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Recent developments in our understanding of how platelet-derived growth factor (PDGF) and its receptors contribute to proliferative vitreoretinopathy

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Abstract

Proliferative vitreoretinopathy, a disease process occurring in the setting of a rhegmatogenous retinal detachment, is thought to develop as a result of exposure of retinal cells to vitreous. Vitreous contains many growth factors, and platelet-derived growth factor (PDGF) has been considered a major contributor to PVR. Evaluation of both PDGF and PDGF receptors (PDGFRs) as potential therapeutic targets in the context of a rabbit model of PVR revealed that PDGFR-based approaches protected from PVR, whereas neutralizing PDGFs was a much less effective strategy. The basis for these observations appears to reflect that fact that the PDGFR could be activated by a wide spectrum of vitreal agents that are outside of the PDGF family. Furthermore, blocking signaling events by which the non-PDGFs indirectly activated PDGF α receptor (PDGFR α) protected rabbits from developing PVR. These studies demonstrate that the best therapeutic targets for PVR are not PDGFs, but PDGFR α and certain signaling events required for indirectly activating PDGFR α .

PVR develops in a small percentage of patients who suffer from a rhegmatogenous retinal detachment

The term proliferative vitreoretinopathy (PVR) is used to describe a condition where traction from sheets of ectopic cells, referred to as vitreous and epiretinal membranes, complicates a rhegmatogenous retinal detachment (RRD) (Campochiaro, P., 2006). Such detachments occur when a tear or hole is created in the retina. They are most commonly preceded by a posterior vitreous detachment which creates traction on the retina and precipitates a full thickness break through which liquefied vitreous enters the subretinal space (Oh, K. et al., 2006). RRD can also be associated with ocular traumas, intraocular inflammation and infections, retinoschisis, macular holes and rarer congenital vitreoretinal disorders such as Stickler syndrome (Byer, N.E., 1976; Cox, M.S. et al., 1966; Kerkhoff, F.T. et al., 2003; Stickler, G.B. et al., 2001; Wolfensberger, T.J. and Gonvers, M., 2000).

PVR is most commonly observed after surgery for RRD. It is estimated to occur in 5 to 11% of such cases making it the most common cause of failed repair of a primary RRD (Han, D., 2008). This occurs because traction induced by the membranes creates new breaks in the retina

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or reopens previously treated ones. It is also important to note that PVR can happen in cases of RRD without any previous surgical intervention. A large number of risk factors for developing PVR have been identified, some more consistently than others. Giant retinal tears, retinal detachments larger than 2 quadrants, vitreous hemorrhage, intraocular inflammation and preoperative choroidal detachment have all been associated with an increased risk of developing PVR (Cowley, M. et al., 1989; Duquesne, N. et al., 1996; Girard, P. et al., 1994; Yanyali, A. and Bonnet, M., 1996; Kerkhoff, F.T. et al., 2003). As previously mentioned, prior retinal detachment repair is the most common factor predisposing an eye to PVR (Thompson, J., 2006).

Several pharmacological approaches to prevent PVR have been tested. A broad specificity inhibitor of cell proliferation such as daunorubicin delivered by intravitreal perfusion was not effective in altering the final anatomical outcome or visual acuity (Wiedemann, P. et al., 1998). A similarly administered combination of 5-fluorouracil and low molecular weight heparin had no impact on visual acuity, but there was a decline in the number of patients needing reoperation (Asaria, R.H. et al., 2001). A molecular approach, involving a DNA-RNA chimeric ribozyme directed against proliferating nuclear antigen, proved unable to prevent recurring PVR (Schiff, W.M. et al., 2007).

The growth factor/cytokine hypothesis for how PVR develops

Several key observations form the foundation for the prevailing growth factor/cytokine hypothesis regarding how PVR develops (Fig 1). First, the break in the retina exposes intra-retinal cells and underlying RPEs to vitreous, which contains many agents including growth factors and cytokines. Second, these vitreal growth factors and cytokines promote all of the key cellular responses that are intrinsic to PVR: cell migration, proliferation, survival, production of extracellular matrix proteins and contraction. Consequently, a simple hypothesis regarding how PVR develops is that the retinal breaks exposes cells to vitreal growth factors and cytokines, which promote the sequence of cellular responses resulting in PVR, i.e. they migrate into vitreous where they proliferate, synthesize extracellular matrix proteins, organize into a membrane and contract, which invariably reopens the retinal tears that were previously treated or creates new ones.

