The Placental Growth Factor Pathway and Its Potential Role in Macular Degenerative Disease

Fiona Cunningham, Tine Van Bergen, Paul Canning, Imre Lengyel, Jean H. M. Feyen & Alan W. Stitt

To cite this article: Fiona Cunningham, Tine Van Bergen, Paul Canning, Imre Lengyel, Jean H. M. Feyen & Alan W. Stitt (2019): The Placental Growth Factor Pathway and Its Potential Role in Macular Degenerative Disease, Current Eye Research, DOI: 10.1080/02713683.2019.1614197

To link to this article: https://doi.org/10.1080/02713683.2019.1614197

Accepted author version posted online: 04 May 2019.
Published online: 24 May 2019.

Submit your article to this journal

Article views: 128

View Crossmark data
The Placental Growth Factor Pathway and Its Potential Role in Macular Degenerative Disease

Fiona Cunningham¹, Tine Van Bergen², Paul Canning³, Imre Lengyel⁴, Jean H. M. Feyen⁵, and Alan W. Stitt³

¹Centre for Experimental Medicine, Queen’s University Belfast, Belfast, Northern Ireland; ²Oxurion NV, Leuven, Belgium

ABSTRACT

There is growing evidence that placental growth factor (PlGF) is an important player in multiple pathologies, including tumorigenesis, inflammatory disorders and degenerative retinopathies. PlGF is a member of the vascular endothelial growth factor (VEGF) family and in the retina, binding of this growth factor to specific receptors is associated with pathological angiogenesis, vascular leakage, neurodegeneration and inflammation. Although they share some receptor signalling pathways, many of the actions of PlGF are distinct from VEGF and this has revealed the enticing prospect that it could be a useful therapeutic target for treating early and late stages of diabetic retinopathy (DR) and neovascular age-related macular degeneration (AMD). Recent research suggests that modulation of PlGF could also be important in the geographic atrophy (GA) form of late AMD by protecting the outer retina and the retinal pigment epithelium (RPE). This review discusses PlGF and its signalling pathways and highlights the potential of blocking the bioactivity of this growth factor to treat irreversible visual loss due to the two main forms of AMD.

Age-related macular degeneration (AMD)

AMD is a leading cause of legal blindness and moderate to severe visual impairment in the Western world. It is predicted that ~196 million people will develop this condition by the year 2020.¹ The progressive loss of central vision and visual acuity impacts on quality of life by hindering facial recognition, and the ability to read or drive. Early clinical hallmarks of AMD are the appearance of multiple drusen located between the retinal pigment epithelium (RPE) and the Bruch’s membrane.² While these deposits also occur in the healthy ageing eye³, in early AMD, they increase in number and size.³

It is hypothesised that multiple factors contribute to progression of early AMD to late AMD, categorised as neovascular AMD (nvAMD) or geographic atrophy (GA). Products of the ageing retina, that include drusen⁴, lipofuscin⁵, and advanced glycation end-products (AGEs)⁶, contribute to a vicious cycle of oxidative stress and inflammation. It is believed that this might be a potential stimulus for pathological angiogenesis that drives nvAMD⁷, or in the case of GA, a driver of retinal cell death⁸,⁹.

Ageing is the greatest risk factor for AMD¹⁰, but factors that exacerbate oxidative stress, including cigarette smoking, also contribute to AMD initiation and progression.¹¹ Several studies have also investigated the role of dietary lipids in AMD¹², with a focus on how different types of lipids may be beneficial¹³, whilst others contribute to disease risk.¹⁴ Genetic risk factors also play a major role, and 52 gene variants associated with AMD have been identified across 34 loci.¹⁵ These include rare variants associated with dysregulated complement activity (CFH, CFI), as well as genes involved in the extracellular matrix (ECM) turnover and cholesterol transport.¹⁵ A significant finding was the identification of a single gene variant upstream of MMP9 that was specific for nvAMD, however, all other variants exhibited association with both subtypes.¹⁵

