PDGF-C and PDGF-D in ocular diseases

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ABSTRACT

PDGFs and their receptors are critical regulators of numerous tissues and organs, including the eye. Extensive studies have shown that PDGFs and their receptors play critical roles in many ocular neovascular diseases, such as neovascular age-related macular degeneration, retinopathy of prematurity, and proliferative vitreoretinopathy. In addition, PDGFs and PDGFRs are also important players in ocular diseases involving the degeneration of retinal neuronal and vascular cells, such as glaucoma and retinitis pigmentosa. Due to their critical roles in the pathogenesis of many blinding ocular diseases, the PDGFs and PDGFRs have been considered as important target molecules for the treatment of eye diseases. PDGF-C and PDGF-D are relatively new members of the PDGF family and are potent angiogenic and survival factors. Recent studies have demonstrated their important roles in different types of eye diseases. Thus, modulating PDGF-C and PDGF-D activities may have therapeutic values for the treatment of ocular neovascular and degenerative diseases. This review mainly summarizes the recent advances on PDGF-C and PDGF-D biology in relationship to some major ocular diseases.

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Introduction

The Platelet-derived growth factor (PDGF) family includes PDGF-A, PDGF-B, PDGF-C and PDGF-D. PDGF-A and PDGF-B were discovered in the 1970s (Betscholtz et al., 1986; Doolittle et al., 1983; Heldin et al., 1986; Johnsson et al., 1982; Kohler and Lipton, 1974; Westermark and Wasteson, 1976) from platelets as peptides that can increase the proliferation of smooth muscle cells (Seifert et al., 1984). PDGF-C and PDGF-D, however, are the relatively new PDGF family members identified in 2000 and 2001 respectively (Andrae et al., 2008; Bergsten et al., 2001; LaRochelle et al., 2001; Li et al., 2000).

The PDGF family members are potent mitogens secreted by different types of cells, including vascular endothelial cells, vascular smooth muscle cells, pericytes, fibroblasts, mesenchymal cells, epithelial cells, astrocytes, and macrophages. Together, the PDGFs make four homodimers, PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD, and a single heterodimeric polypeptide PDGF-AB. The PDGFs use the platelet derived growth factor receptors (PDGFRs) to exert their diverse functions under physiological or pathological conditions (Kazlauskas, 2017; Li and Eriksson, 2003; Reigstad et al., 2005). There are two homodimers of the PDGFRs, PDGFR-αα and PDGFR-ββ, and one heterodimeric form PDGFR-αβ. All PDGF family members and their receptors are expressed in the central nervous system (Hamada et al., 2002), including the retina in the eye, an extended part of the CNS. Particularly, PDGF-C and PDGF-D are abundantly expressed in the retina and retinal pigment epithelial (RPE) cells (Ray et al., 2005).

Extensive studies have shown that the PDGFs and their receptors play critical roles in many ocular neovascular diseases, such as proliferative vitreoretinopathy (PVR), retinopathy of prematurity (ROP), and neovascular age-related macular degeneration (NV-AMD) (Witmer et al., 2003), which are associated with dysfunctions of multiple components, such as blood vessels, inflammatory cells and RPE cells. Moreover, the PDGFs and PDGFRs are also important players in ocular pathologies involving the degeneration of retinal ganglion cells and photoreceptors, such as in glaucoma and retinitis pigmentosa (RP) (Berger et al., 2010). Due to their critical roles in the pathogenesis of many ocular diseases, the PDGFs and PDGFRs have been considered as important target molecules for the treatment of eye diseases.

Roles of PDGF-C and PDGF-D in proliferative vitreoretinopathy

PVR is a blinding disease resulted from rhegmatogenous retinal detachment following retinal reattachment surgery (Pastor et al.,...
PVR is characterized by the outgrowth of the inner and outer membranes of the retina. This leads to the formation of fibrovascular membranes, which ultimately causes retinal scarring and traction (Pennock et al., 2014). PVR can arise before surgery, but is more common after surgical procedures (Claes and Lafeta, 2014). It has been estimated that PVR occurs in about 5–10% of all retinal detachment cases (Pastor et al., 2016).