As mentioned above, a key discovery that supports the growth factor/cytokine hypothesis is that pathological vitreous or the epiretinal membrane contains many growth factors and cytokines. The growth factors include platelet-derived growth factor (PDGF) isoforms, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), transforming growth factor α (TGF α), transforming growth factor β (TGF β), tumor necrosis factor α (TNF α), TNF β , granulocyte colony stimulating factor (G-CSF), fibroblast growth factors (FGF)s, insulin, insulin-like growth factor-1 (IGF-1) and connective tissue growth factor (CTGF) (Asaria, R.H. et al., 2004; Banerjee, S. et al., 2007; Baudouin, C. et al., 1993; Campochiaro, P.A. et al., 1996; Cui, J.Z. et al., 2007; Dieudonne, S.C. et al., 2004; Harada, C. et al., 2006; Hinton, D.R. et al., 2002; Kita, T. et al., 2007; La Heij, E.C. et al., 2002; Lashkari, K. et al., 1999; Lei, H. et al., 2007; Liou, G.I. et al., 2002; Mukherjee, S. and Guidry, C., 2007; Oshima, Y. et al., 2002). The cytokines include interleukin 1 (IL-1), IL-6, IL-8, IL-10, interferon γ (IFN γ), monocyte chemotactic protein and macrophage-colony stimulating factor (Campochiaro, P.A. et al., 1996; Choudhury, P. et al., 1997; El-Ghrably, I.A. et al., 2001; Elner, S.G. et al., 1995; Kon, C.H. et al., 1999; Mukherjee, S. and Guidry, C., 2007). I will focus on studies related to PDGF and its receptors to discuss the evidence supporting the growth factor/cytokine hypothesis.

Clinical studies supporting the idea that PDGF/PDGFR contributes to PVR

Immunohistochemical/immunofluorescent analysis of epiretinal membranes isolated from patients with PVR revealed that both PDGF and PDGFRs were present (Cui, J.Z. et al., 2007; Robbins, S.G. et al., 1994). Both RPE and glial cells expressed PDGFs and their receptors (Cui, J. et al., 2009; Cui, J.Z. et al., 2007; Robbins, S.G. et al., 1994). Subsequent studies indicated that the PDGFRs were activated (Cui, J. et al., 2009), and vitreous contained a high level of PDGF (Lei, H. et al., 2007). PDGF in vitreous was tightly associated with PVR as 8/9 PVR patients had detectable levels of vitreal PDGF, whereas PDGF was present in vitreous of only 1/16 patients with a different type of retinal disease requiring surgery/vitrectomy (Lei, H. et al., 2007). Furthermore, of the 5 PDGF family members, only PDGF-C was detectable (Lei, H. et al., 2007). This form of PDGF must undergo proteolytic processing for activation (Fig 2), and vitreous also contained the proteases capable of accomplishing this conversion (Lei, H. et al., 2008). The major vitreal PDGF-C processing protease was plasmin, and the amount of plasmin activity was higher in rabbits with PVR as compared to the control rabbits (Lei, H. et al., 2008). We conclude that studies with clinical specimens support the idea that a functional relationship between PDGF and its PDGFRs exists and may be contributing to PVR.

Animal studies supporting the idea that PDGF/PDGFR contributes to PVR

Of the 26 animal models of PVR, injecting fibroblasts into vitreous of rabbits is chosen most commonly (Agrawal, R.N. et al., 2007). We used this model to investigate whether experimental PVR reflected the clinical setting. Indeed, while the level of PDGF was undetectable in vitreous of healthy rabbits, there was a high level of PDGF in vitreous of animals that developed PVR. Furthermore, PDGF-C was the predominant isoform (although other PDGF isoforms were also present) (Lei, H. et al., 2007), and vitreous contained the proteases necessary for processing PDGF-C into its biologically active form (Lei, H. et al., 2008). Thus the presence of PDGF-C in vitreous was seen in both clinical and experimental PVR.

