmazie* **67** 1010–1014.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

Finkel T & Holbrook NJ 2000 Oxidants, oxidative stress and the biology of ageing. *Nature* **408** 239–247. (doi:10.1038/35041687)

Fukami K, Ueda S, Yamagishi SI, Kato S, Inagaki Y, Takeuchi M, Motomiya Y, Bucala R, Iida S, Tamaki K *et al.* 2004 AGEs activate mesangial TGF-β–Smad signaling via an angiotensin II type I receptor interaction. *Kidney International* **66** 2137–2147. (doi:10.1111/j.1523-1755.2004.66004.x)

Hammes HP 2005 Pericytes and the pathogenesis of diabetic retinopathy. *Hormone and Metabolic Research* **37** S39–S43. (doi:10.1055/s-2005-861361)

Hammes HP, Alt A, Niwa T, Clausen JT, Bretzel RG, Brownlee M & Schleicher ED 1999 Differential accumulation of advanced glycation end products in the course of diabetic retinopathy. *Diabetologia* **42** 728–736. (doi:10.1007/s001250051221)

He SS, Shi HS, Yin T, Li YX, Luo ST, Wu QJ, Lu L, Wei YQ & Yang L 2012 AAV-mediated gene transfer of human pigment epithelium-derived factor inhibits Lewis lung carcinoma growth in mice. *Oncology Reports* **27** 1142–1148.

Heck TG, Scholer CM & de Bittencourt PI 2011 HSP70 expression: does it a novel fatigue signalling factor from immune system to the brain? *Cell Biochemistry and Function* **29** 215–226. (doi:10.1002/cbf.1739)

Ho TC, Yang YC, Cheng HC, Wu AC, Chen SL & Tsao YP 2006 Pigment epithelium-derived factor protects retinal pigment epithelium from oxidant-mediated barrier dysfunction. *Biochemical and Biophysical Research Communications* **342** 372–378. (doi:10.1016/j.bbrc.2006.01.164)

Ide Y, Matsui T, Ishibashi Y, Takeuchi M & Yamagishi SI 2010 Pigment epithelium-derived factor inhibits advanced glycation end product-elicited mesangial cell damage by blocking NF-κB activation. *Microvascular Research* **80** 227–232. (doi:10.1016/j.mvr.2010.03.015)

Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M & Amano S 2003 Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. *Diabetologia* **46** 284–287.

Ishibashi Y, Matsui T, Ohta K, Tanoue R, Takeuchi M, Asanuma K, Fukami K, Okuda S, Nakamura KI & Yamagishi SI 2013 PEDF inhibits AGE-induced podocyte apoptosis via PPAR-γ activation. *Microvascular Research* **85** 54–58. (doi:10.1016/j.mvr.2012.10.007)

Kociok N & Joussen A 2007 Varied expression of functionally important genes of RPE and choroid in the macula and in the periphery of normal human eyes. *Graefe's Archive for Clinical and Experimental Ophthalmology* **245** 101–113. (doi:10.1007/s00417-006-0266-x)

Kozulin P, Natoli R, Bumsted O'Brien KM, Madigan MC & Provis JM 2010 The cellular expression of antiangiogenic factors in fetal primate macula. *Investigative Ophthalmology & Visual Science* **51** 4298–4306. (doi:10.1167/iovs.09-4905)

Krause MS & de Bittencourt PI Jr 2008 Type 1 diabetes: can exercise impair the autoimmune event? The L-arginine/glutamine coupling hypothesis *Cell Biochemistry and Function* **26** 406–433. (doi:10.1002/cbf.1470)

Milne R & Brownstein S 2013 Advanced glycation end products and diabetic retinopathy. *Amino Acids* **44** 1397–1407. (doi:10.1007/s00726-011-1071-3)

Min C, Kang E, Yu SH, Shinn SH & Kim YS 1999 Advanced glycation end products induce apoptosis and procoagulant activity in cultured human umbilical vein endothelial cells. *Diabetes Research and Clinical Practice* **46** 197–202. (doi:10.1016/S0168-8227(99)00094-7)

Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y & Nishihira J 2001 Monocyte chemotactic protein-1 in the vitreous of patients with proliferative diabetic retinopathy. *Ophthalmologica* **215** 415–418. (doi:10.1159/000050900)

Murakami Y, Ikeda Y, Yonemitsu Y, Onimaru M, Nakagawa K, Kohno R, Miyazaki M, Hisatomi T, Nakamura M, Yabe T *et al.* 2008 Inhibition of nuclear translocation of apoptosis-inducing factor is an essential mechanism of the neuroprotective activity of pigment epithelium-derived factor in a rat model of retinal degeneration. *American Journal of Pathology* **173** 1326–1338. (doi:10.2353/ajpath.2008.080466)

Nakamura K, Yamagishi SI, Matsui T, Yoshida T, Takenaka K, Jinnouchi Y, Yoshida Y, Ueda SI, Adachi H & Imaizumi T 2007 Pigment epithelium-derived factor inhibits neointimal hyperplasia after vascular injury by blocking NADPH oxidase-mediated reactive oxygen species generation. *American Journal of Pathology* **170** 2159–2170. (doi:10.2353/ajpath.2007.060838)

Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, Oliveira-Emilio HC, Carpinelli AR & Curi R 2007 Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. *Journal of Physiology* **583** 9–24. (doi:10.1113/jphysiol.2007.135871)

Newsholme P, Rebelato E, Abdulkader F, Krause M, Carpinelli A & Curi R 2012 Reactive oxygen and nitrogen species generation, antioxidant defenses, and β-cell function: a critical role for amino acids. *Journal of Endocrinology* **214** 11–20. (doi:10.1530/JOE-12-0072)

Nishikawa T, Edelstein D, Du XL, Yamagishi SI, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP *et al.* 2000 Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* **404** 787–790. (doi:10.1038/35008121)

Pai S, Tilton R & Przybycien T 2009 Poly(ethylene glycol)-modified proteins: implications for poly(lactide-co-glycolide)-based microsphere delivery. *AAPS Journal* **11** 88–98. (doi:10.1208/s12248-009-9081-8)

Park K, Jin J, Hu Y, Zhou K & Ma JX 2011 Overexpression of pigment epithelium-derived factor inhibits retinal inflammation and neovascularization. *American Journal of Pathology* **178** 688–698. (doi:10.1016/j.ajpath.2010.10.014)

Pfister F, Feng Y, Hagen FV, Hoffmann S, Molema G, Hillebrands JL, Shani M, Deutsch U & Hammes HP 2008 Pericyte migration: a novel mechanism of pericyte loss in experimental diabetic retinopathy. *Diabetes* **57** 2495–2502. (doi:10.2337/db08-0325)

Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR & Bluher M 2009 Antioxidants prevent health-promoting effects of physical exercise in humans. *PNAS* **106** 8665–8670. (doi:10.1073/pnas.0903485106)

Romeo G, Liu WH, Asnaghi V, Kern TS & Lorenzi M 2002 Activation of nuclear factor-κB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* **51** 2241–2248. (doi:10.2337/diabetes.51.7.2241)

Sanagi T, Yabe T & Yamada H 2005 The regulation of pro-inflammatory gene expression induced by pigment epithelium-derived factor in rat cultured microglial cells. *Neuroscience Letters* **380** 105–110. (doi:10.1016/j.neulet.2005.01.035)

Sanchez A, Tripathy D, Yin X, Luo J, Martinez J & Grammas P 2012 Pigment epithelium-derived factor (PEDF) protects cortical neurons *in vitro* from oxidant injury by activation of extracellular signal-regulated kinase (ERK) 1/2 and induction of Bcl-2. *Neuroscience Research* **72** 1–8. (doi:10.1016/j.neures.2011.09.003)

Sato T, Iwaki M, Shimogaito N, Wu X, Yamagishi SI & Takeuchi M 2006 TAGE (toxic AGEs) theory in diabetic complications. *Current Molecular Medicine* **6** 351–358. (doi:10.2174/156652406776894536)