Current therapeutic interventions for PVR include surgeries such as scleral buckling, membrane peeling, pars plana vitrectomy, and pneumatic retinopexy. However, all these procedures impair visual acuity significantly due to damage to the retinae (Coffee et al., 2014; Sadaka and Giuliari, 2012). Other therapeutic options include anti-inflammatory drugs (Cheema et al., 2007), anti-proliferative reagents (Charteris et al., 2004), and antineoplastic drugs (Hou et al., 2015). However, these drugs are not able to impede the progression of the disease effectively.

Numerous studies have shown that the pathogenesis of PVR is orchestrated by multiple aspects, such as growth factors (Charteris, 1998; Wubben et al., 2016), cytokines (Harada et al., 2006; Limb et al., 1991), extracellular matrix proteins (Feist et al., 2014) and various cellular interactions (Pennock et al., 2011). Importantly, clinical studies have demonstrated the presence of PDGFs in human vitreous samples of PVR patients (Akiyama et al., 2006; Andrews et al., 1999; Lei et al., 2007, 2011; Lei and Kazlauskas, 2014; Mori et al., 2002; Pastor et al., 2016; Si et al., 2013). Moreover, activated PDGF receptors have been found on the epiretinal membranes of PVR patients (Cui et al., 2009). Noteworthy, one of the most abundant vitreal growth factors detected in both experimental PVR models and PVR patients is PDGF-C (Lei et al., 2007). Indeed, both PDGF-C and PDGF-D have been shown to promote the proliferation and migration of RPE cells (Li et al., 2007) (Fig. 1). Moreover, studies using animal model have shown that PDGF-C promotes ECM production and remodeling via PDGFR-alpha (Wiradjaja et al., 2013). In addition, in PVR, a critical pathology is the transformation of RPE cells into fibroblast-like cells via epithelial-mesenchymal transition (EMT) (Bastiaans et al., 2013).

Fig. 1. PDGF-C signaling in proliferative vitreoretinopathy (PVR).

The human retina is highly laminated. Inset box a shows that in a cross section view, the retina is embraced with superior nerve fibre layer, retinal ganglion cells, radial Müller cells, amacrine cells, bipolar cells, horizontal cells, rod cells, cone cells, retinal pigment epithelial cells, and choriocapillaris. Repeating retinal detachment steers the retinal tear and compromises the retinal integrity, which subsequently triggers the production of various growth factors and proteases to initiate the wound healing process with damaged gliotic MCs, apoptotic RGCs, invading RPE cells and other retinal cells. Inset box b shows that elevated levels of PDGF-C activate PDGF receptor alpha signaling, which promotes transcriptional changes to enforce cell differentiation, proliferation, migration, survival, epithelial mesenchymal transition and extracellular remodeling. Inset box c shows that in a cross section view of the retina with PVR, the otherwise stationary RPE cells are highly differentiated and migrate towards the nerve fibre layer. The presence and potential role of PDGF-D in PVR remain to be investigated. NFL: nerve fibre layer, RGC: retinal ganglion cell, AC: amacrine cell, MC: Müller cell, BC: bipolar cell, HC: horizontal cells, R: rod cell, C: cone cell, RPE: retinal pigment epithelial cell, CC: choriocapillaris.

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PDGF family members have been shown to promote EMT alone (Smith et al., 2011; Yang et al., 2006) or involving transforming growth factor-β signaling, which is a known potent EMT inducer (Li et al., 2011). The potential role of PDGF-C in EMT remains to be investigated. However, by enhancing the proliferation and migration of RPE cells into the inner neurosensory retina (Fig. 1), by promoting fibrovascular growth, and by increasing the production of extracellular matrix, PDGF-C plays an important role in PVR.

PDGF-C has been shown to display pleiotropic effects on multiple cellular targets, including both vascular and non-vascular cells, such as endothelial cells, mural cells, astrocytes, fibroblasts, and macrophages (Lee et al., 2013). Indeed, immunohistochemical analysis of PVR membranes highlighted the presence of both PDGFR-α and PDGFR-β on them (Cui et al., 2009; Robbins et al., 1994). Moreover, in PVR, phosphorylated PDGFR-α has been shown to be more abundant compared with phosphorylated PDGFR-β, suggesting the involvement of PDGF-C and PDGFR-β signaling in the pathogenesis of PVR (Lei and Kazlauskas, 2014). Interestingly, the presence of plasmin has been shown in the vitreous of rabbits and patients that underwent surgery (Lei and Kazlauskas, 2008). Since plasmin can proteolytically generate the active form of PDGF-C from its latent form, this further supports the conclusion that PDGF-C is involved in the pathogenesis of PVR (Fredriksson et al., 2004; Lei et al., 2008). Moreover, it has been also shown that PDGFR-α can be highly activated by the reactive oxygen species generated during PVR (Lei and Kazlauskas, 2014). Therefore, studies have shown that PDGF-C and its cognate receptor PDGFR-β may be effective therapeutic targets for the treatment of PVR. PDGF-D binds to and activates PDGFR-β, which is highly expressed in PVR. Therefore, PDGF-D may play a role in the pathogenesis of PVR as well. However, future studies are needed to verify this.