As in the rabbit model of PVR, plasmin accounted for the majority of the PDGF-C processing activity in vitreous from patients with PVR (Lei, H. et al., 2008). This phenomenon did not appear to be diagnostic for PVR, since a comparable amount of plasmin activity was present in vitreous of patients undergoing retinal surgery to correct either PVR, or issues unrelated to PVR (Lei, H. et al., 2008). In light of the fact that there is a high level of plasmin in the circulation, the bloodstream is a likely source of plasmin that is present in patient vitreous.

Consistent with the clinical observation that activated PDGFRs were present in the epiretinal membranes isolated from PVR patients, PDGFRs were an essential component of experimental PVR. Using a panel of genetically modified fibroblasts we found that expression of functional PDGFRs was essential for experimental PVR. The PVR potential of cells null for PDGFR genes was low, and re-expressing the wild type PDGFR, but not a kinase inactive mutant dramatically increased the ability of the cells to induce PVR (Andrews, A. et al., 1999; Ikuno, Y. et al., 2002). Similarly, both molecular and pharmacological approaches to poison naturally expressed PDGFRs reduced the PVR potential of even the most potent PVR-inducing cells (Ikuno, Y. et al., 2000; Zheng, Y. et al., 2003). Thus, the commonly used fibroblast injection model of PVR reflects a number of features of clinical PVR, which support the idea of an involvement of PDGF/PDGFR in PVR.

PDGFR α is better than PDGFR β at inducing experimental PVR

Two genes encode PDGF receptor (PDGFR) subunits, which hetero or homodimerize into 3 different PDGFRs: PDGFR α ($\alpha\alpha$ homodimers), PDGFR β , ($\beta\beta$ homodimers) and PDGFR $\alpha\beta$, ($\alpha\beta$ heterodimers) (Heldin, C.-H. and Westermark, B., 1990; Raines, E.W. et al., 1990). Each

half of the dimeric PDGF ligand recruits one receptor subunit to assemble the PDGFR dimer (Fig 3). The intrinsic affinity of PDGF family members for the two receptor subunits determines the composition of the assembled PDGFR (Fig 3) (Fredriksson, L. et al., 2004a; Reigstad, L.J. et al., 2005).

We compared the PVR potential of different PDGFRs using a panel of genetically engineered cells re-expressing either/or both of the PDGFR subunits. Cells expressing PDGFR α induced PVR much more effectively than PDGFR β -expressing cells, whereas an intermediate response was observed in the rabbits injected with cells expressing both PDGFR subunits (Andrews, A. et al., 1999). The difference in potency of PDGFR α and PDGFR β was a bit of surprise because previous studies with cultured cells expressing either of the PDGFR genes did not reveal overt differences in their ability to promote PDGF-dependent cellular responses intrinsic to PVR (Andrews, A. et al., 1999; Matsui, T. et al., 1989). However, subsequent studies investigating the contribution of PDGFRs during development indicated that the two PDGFRs were not interchangeable, even after taking into account the fact that they are expressed at different times and locations, and are activated by non-identical sets of the PDGF isoforms (Tallquist, M. and Kazlauskas, A., 2004).

Analysis of clinical specimens also supports the idea that PDGFR α is more important than PDGFR β (Cui, J. et al., 2009). While both receptors are expressed in epiretinal membranes, a greater percentage of PDGFR α is activated (Cui, J. et al., 2009). Furthermore, the vitreal PDGF isoforms preferentially activate PDGFR α (Lei, H. et al., 2007).

Taken together, the working hypothesis that emerges for both clinical and experimental PVR is that vitreal PDGFs activate PDGFRs and thereby drive the cellular events leading to PVR.

Neutralizing vitreal PDGFs did not protect rabbits from PVR

The availability of an animal model enables experiments testing whether PDGF-dependent activation of PDGFR was a required event in PVR. In transgenic mice that overexpress PDGF-B (the universal ligand) in photoreceptors, intravitreal injection of an aptamer directed against PDGF-B protected from retinal detachment that these mice are prone to develop (Akiyama, H. et al., 2006). In light of the data discussed above, we anticipated that antibodies and/or traps that neutralized PDGFs would prevent PVR in the rabbit model of the disease. Surprisingly, they did not, despite the fact that they effectively inhibited vitreal PDGFs (Lei, H. et al., 2009b). These findings presented an intriguing puzzle: experimental PVR was dependent on PDGFR (Andrews, A. et al., 1999; Ikuno, Y. and Kazlauskas, A., 2002; Ikuno, Y. et al., 2000; Zheng, Y. et al., 2003), but not PDGF (Lei, H. et al., 2009b). A potential answer was that in the context of PVR, the PDGFR was being activated by agents other than PDGFs.