Schmidt AM, Yan SD, Yan SF & Stern DM 2001 The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *Journal of Clinical Investigation* **108** 949–955. (doi:10.1172/JCI200114002)

Sethi G, Sung B & Aggarwal BB 2008 Nuclear factor-κB activation: from bench to bedside. *Experimental Biology and Medicine* **233** 21–31. (doi:10.3181/0707-MR-196)

Sorescu D, Weiss D, Lassègue B, Clempus RE, Szöcs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD *et al.* 2002 Superoxide production and expression of Nox family proteins in human atherosclerosis. *Circulation* **105** 1429–1435. (doi:10.1161/01.CIR.0000012917.74432.66)

Streck CJ, Zhang Y, Zhou J, Ng C, Nathwani AC & Davidoff AM 2005 Adeno-associated virus vector-mediated delivery of pigment epithelium-derived factor restricts neuroblastoma angiogenesis and

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

growth. *Journal of Pediatric Surgery* **40** 236–243. (doi:10.1016/j.jpedsurg.2004.09.049)

Ta HT, Dass CR, Larson I, Choong PF & Dunstan DE 2009 A chitosan hydrogel delivery system for osteosarcoma gene therapy with pigment epithelium-derived factor combined with chemotherapy. *Biomaterials* **30** 4815–4823. (doi:10.1016/j.biomaterials.2009.05.035)

Tan ML, Choong PF & Dass CR 2010 Anti-chondrosarcoma effects of PEDF mediated via molecules important to apoptosis, cell cycling, adhesion and invasion. *Biochemical and Biophysical Research Communications* **398** 613–618. (doi:10.1016/j.bbrc.2010.05.098)

Taniwaki T, Becerra SP, Chader GJ & Schwartz JP 1995 Pigment epithelium-derived factor is a survival factor for cerebellar granule cells in culture. *Journal of Neurochemistry* **64** 2509–2517. (doi:10.1046/j.1471-4159.1995.64062509.x)

Taniwaki T, Hirashima N, Becerra SP, Chader GJ, Etcheberrigaray R & Schwartz JP 1997 Pigment epithelium-derived factor protects cultured cerebellar granule cells against glutamate-induced neurotoxicity. *Journal of Neurochemistry* **68** 26–32. (doi:10.1046/j.1471-4159.1997.68010026.x)

Tsao YP, Ho TC, Chen SL & Cheng HC 2006 Pigment epithelium-derived factor inhibits oxidative stress-induced cell death by activation of extracellular signal-regulated kinases in cultured retinal pigment epithelial cells. *Life Sciences* **79** 545–550. (doi:10.1016/j.lfs.2006.01.041)

Wang SH, Liang CJ, Wu JC, Huang JJ, Chien HF, Tsai JS, Yen YS, Tseng YC, Lue JH & Chen YL 2012 Pigment epithelium-derived factor reduces the PDGF-induced migration and proliferation of human aortic smooth muscle cells through PPARγ activation. *International Journal of Biochemistry & Cell Biology* **44** 280–289. (doi:10.1016/j.biocel.2011.10.023)

Yabe T, Wilson D & Schwartz JP 2001 NFκB activation is required for the neuroprotective effects of pigment epithelium-derived factor (PEDF) on cerebellar granule neurons. *Journal of Biological Chemistry* **276** 43313–43319. (doi:10.1074/jbc.M107831200)

Yabe T, Sanagi T, Schwartz JP & Yamada H 2005*a* Pigment epithelium-derived factor induces pro-inflammatory genes in neonatal astrocytes through activation of NF-κB and CREB. *Glia* **50** 223–234. (doi:10.1002/glia.20171)

Yabe T, Kanemitsu K, Sanagi T, Schwartz JP & Yamada H 2005*b* Pigment epithelium-derived factor induces pro-survival genes through cyclic AMP-responsive element binding protein and nuclear factor κB activation in rat cultured cerebellar granule cells: Implication for its neuroprotective effect. *Neuroscience* **133** 691–700. (doi:10.1016/j.neuroscience.2005.03.007)