**PDGF-C and PDGF-D in ischemia-induced retinopathy**

Blood vessels are essential for the normal function and homeostasis of the retinæ by providing oxygen, nutrients, and metabolic exchange (Anand-Apte et al., 2010). Any defect or disruption of the vascular system will impair the delivery of oxygen and nutrients through the blood supply to the energy utilizing retinal cells and leads to retinal ischemia (Moran et al., 2016). As a consequence, the oxygen-deprived and starving retinal cells upregulate angiogenic and growth factors to promote blood vessel growth, which can in turn result in ischemia-induced retinopathies, such as diabetic retinopathy, NV-AMD, and ROP.

ROP is a potentially blinding disease that occurs primarily in premature infants. In ROP, abnormal blood vessels grow into the retina and cause it to detach from the back of the eye, when uncontrollable, leading to blindness. ROP is one of the most common causes of visual loss in childhood globally and can lead to lifelong vision impairment or blindness (Lieg et al., 2016; Zin and Gole, 2013). In middle- and low-income countries, the incidence of ROP is over 40% (Rivera et al., 2017; Zin and Gole, 2013). However, in high-income countries, the incidence of ROP is reported to be less than 10%. The development of ROP is characterized initially by delayed retinal vascular development (Cayabyab and Ramanathan, 2016). This results in retinal ischemia, which subsequently leads to vascular proliferation and abnormal growth of retinal blood vessels (Smith, 2004). Clinical studies using ocular fluid from ROP patients have implicated roles of proangiogenic factors in the development of ROP (Lieg et al., 2016). Such proangiogenic factors include cytokines (Hartnett, 2007), chemokines (Powers et al., 2005), and growth factors, including vascular endothelial growth factor (VEGF) (Kandasamy et al., 2017), fibroblast growth factor (Sato et al., 2009) and PDGFs (Tolentino, 2009).

Current treatments for ROP include laser therapy and cryotherapy (Hartnett, 2003; Long, 1989; McNamara et al., 1991). Both procedures are invasive and destroy the peripheral retinae, thus impairing some side vision. For late stage ROP, available treatments include scleral buckle and vitrectomy. When the center of the retina or the entire retina detaches, surgery is needed to reattach the retina. In recent years, researchers have tested anti-VEGF therapies for the treatment of ROP, and inhibition of retinal neovascularization has been observed (Jin et al., 2017; Sankar et al., 2016). However, the data are not sufficient to draw conclusions on other critical outcomes and potential long-term systemic adverse effects. Moreover, it is known that VEGF is required for the normal development of the neurovascular system of preterm infants (Patel et al., 2016). Therefore, inhibition of VEGF requires caution since it might impede the normal development of retinal vasculature and neurons. In summary, further studies are required to investigate the effects of anti-VEGF drugs on the retinae and any potential delayed systemic side effects (Sankar et al., 2016).

The roles of the PDGs in ROP have been reported by several studies. It has been shown that intracocular injection of anti-PDGF-BB DARPin (designed ankyrin repeat proteins), a recombinant protein with ankyrin repeats of conserved and variable amino acids, strongly suppressed ischemia-induced retinal neovascularization (Dong et al., 2014). However, another study showed different results. In a rat model of ischemic retinopathy, inhibition of PDGF receptor tyrosine kinase (RTK) by ST571 (Gleevec), a potent inhibitor of PDGF RTK, led to pericyte loss and increased retinal angiogenesis (Wilkinson-Berka et al., 2004), supporting that PDGFs are required for the survival of pericytes, which are required to prevent VEGF/VEGFR-2 upregulation in ROP.