Indirect activation of PDGFR α was sufficient for experimental PVR

While PDGF is by far the best-known agonist for the PDGFR, it is not the only agent capable of activating the PDGFR. Agonists of G protein-coupled receptors transactivate receptor tyrosine kinases, including PDGFR β (Heeneman, S. et al., 2000; Herrlich, A. et al., 1998; Linseman, D.A. et al., 1995; Liu, Y. et al., 2007; Siegbahn, A. et al., 2008; Tanimoto, T. et al., 2004). Since vitreous contains many growth factors outside of the PDGF family (non-PDGF), and certain non-PDGF-induced signaling events were potentiated by expression of PDGFRs (Zhang, H. et al., 2007), we considered whether non-PDGFs present in vitreous activated PDGFR α . Indeed, vitreous, as well as purified versions of four vitreal growth factors, induced tyrosine phosphorylation of PDGFR α (Lei, H. et al., 2009b). To test if non-PDGFs used the PDGFR's extracellular domain to activate PDGFR α , we compared this event in cells expressing either full length PDGFR α , or a mutant that lacked the extracellular domain. Non-PDGFs activated both receptors to a comparable extent (Lei, H. and Kazlauskas, A., 2009);

we concluded that the extracellular domain was dispensable and therefore focused our quest to elucidate the mechanism by which non-PDGFRs activated PDGFR α on intracellular-based events. We found that activation of PDGFR α by non-PDGFRs involved elevation of the level of intracellular reactive oxygen species (ROS), which led to activation of Src family kinases (SFK)s that directly or indirectly promoted phosphorylation of PDGFR α (Lei, H. and Kazlauskas, A., 2009). This indirect route to activate PDGFR α was fundamentally different than direct (i.e. PDGF-driven) activation of PDGFR, which requires the receptor's ligand binding domain, and is independent of both ROS and SFK (Klinghoffer, R.A. et al., 1999; Lei, H. and Kazlauskas, A., 2009). The distinction between the direct and indirect mechanisms to activate PDGFRs may not always readily discernable since in certain cell types (such as vascular smooth muscle cells) ROS is required for direct activation of PDGFR (Sundaresan, M. et al., 1995).

A curious feature of vitreous-dependent (i.e. indirect) activation of PDGFR α was that the magnitude of phosphorylation of the receptor was approximately an order of magnitude below the level of receptor phosphorylation induced by direct activation (Lei, H. and Kazlauskas, A., 2009; Lei, H. et al., 2009b). Yet cells expressing PDGFR α that lacked the extracellular domain (and hence cannot be activated directly) were only slightly compromised (as compared with cells expressing full-length PDGFR α) in their ability to induce PVR (Lei, H. et al., 2009b). We conclude that modest activation of PDGFR α accomplished by vitreal agents that are outside of the PDGF family was sufficient to drive PVR. Ongoing studies are focused on elucidation of the signaling events that an indirectly activated PDGFR α transmits in order to induce PVR.

Emerging therapeutic opportunities

One of the translational benefits of elucidating signaling events required for disease progression is that it identifies potential therapeutic targets. This is especially desirable for disease such as PVR, for which there are no pharmacological treatment options (Charteris, D.G., 1998). Our work thus far provides guidance in this regard. While vitreal PDGFRs are not likely to be a good therapeutic target, the non-PDGFRs may be worth considering. While vitreous contains a large number of non-PDGFRs, and all of those that have been tested are able to indirectly activate PDGFR α , they do not do so with equal potency (Lei, H. et al., 2009b). Identifying those that activate PDGFR α best, and then testing if simultaneously neutralizing them prevents experimental PVR is an ongoing effort in the lab.