Yamagishi S & Matsui T 2011 Advanced glycation end products (AGEs), oxidative stress and diabetic retinopathy. *Current Pharmaceutical Biotechnology* **12** 362–368. (doi:10.2174/138920111794480534)

Yamagishi SI, Hsu CC, Kobayashi KI & Yamamoto H 1993*a* Endothelin 1 mediates endothelial cell-dependent proliferation of vascular pericytes. *Biochemical and Biophysical Research Communications* **191** 840–846. (doi:10.1006/bbrc.1993.1293)

Yamagishi SI, Kobayashi KI & Yamamoto H 1993*b* Vascular pericytes not only regulate growth, but also preserve prostacyclin-producing ability and protect against lipid peroxide-induced injury of co-cultured endothelial cells. *Biochemical and Biophysical Research Communications* **190** 418–425. (doi:10.1006/bbrc.1993.1064)

Yamagishi SI, Edelstein D, Du XL & Brownlee M 2001 Hyperglycemia potentiates collagen-induced platelet activation through mitochondrial superoxide overproduction. *Diabetes* **50** 1491–1494. (doi:10.2337/diabetes.50.6.1491)

Yamagishi SI, Inagaki Y, Amano S, Okamoto T, Takeuchi M & Makita Z 2002*a* Pigment epithelium-derived factor protects cultured retinal pericytes from advanced glycation end product-induced injury through its antioxidative properties. *Biochemical and Biophysical Research Communications* **296** 877–882. (doi:10.1016/S0006-291X(02)00940-3)

Yamagishi SI, Amano S, Inagaki Y, Okamoto T, Koga K, Sasaki N, Yamamoto H, Takeuchi M & Makita Z 2002*b* Advanced glycation end products-induced apoptosis and overexpression of vascular endothelial growth factor in bovine retinal pericytes. *Biochemical and Biophysical Research Communications* **290** 973–978. (doi:10.1006/bbrc.2001.6312)

Yamagishi SI, Okamoto T, Amano S, Inagaki Y, Koga K, Koga M, Choei H, Sasaki N, Kikuchi S, Takeuchi M *et al.* 2002*c* Palmitate-induced apoptosis of microvascular endothelial cells and pericytes. *Molecular Medicine* **8** 179–184.

Yamagishi SI, Amano S, Inagaki Y, Okamoto T, Takeuchi M & Inoue H 2003 Pigment epithelium-derived factor inhibits leptin-induced angiogenesis by suppressing vascular endothelial growth factor gene expression through anti-oxidative properties. *Microvascular Research* **65** 186–190. (doi:10.1016/S0026-2862(03)00005-0)

Yamagishi SI, Nakamura K, Ueda S, Kato S & Imaizumi T 2005 Pigment epithelium-derived factor (PEDF) blocks angiotensin II signaling in endothelial cells via suppression of NADPH oxidase: a novel anti-oxidative mechanism of PEDF. *Cell and Tissue Research* **320** 437–445. (doi:10.1007/s00441-005-1094-8)

Yamagishi SI, Matsui T, Nakamura K, Yoshida T, Shimizu K, Takegami Y, Shimizu T, Inoue H & Imaizumi T 2006*a* Pigment-epithelium-derived factor (PEDF) inhibits angiotensin-II-induced vascular endothelial growth factor (VEGF) expression in MOLT-3 T cells through anti-oxidative properties. *Microvascular Research* **71** 222–226. (doi:10.1016/j.mvr.2006.03.001)

Yamagishi SI, Nakamura K, Matsui T, Inagaki Y, Takenaka K, Jinnouchi Y, Yoshida Y, Matsuura T, Narama I, Motomiya Y *et al.* 2006*b* Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. *Journal of Biological Chemistry* **281** 20213–20220. (doi:10.1074/jbc.M602110200)