PDGF-C and PDGF-D are produced by multiple types of ocular cells, including RPE cells (Li et al., 2007), endothelial cells (Karvinen et al., 2009), fibroblasts (Crawford et al., 2009), macrophages (Wagsater et al., 2009), pericytes and vascular smooth muscle cells (Gilbertson et al., 2001). The cognate tyrosine kinase receptors for PDGF-C and PDGF-D, PDGFR-α and PDGFR-β, are expressed by mural cells (Sundberg et al., 1993), RPE cells (Vinores et al., 1995), fibroblasts (Borkham-Kamphorst et al., 2015), macrophages (Savikko and von Willebrand, 2001), and retinal neuronal cells (Tang et al., 2010). Thus, the expression of PDGF-C and PDGF-D and their cognate receptors on many types of ocular cells suggests that they may have important functions in ROP.

Indeed, the roles of PDGF-C and PDGF-D in ROP have been reported (Hou et al., 2010; Kumar et al., 2010). In an oxygen-induced retinopathy model (Fig. 2), treatment of the neonatal mice from postnatal day 7–12 (P7-P12) with hyperoxia leads to a severe regression of retinal blood vessels (Smith et al., 1994). This subsequently results in retinal hypoxia and retinal neovascularization during P12-P17. In this model, targeting PDGF-C and PDGF-D by neutralizing antibodies or shRNA reduced the formation of retinal vascular tufts (Hou et al., 2010; Kumar et al., 2010). Indeed, this observation was confirmed in a mouse model of ROP using PDGF-C deficient mice (Hou et al., 2010). At a gene expression level, loss of PDGF-C decreased the expressions of many proangiogenic genes, such as fibroblast growth factor-2, VEGF-b, placental growth factor, and Vegf, and increased the expressions of many proapoptotic genes, including decorin and tumor necrosis factor-α, which inhibits PDGF-induced proliferation and migration of vascular cells (Hou et al., 2010). Moreover, PDGF-D knockdown by shRNA inhibited retinal neovascularization and decreased the expressions of HIF1α, Hes, and Vegf-b in mouse eyes (Kumar et al., 2010). Furthermore, gene silencing of PDGF-D decreased the expressions of many proapoptotic genes, such as Olr-1, a potent vascular apoptosis inducer, tumor necrosis factor-α, and decorin (Kumar et al., 2010). Mechanistically, it has been shown that glycogen synthase kinase-3β acts as a downstream effector mediating the angiogenic effects.
of PDGF-C and PDGF-D (Hou et al., 2010; Kumar et al., 2010). Importantly, since the cellular targets of PDGF-C are largely different from those of VEGF, the PDGF-C-induced angiogenesis is therefore to a great extent independent of VEGF pathway (Li et al., 2010). Thus, targeting PDGF-C and PDGF-D may have therapeutic usage for the treatment of ROP.

Roles of PDGF-C and PDGF-D in neovascular age-related macular degeneration (NV-AMD)

AMD is the leading cause of irreversible blindness in aged population worldwide (Bird, 2010; Gelfand and Ambati, 2016; Miller, 2013). Results of a meta-analysis show that about 8.7% of the world’s population has AMD (Wong et al., 2014). By 2020, the number of people with AMD is expected to reach 196 million worldwide and has been estimated to touch 288 million by 2040 (Wong et al., 2014). Numerous factors are involved in the pathogenesis of AMD. However, age is the most prominent risk factor associated with AMD, followed by genetic and environmental factors and lifestyle (Fritsche et al., 2014; Hammond et al., 2002; Rudnicka et al., 2015; Sobrin and Seddon, 2014). People between 50 and 59 years old has a 2% risk of developing AMD. For those over 75 years old, the risk increases to 30% (Klein et al., 1992, 2010).

AMD can be classified into early and late stages (Ambati et al., 2003; Ferris et al., 2013). Early AMD is also known as dry AMD (Bowes Rickman et al., 2013; Narayanan and Kuppermann, 2017) and is characterized by the accumulation of lipoproteins and cellular debris between the basal lamina of RPE cells and the inner layer of Bruch’s membrane, which ultimately leads to photoreceptor degeneration (Curcio et al., 2005, 2011; Wang et al., 2010). Dry AMD accounts for about 90% of the AMD cases diagnosed. About 10% of dry AMD develop into late stage AMD (Ferris et al., 2013). Clinically, late stage AMD can be either exudative (wet AMD) or has geographic atrophy (Brucker, 2009; Ferris et al., 2013;
Wet AMD is also called neovascular AMD (NV-AMD). In NV-AMD, abnormal blood vessels grow from the choroids into the subretinal space in response to the extensive damage of the pigment epithelial layer of the macula due to the presence of drusen. The fragile neovessels are disorganized and leaky, leading to swelling and damage of the macula (Hartnett et al., 1992; Miller, 2013). Even though NV-AMD accounts for only about 10% of AMD patients, they are responsible for most cases of blindness.