NvAMD is characterised by choroidal neovascularisation (CNV), and in some cases retinal angiomatous proliferation (RAP), where proliferating retinal capillaries Anastomose with CNV lesions.¹⁶ In CNV, inflammatory and hypoxia-related stimuli drive the growth of fragile new choroidal vessels, and weaken the barriers formed by Bruch’s membrane and the RPE monolayer, allowing the neovascularure to invade sub-RPE and sub-retinal spaces in the macula.¹⁷ These stimuli include a range of pro-angiogenic growth factors and cytokines of which vascular endothelial growth factor (VEGF) is the most prominent. Fortunately, it is possible to stop progression or even reverse CNV with repeated intravitreal injections of antibodies that ‘trap’ VEGF. These drugs have transformed the care of nvAMD¹⁸, although there are some growing concerns that prolonged therapy may accelerate retinal and neuronal atrophy, thus progressing GA whilst aiming to treat nvAMD.¹⁹,²⁰

GA is characterised by progressive modifications of Bruch’s membrane, patchy loss of the RPE, involution of the neighbouring choriocapillaris and degeneration of photoreceptors. The precise pathological mechanisms underpinning GA remain unclear and unlike nvAMD, no adequate treatments are available, although clinical trials have or are currently investigating the potential of antioxidant therapy²¹, Complement system inhibition²² and
choroidal reperfusion. To date, many of these trials have been unsuccessful and there is a pressing need for new options. To this end, a recent clinical trial has investigated RPE cell replacement therapy with promising results. However, the impact of an abnormal microenvironment on the health of transplanted RPE has not been addressed, and cell replacement therapies may need to be combined with compound-based therapeutics for long-term success.

Altered growth factor signalling in AMD

In both forms of late AMD, RPE dysfunction plays a central role. This monolayer of neuroepithelium, together with the underlying choroidal microcapillaries, forms the outer blood-retinal-barrier (oBRB) by virtue of intercellular tight junctions. These junctions regulate the tissue-specific polarisation of RPE that is crucial for their proper function. The RPE also plays a crucial supportive role in vision by phagocytosing spent photoreceptor outer segments and participating in the retinoid cycle, transporting metabolites between the choriocapillaris and photoreceptors maintaining water homeostasis and expelling waste products of metabolism.

Through the paracrine secretion of growth factors, the RPE also plays a key role in maintaining the integrity and fene-

stromed phenotype of the choriocapillaris.

The choriocapillaris exhibits phenotypic characteristics of VEGF-dependent capillaries, including fenestrae and cognate receptor expression, both primarily at the RPE-facing side of the endothelium. Loss of RPE-derived VEGF is detrimental to the choriocapillaris and results in reduced fenestration and vessel atrophy. In nvAMD, overexpression of VEGF-A is associated with CNV, vessel leakage and inflammatory cell infiltration. Inhibition of retinal VEGF-A signalling via intravitreal antibody delivery can partially halt some of these changes, and improve visual function in patients with active nvAMD. These benefits have also extended to patients with RAP, suggesting that VEGF-A overexpression is also associated with this pathology.

RPE cells secrete VEGF family proteins and express these proteins’ receptors, including the R1 receptor of VEGF (VEGFR1), VEGFR2, VEGFR3 and neuropilins (NP-1 and NP-2). VEGF-A is upregulated in RPE cells under conditions of stress (hypoxia, oxidative stress, inflammation) associated with AMD, and is thought to contribute to CNV. The functional response of RPE to VEGF-A is increased tight junction permeability, likely via ZO-1 phosphorylation and subsequent internalisation and degradation of junction proteins such as occludin, as is observed in endothelial cells. This VEGF-induced response permits the growth of neovascular vessels from the choroid into the sub-retinal spaces, progressing CNV.

Placental growth factor

Placental growth factor (PIGF) is a member of the VEGF family, first discovered in the placenta, where its elevation corresponds with placental angiogenesis. The human PIGF gene encodes four isoforms with distinct properties. PIGF-1 and -3 are diffusible isoforms, while PIGF-2 and -4 possess heparin-binding domains, conferring them additional ligand–receptor interactions. All four isoforms bind VEGFR1, while PIGF-2 and -4 additionally bind to neuropilins (NP-1 and NP-2), which are the VEGFR1 co-receptors highly expressed by neurons. PIGF-activated VEGFR1 signalling is distinct from VEGF-A-mediated signalling, due to the phosphorylation of different tyrosine residues on the receptors, leading to specific regulation of downstream targets.