Yamagishi SI, Matsui T, Nakamura K, Takeuchi M & Imaizumi T 2006*c* Pigment epithelium-derived factor (PEDF) prevents diabetes- or advanced glycation end products (AGE)-elicited retinal leukostasis. *Microvascular Research* **72** 86–90. (doi:10.1016/j.mvr.2006.04.002)

Yamagishi SI, Ueda S & Okuda S 2007*a* Food-derived advanced glycation end products (AGEs): a novel therapeutic target for various disorders. *Current Pharmaceutical Design* **13** 2832–2836. (doi:10.2174/138161207781757051)

Yamagishi SI, Abe R, Jinnouchi Y, Matsui T, Imaizumi T & Inoue H 2007*b* Pigment epithelium-derived factor inhibits vascular endothelial growth factor-induced vascular hyperpermeability both *in vitro* and *in vivo*. *Journal of International Medical Research* **35** 896–899. (doi:10.1177/147323000703500619)

Yamagishi SI, Matsui T, Takenaka K, Nakamura K, Takeuchi M & Inoue H 2009 Pigment epithelium-derived factor (PEDF) prevents platelet activation and aggregation in diabetic rats by blocking deleterious effects of advanced glycation end products (AGEs). *Diabetes/Metabolism Research and Reviews* **25** 266–271. (doi:10.1002/dmrr.906)

Yang SL, Chen SL, Wu JY, Ho TC & Tsao YP 2010 Pigment epithelium-derived factor induces interleukin-10 expression in human macrophages by induction of PPARγ. *Life Sciences* **87** 26–35. (doi:10.1016/j.lfs.2010.05.007)

Yokoi M, Yamagishi SI, Saito A, Yoshida Y, Matsui T, Saito W, Hirose S, Ohgami K, Kase M & Ohno S 2007 Positive association of pigment epithelium-derived factor with total antioxidant capacity in the vitreous fluid of patients with proliferative diabetic retinopathy. *British Journal of Ophthalmology* **91** 885–887. (doi:10.1136/bjo.2006.110890)

Yoshida T, Yamagishi SI, Nakamura K, Matsui T, Imaizumi T, Inoue H, Ueno T & Sata M 2006 Pigment epithelium-derived factor (PEDF) blocks the interleukin-6 signaling to C-reactive protein expression in Hep3B cells by suppressing Rac-1 activation. *Life Sciences* **79** 1981–1987. (doi:10.1016/j.lfs.2006.06.034)

Yoshida Y, Yamagishi SI, Matsui T, Jinnouchi Y, Fukami K, Imaizumi T & Yamakawa R 2009 Protective role of pigment epithelium-derived factor (PEDF) in early phase of experimental diabetic retinopathy. *Diabetes/Metabolism Research and Reviews* **25** 678–686. (doi:10.1002/dmrr.1007)

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.

Zhang SX, Wang JJ, Dashti A, Wilson K, Zou MH, Szweda L, Ma JX & Lyons TJ 2008 Pigment epithelium-derived factor mitigates inflammation and oxidative stress in retinal pericytes exposed to oxidized low-density lipoprotein. *Journal of Molecular Endocrinology* **41** 135–143. (doi:10.1677/JME-08-0011)

Zhang Y, Han J, Yang X, Shao C, Xu Z, Cheng R, Cai W, Ma J, Yang Z & Gao G 2011 Pigment epithelium-derived factor inhibits angiogenesis and growth of gastric carcinoma by down-regulation of VEGF. *Oncology Reports* **26** 681–686.

Zheng Z, Chen H, Zhao H, Liu K, Luo D, Chen Y, Chen Y, Yang X, Gu Q & Xu X 2010 Inhibition of JAK2/STAT3-mediated VEGF upregulation under high glucose conditions by PEDF through a mitochondrial ROS pathway *in vitro*. *Investigative Ophthalmology & Visual Science* **51** 64–71. (doi:10.1167/iovs.09-3511)

Received in final form 5 June 2014
Accepted 11 June 2014
Accepted Preprint published online 13 June 2014

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-14-0065

Published by Bioscientifica Ltd.