There are several therapeutic options for AMD management. The first generation of therapies include laser and photodynamic treatments (study, 1986). In 2004, the first antiangiogenic drug for AMD treatment, Pegaptanib (Macugen), was approved by FDA. This symbolizes the beginning of the second generation of therapies for AMD using anti-VEGF treatment. Pegaptanib is a single strand RNA aptamer that binds specifically to VEGF and inhibits its function (Ng and Adamis, 2006; Ng et al., 2006). Encouraged by this success, other types of VEGF inhibitors were soon developed and have been shown to be more effective in inhibiting choroidal neovascularization in NV-AMD. Ranibizumab (Lucentis) is a monoclonal antibody fragment that binds to all VEGF isoforms (Bressler et al., 2010; Rosenfeld et al., 2009). Affiberaic (Eylea) is a recombinant protein containing the extracellular domains of VEGFR1 and VEGFR2. Moreover, PDGF-D has been shown to induce the proliferation and migration of multiple cell types, including choroidal fibroblasts and vascular pericytes. Thus, plenty of data have suggested important roles of PDGF-D in NV-AMD. Indeed, PDGF-D has been shown to be a potent chemoattractant to macrophages (Uutela et al., 2004). Moreover, the receptors used by PDGF-D, PDGFR-β and neuropilin (NRP) 1, are expressed by macrophages and microglia (Dai et al., 2017; Dejda et al., 2016; Muhl et al., 2017). PDGF-D thus has a direct effect on macrophages and microglia (Dai et al., 2017; Dejda et al., 2016; Muhl et al., 2017). PDGF-D has been shown to induce the proliferation and migration of multiple cell types, including choroidal fibroblasts and vascular pericytes. Thus, plenty of data have suggested important roles of PDGF-D in NV-AMD. Indeed, PDGF-D has been shown to be up-regulated in the neovascular choroid and retina (Kumar et al., 2010). Importantly, in an experimental NV-AMD mouse model, silencing of PDGF-D by short hairpin (sh) RNA or its neutralizing antibody suppressed both retinal and choroidal neovascularization in mice (Kumar et al., 2010). In addition, inhibition of PDGF-D decreased the number of infiltrated macrophages during CNV formation. Mechanistically, it has been shown that PDGF-D regulates the phosphorylation of its downstream effector, glycogen synthase kinase-3β, via PDGFR-β (Kumar et al., 2010). Thus, based on the potent angiogenic effect of PDGF-D, it may serve as a promising target molecule for antiangiogenic therapy for the treatment of NV-AMD.

**Neuro- and vaso-protective effects of PDGF-C and PDGF-D in retinitis pigmentosa**

RP is an inherited and heterogeneous degenerative retinal dystrophy that causes severe vision impairment due to the progressive degeneration of the rod photoreceptor cells in the retina (Hartong et al., 2006). In the early stage of RP, peripheral and dim light vision compromise due to the decline of rod photoreceptors. The progressive rod degeneration is later followed by abnormalities in the retinal blood vessels (Adams et al., 2012; Liu et al., 2016), retinal pigment epithelium and the deterioration of cone photoreceptor cells (Ali et al., 2017). As peripheral vision becomes increasingly compromised, patients experience progressive “tunnel vision” and eventual blindness. The incidence of RP is about 1 in 3000–7000 people. More than 1.5 million individuals are affected worldwide (Ferrari et al., 2011). RP can be inherited as an autosomal dominant, autosomal recessive, or X-linked disease (Daiger et al., 2007; Hartong et al., 2011). More than 250 genes have been identified to be associated with this progressive degenerative disease (Retinal information network, https://sph.uth.edu/retinet/sum-dis.html#A-genes).