PIGF, endothelial cells and pathological angiogenesis

It has been postulated that PIGF competently binds VEGFR1 and leads to the increased availability of VEGF-A to bind and activate VEGFR2, producing a stronger angiogenic stimulus than VEGFR1. While this synergy could be potentially important, recent computational analysis suggests this may not be physiologically significant. By using simulations, the authors showed that PIGF does not displace VEGF-A from VEGFR1 to increase VEGFR2 phosphorylation at physiological concentrations, however when overexpressed PIGF did slightly increase VEGFR2 phosphorylation. PIGF and VEGF-A can also form biologically inactive heterodimers, however other publications suggest that PIGF/VEGF-A heterodimers can act synergistically to promote angiogenesis and consequently drive neovascular pathology in the retina. PIGF activation of neuropilin receptors could also promote angiogenesis and endothelial cell survival, independent of VEGFR signalling. However, evidence suggests that PIGF blockade does not exhibit deleterious effects on the vascular physiology of healthy mice. On the contrary, in PIGF knockout rodent models with superimposed disease conditions, such as ischaemia, lack of PIGF reduces disease-related angiogenesis, suggesting that PIGF contributes to pathological angiogenesis.

PIGF signalling in endothelial cells is illustrated in Figure 1.

PIGF and its receptors are upregulated in DR, and in nvAMD, where PIGF has been found in CNV membranes. In murine models of laser-induced CNV, VEGFR1 and PIGF are upregulated in the CNV lesion, and in neighbouring tissues. Neuropilin is also expressed in the retina, and in nvAMD these co-receptors are expressed in CNV lesions, and localise with VEGF receptors. These findings contribute to the understanding of nvAMD pathophysiology. The fragile neovascular structures observed in nvAMD are highly permeable and haemorrhage into the sub-RPE and sub-retinal spaces. The resulting oedema upsets outer retinal anatomy and photoreceptor function and can result in irreversible loss of central vision. The changes in expression of PIGF and its receptors observed in CNV models suggest this may contribute to neovascularisation and vascular permeability in nvAMD. These findings suggest that localised modulation of PIGF may have the potential to alleviate the major pathological changes observed in nvAMD. Indeed, studies have reported a protective effect of PIGF knockout in mice with laser-induced CNV, where the lesions were smaller when compared to wild type mice subjected to the same insult. Similar results have been reported with antibody-mediated blockade of PIGF in the same mouse model, while genetic deletion of PIGF in diabetic mice protects the retinal vasculature and increases expression of tight junction proteins.
These data highlight that PlGF can be upregulated in retinal pathologies with detrimental effects that can be reversed by its ablation. There is currently no approved therapeutic that solely targets PlGF to treat retinopathies, although a recombinant humanised anti-PlGF antibody (THR-317; Oxurion N.V.) is currently under clinical investigation for the treatment of DME. On the other hand, aflibercept, although primarily an anti-VEGF-A agent, also inhibits bioactivity of other VEGF family members including PlGF. The efficacy of aflibercept has been compared to the anti-VEGF-A agent ranibizumab and while there is no difference in nvAMD outcomes overall, there may be enhanced benefit from aflibercept in some patients, with the potential added benefit of less frequent intravitreal injections. Indeed, it has been reported that aflibercept may be a more effective treatment option for nvAMD patients who present with greater disease severity and/or fail to respond to anti-VEGF-A monotherapy. This is consistent with a 2018 observational study of nvAMD patients who responded poorly to ranibizumab therapy, which indicated that these patients had higher aqueous levels of PlGF as compared to responders, suggesting that VEGF-A was not the sole contributing factor to their disease progression. It should be noted, however, that when switched to aflibercept, these patients did not experience improved visual acuity, despite a significant improvement in macular anatomy as evaluated by optical coherence tomography. Such observations further highlight the complex, multifactorial nature of AMD, patient-to-patient variation and the subtle balance of pathogenic factors that can contribute to AMD pathology.