Kinases are a readily druggable target, and a large number of kinase inhibitors are at various stages of development for a wide variety of diseases. SFKs are required for indirect activation of PDGFR, and hence drugs capable of inhibiting SFKs have the potential to prevent PVR. Since the kinase activity of the PDGFR is required for experimental PVR, agents that selectively inhibit PDGFRs kinase are also of interest. Many of the available inhibitors are not selective for a single kinase; perhaps one could leverage this feature of kinase inhibitors by choosing those that inhibited both SFKs and PDGFRs.

It seems likely that more therapeutic targets will emerge as we gain additional insights into the signaling events by which PDGFR α instructs cells to undergo the necessary cellular events that culminate in PVR. For instance, while kinase inactive PDGFR α is unable to drive PVR, it does undergo phosphorylation in response to vitreal non-PDGFRs. These findings suggest that there are two components to triggering the PDGFR α in the context of PVR. The first involves vitreal non-PDGFRs that increase phosphorylation of PDGFR α . The second is a consequence of this phosphorylation and dependent on the receptor's kinase activity. For instance, phosphorylation of PDGFR α enables it to stably associate with potent signaling enzymes such as PI3K (Rosenkranz, S. and Kazlauskas, A., 1999), which are only partially activated by associating with the PDGFR. Additional events, such as activation/association with Ras, are

necessary and dependent on the kinase activity of the PDGFR (Rodriguez-Viciano, P. et al., 1994; Rodriguez-Viciano, P. et al., 1996). Thus signal events that are downstream of the activated PDGFR are likely to constitute additional therapeutic targets.

In light of the fact that SFKs contribute to a wide spectrum of cellular responses, would the side effects of an inhibitor of SFKs, even one that is absolutely specific for SFKs, be tolerable? Rapamycin, which targets mTORC1 (mammalian target of rapamycin complex 1), an enzyme that appears to regulate at least as many cellular functions as SFKs, is well tolerated when administered systemically. Thus agents directed against broadly acting signaling agents are not necessarily doomed. Furthermore, anti-PVR drugs could be administered vitreally and thereby greatly reduce systemic exposure and the severity of side effects.

Because indirect activation of PDGFR α required an increase in the level of ROS, we considered if antioxidants could protect rabbits from PVR. Of the many possible antioxidant choices, we selected N-acetyl cysteine (NAC) because it is currently used in many clinical setting and is well tolerated. Furthermore, NAC effectively prevented indirect activation of PDGFR α (Lei, H. and Kazlauskas, A., 2009) and a number of cellular responses intrinsic to PVR at a concentration that was below the dose that induced overt retinal toxicity (Lei, H. et al., 2009a). Three vitreal injections of NAC prevented rabbits from undergoing retinal detachment, the sight-threatening phase of PVR (Lei, H. et al., 2009a). These initial studies indicate that antioxidant-directed approaches have the potential to protect rabbits from developing PVR.

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Figure 1. The growth factor/cytokine hypothesis for how PVR develops

The break in the retinal exposes cells within the retina and beneath it to the various agents within vitreous. These agents include growth factors and cytokines that promote cellular responses intrinsic to PVR such as migration, survival, proliferation and extracellular matrix production. The consequence of such cellular responses is the formation of an epiretinal membrane. Growth factors and cytokines within vitreous can stimulate cells within this epiretinal membrane to contract and thereby detach the retina.

Figure 2. PDGF-C is secreted in a latent state

PDGF-C is secreted in a biologically inactive (latent) form. Proteolytic cleavage of the CUB domain permits the core domain to bind and activate PDGFRs. The diagram shows an autocrine relationship between cells that produce and respond to PDGF; this appears to be the case for mesenchymal cells (Fredriksson, L. et al., 2004b; Gilbertson, D.G. et al., 2001). PDGF-C is also likely to activate cells in a paracrine manner.

Figure 3. The PDGF/PDGFR family

There are two receptor subunits, and they are either homo-, or heterodimerized by PDGF to form a functional PDGFR. PDGF-B is the universal ligand. PDGF-AB and PDGF-C assemble and activate both PDGFR α and PDGFR $\alpha\beta$. PDGF-D activates PDGFR β , and under certain circumstances PDGFR $\alpha\beta$. PDGF-A is the most selective member of the PDGF family and activates PDGFR α exclusively. There is no evidence that PDGF-AC, PDGF-BC, PDGF-AD, PDGF-BD or PDGF-CD exist.