Currently, there are no effective treatments for RP. Dietary supplementation with vitamin A palmitate and fish oil, which is rich in omega-3 fatty acids, have been recommended to help slow down the progression of the disease (Parmeggiani et al., 2011). In addition, preclinical studies have shown that gene therapy might be a plausible approach for the treatment of RP. For example, in AiPI
mutant mice, successful rescuing of retinal denegation by AIPL1 gene delivery using AAV virus (AAV-hAIPL1) has been shown (Ku et al., 2015). Moreover, using dogs with Pde6a mutation, it has been shown that subretinal injection of virus encoding the gene Pde6a (AAV-Pde6a) improved dim light vision and cone photoreceptor survival (Mowat et al., 2017). This success of Pde6a gene therapy for retinitis pigmentosa in a large animal model prompts further studies on gene therapy in RP patients.

Although PDGFs and PDGFRs are abundantly expressed in the retina (Andrae et al., 2008; Biswas et al., 2008; Kanamoto et al., 2011; Wilkinson-Berka et al., 2004), their potential roles in RP have not been well studied. However, studies have shown that PDGF-C is a potent neuroprotective factor and a vascular survival factor (Cai et al., 2016; Lee et al., 2013; Peng et al., 2012; Tang et al., 2010). In addition, PDGF-C has been shown to act on multiple cell types by regulating the expression of heme oxygenase-1, which is a potent antioxidant and anti-inflammatory factor (Chung et al., 2008; Wegiel et al., 2014). These observations thus have suggested protective and surviving roles of PDGF-C in RP. Indeed, in a mouse model of RP, it has been shown that the degeneration of both vascular and non-vascular cells of the neurosensory retina can be partially restored by PDGF-CC protein treatment (Fig. 4)(He et al., 2014). Additionally, PDGF-CC delivery preserved the architecture of retinal layers by reducing focal retinal lesions and attenuating the loss of photoreceptors in a mouse model of RP (He et al., 2014). This effect of PDGF-CC is at least partially mediated by downregulating the expression of some pro-apoptotic molecules such as Fas ligand and Bax (He et al., 2014). Consistently, it has also been shown that in a mouse model of
oxygen-induced blood vessel regression, PDGF-CC treatment prevented the regression of retinal blood vessels (He et al., 2014). Taken together, these data show that PDGF-CC may have a therapeutic potential in RP treatment by promoting neuronal and vascular cell survival.

**Anti-apoptotic effect of PDGF-C and PDGF-D in glaucoma**

Glaucoma is the second leading ocular disease worldwide (Kingman, 2004; Quigley, 1996). The global prevalence of glaucoma is over 57 million individuals in 2015. It is estimated to reach over 111 million by 2040 (Kapetanakis et al., 2016). The increase of intraocular pressure (IOP) is believed to be the main cause of this progressive and chronic neurodegenerative disorder. In glaucoma, extensive extracellular remodeling of the trabecular meshwork is one of the reasons leading to increased IOP. Also, myofibroblasts are activated in glaucoma and drive the formation of tissue fibrosis and stiffening (Liu et al., 2017). Loss of retinal ganglion cells and deformation of the optic nerve head are the hallmarks of glaucoma (Burgoyne et al., 2005).

Clinically, glaucoma can be classified into four categories, including primary open angle glaucoma, secondary open angle glaucoma, primary angle closure glaucoma and primary congenital glaucoma (Foster et al., 2002). Currently, topical eye drops and oral medications that can lower IOP are used to treat glaucoma. Such drugs include β-adrenergic antagonists, prostaglandin analogues, adrenergic agonists, carbonic anhydrase inhibitors and cholinergic agonists (Alward, 1998). Despite the existing drugs, therapies that can lower IOP more effectively and rescue the progressive neurodegeneration without adverse effects are yet to be realized. In addition to IOP-lowering drugs, surgical procedures including laser surgery, trabeculectomy (Rosenquist et al., 1989) and drainage implant surgery (Guerrero and Latina, 2000) are being used for the long-term management of glaucoma. Furthermore, various approaches aiming at rescuing neurodegeneration in glaucoma have been tested in preclinical models. These include intravitreal delivery of stem cells (Venugopalan et al., 2016), neurotrophic factors such as BDNF (Ko et al., 2001), CNTF (Ji et al., 2004) and gene therapy (Wilson and Di Polo, 2012).