**PIGF in non-ocular epithelium and RPE function**

Beyond its effects on vascular angiogenesis, PlGF signalling also affects epithelial function. Within the lung, PlGF acts on alveolar epithelial cells (AECs), with implications for emphysema, and chronic obstructive pulmonary disorder. These studiesimplicate reactive oxygen species in the induction of PlGF airway expression and report that AECs exposed to PlGF undergo epithelial-to-mesenchymal transition (EMT), while PlGF blockade prevents this phenotypic switch. These findings have also been reported in epithelial-derived cancer cell lines, where PlGF-induced EMT was associated with cell migration. Furthermore, it has been reported that PlGF can induce autophagy and apoptosis of AECs, via activation of MAPK signalling. PlGF is capable of inducing atrophy of epithelial monolayers, which can be induced by oxidative stress and inhibited by PlGF blockade. Figure 2 summarises the findings from these published studies.

RPE atrophy is one of the defining characteristics of GA and is associated with subsequent degeneration of photoreceptors, and involution of the choriocapillaris, as demonstrated in patient studies, and further supported by preclinical studies using sodium iodate for RPE-selective degeneration. These studies highlight an angiopathic aspect to GA and suggest the progressive development of a hypoxic microenvironment.

Human RPE reportedly produces PlGF in low concentrations although its expression can be upregulated during hypoxia, which has been demonstrated in the human RPE cell line ARPE-19. RPE also responds to PlGF, and its overexpression is associated with altered RPE morphology, disrupted tight junctions and elevated monolayer permeability. These changes are important pathological features of AMD and indicate compromised RPE function.

In nvAMD, it is thought that compromised RPE tight junctions allow blood vessels to breach the oBRB and infiltrate the sub-retinal space, synergising with pathological angiogenesis. With regards to GA, it has been demonstrated that RPE dysfunction and retinal atrophy induced by light exposure in mice can be significantly attenuated by PlGF inhibition.
This study was the first to propose that retinal damage induces PlGF expression and adds to in vitro RPE studies that suggest light-induced oxidative stress in the RPE is associated with altered cell signalling and reorganisation of cell-cell junctions. More recently, similar outcomes have been replicated using aflibercept in the light-induced retinal atrophy model although an explicit confirmation of altered PlGF expression would be helpful to support this research. These retina-specific studies suggest that light-induced oxidative stress upregulates PlGF in the retina/RPE and may contribute to GA-related retinal atrophy (see Figure 2). More research is needed to establish the role of PlGF in all aspects of AMD, especially in GA-related pathology.

**PIGF in inflammatory pathology**

PIGF has been associated with the induction of inflammation in various pathologies, and VEGFR1-expressing monocytes and macrophages respond to this growth factor. VEGFR1-mediated PIGF signalling promotes macrophage accumulation in atherosclerotic plaques and is a pro-inflammatory mediator of rheumatoid arthritis, airway hyper-responsiveness and liver fibrosis. PIGF silencing in these conditions is associated with reduced inflammation and downregulation of macrophage markers, suggesting that PIGF is an attractive target for inflammatory-driven diseases.

Normal parainflammatory measures exist in the retina for homeostasis. However, chronic inflammation is considered a major player in the pathogenesis of AMD, where parainflammation is dysregulated. Dysregulated activation of the complement cascade and subsequent formation of membrane attack complexes on the choroid and RPE has been associated with cell lysis, atrophy and release of pro-inflammatory cytokines, generating local retinal inflammation. Environmental factors and oxidative stress contribute to retinal inflammation, as do by-products of ageing and cell dysfunction.