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**Fig. 4. Neuroprotective effect of PDGF-C in retinitis pigmentosa.**

(a) Normal retina is well organised into several layers containing blood vessels, neuronal cells and other cell types. Different retinal cells are functionally and structurally interconnected to process the visual perception. (b) In retinitis pigmentosa, photoreceptor degeneration occurs, leading to the impairment of retinal structure, tissue remodeling, displacement of inner retinal cells, gliosis of MC and vascular degeneration. (c) Results from preclinical studies show that intravitreal injection of PDGF-CC protein rescues photoreceptor and vascular degeneration in a RP model. The potential role of PDGF-D in RP remains to be investigated. BV: blood vessel, RGC: retinal ganglion cell, AC: amacrine cell, MC: Müller cell, BC: bipolar cell, Mi: microglia, HC: horizontal cells, R: rod cell, C: cone cell, RPE: retinal pigment epithelial cell, RP: retinitis pigmentosa.
Studies have shown the roles of PDGFs and PDGFRs in glaucoma. It is reported that PDGFR-α is expressed in retinal ganglion cell layer and is critical for the survival of retinal cells under oxidative stress (Kanamoto et al., 2011). Recently, it has been shown that PDGF-AA protein treatment rescues retinal ganglion cells from death in mice expressing enhanced green fluorescent protein (EGFP) under PDGFR-α promoter (Takahama et al., 2017). It has also been shown that PDGFR-α is expressed in the inner nuclear layer and ganglion cell layer (Takahama et al., 2017). Moreover, PDGF signaling pathway has been shown to be implicated in the pathogenesis of primary open angle glaucoma (Colak et al., 2012). Additionally, it has been reported that PDGF-AB and PDGF-BB are detected in the aqueous humor of patients with uveitic glaucoma, also suggesting their potential roles in glaucoma (Ohira et al., 2016).

PDGF-C and its receptors are highly expressed in the retina (Tang et al., 2010). Preclinical studies using animal models have shown that PDGF-CC protein or gene delivery rescued ganglion cells in the retina in an axotomy-induced neuronal injury model and a neurotoxin-induced retinal neuronal death model (Tang et al., 2010). Preclinical studies using animal models have also suggested their potential roles in glaucoma (Takahama et al., 2017). The neuroprotective effect of PDGF-CC, at least in part, is exerted by regulating the expression of numerous apoptotic, cell survival and neurotrophic genes. At a molecular level, it has been shown that the phosphorylation of GSK3β is the downstream signaling employed by PDGF-CC for its neuroprotective effect. These findings thus highlight the importance of the neurovascular protective effects of PDGF-CC. The potential role of PDGF-D in glaucoma is thus far unclear. However, it has been recently shown that PDGF-D binds to NRPI, which plays an important role in the neurovascular system (Muhl et al., 2017), thereby suggesting a possible function of PDGF-D in glaucoma. Future studies are needed to verify this.

Perspectives and outstanding questions

Decades of extensive studies have shown that PDGFs and their receptors are critical players in numerous biological processes. Particularly, they are key regulators of vascular growth and function, neuronal survival, and fibrosis, all of which are critical components of many blinding diseases. Thus, the PDGFs and their receptors have been considered as important therapeutic targets for neurovascular and fibrotic diseases. Indeed, numerous preclinical studies have demonstrated the effectiveness of modulating PDGF pathway in different animal models. However, despite the significant advances, promising laboratory results have not been translated into clinically useful therapies, suggesting that a better and deeper understanding of the mechanisms and the underlying mechanisms are still needed.

Indeed, many outstanding questions remain to be answered. What are the functional differences among the different PDGFs in ocular diseases? What are the relative contributions of the different PDGFs and PDGFRs to different ocular pathologies, particularly, to ocular neovascularization? Combination therapy using Fovista targeting PDGF-B together with anti-VEGF therapy has failed. Would combination therapy targeting other PDGFs together with anti-VEGF treatment work?

How are the expressions of the PDGFs and PDGFRs regulated or dysregulated in different ocular diseases? Do current therapies affect their expression and function? How are the functions and signaling pathways of the PDGFs related to the VEGF pathway? Are they functionally independent of each other? Are the angiogenic activities of the PDGFs related to the development of drug resistance to anti-VEGF therapy?

Can the PDGFs be used as biomarkers for the states of ocular diseases or therapeutic outcomes? Are there new receptors or new signaling pathways for the relatively new PDGFs, PDGF-C and PDGF-D? Can the protective and survival effects of PDGFs be used to treat retinal degenerative diseases to preserve different types of retinal cells? Answers to these questions will not only help understand the basic biology of the PDGF family but will also facilitate the process of translating laboratory observations into clinically useful therapies.

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