In the outer retina activated macrophages and microglia accumulate and secrete inflammatory and angiogenic factors that contribute to RPE atrophy, and CNV. PIGF is a potent chemoattractant for VEGFR1-expressing macrophages and reactive microglia. In CNV models, PIGF recruits microglia and macrophages to lesions at early timepoints (day 3 post-laser injury reported), and this effect can be prevented through inhibition with a PIGF antibody or anti-VEGFR1. This is additionally associated with reduced expression of inflammatory markers and cytokines, including Iba1, IL-6 and IL-1β. The resultant reduced macrophage infiltration alleviates chronic inflammation and tissue destruction, thus potentially benefiting both subtypes of late AMD (see Figure 3).

Previous studies have investigated the interplay between VEGF-A and complement in the eye and suggest that VEGF-A positively regulates the expression of complement factor H, and that its ablation may leave the retina susceptible to uncontrolled activation of the complement cascade. However, it is not clear whether PIGF plays a role in this process, and future studies are required to investigate this.

**PIGF and metabolism**

VEGFR system regulation of cell metabolism has been widely investigated. VEGF blockade is known to shift tumour cell metabolism in favour of glycolysis, a response that contributes to treatment resistance. It has also been demonstrated that VEGF-A inhibition reduces the insulin resistance of murine
liver cells, and supports a role for VEGFR signalling in glucose metabolism.\textsuperscript{111}

Endothelial cells, despite their proximity to circulating oxygen, preferentially rely on glycolytic metabolism in preference to mitochondrial respiration.\textsuperscript{112} However, in conditions of impaired VEGF signalling in endothelial cells there is suppression of glucose metabolism, alongside induced autophagy and cell death.\textsuperscript{113} Domigan et al. reported that VEGF-A signalling in endothelial cells downregulates the transcription factor FOXO1, to maintain normal metabolism.\textsuperscript{113} The proangiogenic signalling via VEGFR2 upregulates the expression of the \textit{PFKB3} gene, driving glycolysis.

In ischaemic retinopathies, low oxygen tension stimulates the upregulation of proangiogenic factors, including VEGF-A. Potent angiogenic signalling is thought to favour a shift towards glycolysis, drive endothelial cell motility and propagate pathological angiogenesis. In nvAMD, inflammatory signals activate glycolytic enzymes and correlate with increased VEGF-A expression\textsuperscript{114}, driving CNV.\textsuperscript{115} With regards to GA, it has been suggested that the RPE is metabolically flexible.\textsuperscript{116} For example, in hypoxia resulting from reduced perfusion of the choriocapillaris, glycolysis is increased with a concomitant rise in RPE glucose consumption, which can subsequently deprive photoreceptors of their energy substrate and evoke outer retinal degeneration.\textsuperscript{116} A role for VEGFR signalling in GA metabolism has not been extensively investigated; however, it has been reported that silencing VEGFR2 in the ARPE-19 cell line can protect against stress and death induced by glucose-deprivation\textsuperscript{117}, suggesting that VEGFR signalling influences RPE metabolism under conditions of stress.

Altered metabolic activity of RPE has also been associated with EMT and cell migration.\textsuperscript{118} Migration of RPE cells into the inner retina has been observed in GA patients and may contribute to atrophy of the RPE monolayer.\textsuperscript{119} Additionally, RPE EMT could contribute to nvAMD, by increasing the oBRB permeability and contributing to scar formation via wound healing.

The effect of PIGF on retinal cell metabolism has not been widely investigated; however, PIGF signalling in DR and nvAMD may modulate alterations in retinal endothelial cell metabolism. PIGF knockout in diabetic mice has been associated with decreased insulin resistance through regulating expression of proteins involved in glucose metabolism.\textsuperscript{72} Additionally, proteomic analysis of this PIGF knockout Akita diabetic mouse model revealed increased expression of the antioxidant enzyme Prdx6 and neuronal survival protein Map2.\textsuperscript{72} This has potential implications for oxidative stress and photoreceptor death in AMD. A 2018 report from Zhang et al. suggests that PIGF signalling in hypoxic ARPE-19 induces EMT-like changes\textsuperscript{120}, in line with previous observations from lung epithelial cells. This change in RPE phenotype may contribute to both forms of late AMD. Further investigations are needed to determine the definitive role of PIGF in retinal cell metabolism and the repercussions of this for AMD.

**Future directions**

Future research should further assess the potential for inhibition of PIGF to attenuate oxidative stress-associated retinal atrophy\textsuperscript{89,90,92}, especially in the context of GA in AMD. Effort should be placed on providing a clearer understanding of the effects of PIGF on RPE EMT in both pre-clinical systems and in clinical examples.\textsuperscript{121} The association between PIGF and autophagy in the lung epithelium\textsuperscript{80} suggests that PIGF may have similar effects on RPE autophagy in the eye. As changes in RPE autophagy have been extensively reported in AMD\textsuperscript{122} this process could be further investigated. Despite the studies on the influence of PIGF on inflammation in nvAMD\textsuperscript{66,68}, the
effect of PlGF signalling on retinal inflammatory cells in the context of retinal and RPE atrophy has not been sufficiently studied. Future studies utilising a range of animal models of AMD may be able to elucidate the contribution of PlGF-induced inflammation to retinal atrophy.

The impact of altered metabolism on these aforementioned processes also warrants further investigation, as the involvement of the VEGFR axis in retinal cell metabolism is becoming increasingly apparent. Collectively, studies of RPE suggest that a pathological microenvironment (e.g., hypoxia) can upregulate PlGF expression and induce EMT, potentially via metabolic changes. Going forward, studies should aim to elucidate the exact signalling pathways involved, and investigate both in vitro and in vivo the effect of PlGF on cell metabolism, analysing glycolysis and mitochondrial respiration, and the resulting functional and morphological changes that may occur.

Although studies of aflibercept’s efficacy for the treatment of nAMD have reported variable results, the results suggest aflibercept could become an effective alternative for patients who do not respond to anti-VEGF-A monotherapy or have poorer baseline visual outcomes. In addition, VEGF-A inhibition via intravitreal injection has been associated with the development of secondary GA in nAMD patients, suggesting that downregulation of constitutively expressed VEGF-A alters the progression of late AMD. Therefore, anti-PlGF monotherapy may provide another alternative to aflibercept where patients are at risk of developing secondary GA. It is possible that further ‘repurposing’ of an anti-PlGF therapeutic for the treatment of GA may follow in future clinical studies, if preclinical studies suggest PlGF inhibition is an effective method of attenuating retinal atrophy.

Summary and conclusions

PlGF has a range of effects on various retinal cells and in some disease conditions such as AMD, upregulated expression of this growth factor may drive significant pathogenic processes. In the retina, PlGF signalling via the VEGFR system is often associated with pathological angiogenesis, vascular leakage, and the induction of inflammatory sequelae. Beyond its effects on endothelial and immune cells in the retina, PlGF also has documented effects on the RPE, especially its normal barrier activity which is compromised in many retinal diseases. A hypoxic microenvironment in the retina due to choroidal degeneration may induce PlGF expression and contribute to RPE dysfunction and atrophy via altered cell metabolism and EMT. The role of PlGF in the loss of RPE integrity and atrophy of the oBRB and how this contributes to AMD subtypes warrants further research. This is especially worthwhile with the already existing therapeutic option to effectively block bio-activity of PlGF to protect against sight-threatening retinal disease.

Disclosure statement

Oxurion NV. In accordance with Taylor & Francis policy ethical obligations, the corresponding author discloses that he receives funding from Oxurion NV.

Funding

This project is funded through the Department for Education (DfE) CAST/CASE Scheme in Northern Ireland with enhanced running costs provided by Oxurion NV.

References


The image contains a page of a scientific document with multiple references and abstracts. The text is in English and pertains to various topics related to the eye, including mechanisms of age-related macular degeneration, angiogenesis, photoreceptor cell death, and retinal pigment epithelial cells. The document appears to be a collection of research papers and reviews, with references to other works, indicating a comprehensive approach to understanding these topics. However, without the full context of the document, it is challenging to provide a comprehensive summary or cross-references to other parts of the document. The page includes references to studies published in journals like Ophthalmology, Vision Research, and Retina, among others. The authors range from multiple institutions, suggesting a collaborative and interdisciplinary approach to these investigations.


