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PDGF Receptors Are Activated in Human Epiretinal Membranes

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Abstract

Previous investigators reported that epiretinal membranes isolated from patients with proliferative vitreoretinopathy (PVR) express various platelet-derived growth factor (PDGF) family members and PDGF receptors (PDGFRs) (Cui, J.Z. et al., 2007; Robbins, S.G. et al., 1994). Co-expression of ligand and receptor raises the possibility of an autocrine loop, which could be of importance in the pathogenesis of PVR. To begin to address this issue we determined whether the PDGFRs in epiretinal membranes isolated from PVR patient donors were activated. Indeed, immunohistochemical staining (using pan- and phospho-PDGFR antibodies) revealed that both PDGFR subunits were activated. Quantification of these data demonstrated that a greater percentage of cells expressed the PDGFR α subunit as compared with the β subunit (44 $\pm$ 13% versus 32 $\pm$ 6.5%). Staining with phospho-PDGFR antibodies indicated that 36 $\pm$ 10% of the PDGFR α subunits were activated, whereas only 16 $\pm$ 5.5% of the PDGFR β subunits were activated. Thus, a 2.25 fold greater percentage of the PDGFR α subunits were activated. Co-staining with diagnostic cell-type antibodies indicated that both retinal pigment epithelial and glial cells expressed activated PDGFR α subunits. These findings support the recent discovery that PDGF-C is the major vitreal isoform because PDGF-C is 3 times more likely to activate a PDGFR α subunit as compared with a PDGFR β subunit. We conclude that PDGFRs are activated in epiretinal membranes of patients with PVR, and that the profile of active PDGFR subunits functionally supports the idea that PDGF-C is the predominant PDGF isoform present in the vitreous of patients with PVR. These findings identify PDGF-A, -AB and C as the best therapeutic targets within the PDGF family.

1. Introduction

While the vast majority of surgeries to re-attach a detached retina are successful, 5–10% of such patients develop proliferative vitreoretinopathy (PVR) (Glaser, B.M. et al., 1987; Laqua, H. and Machemer, R., 1975; Ryan, S.J., 1993). PVR is characterized by the growth and contraction of cellular membranes on both inner and outer surfaces of the retina. The PVR membrane consists of extracellular matrix proteins and cells originating from retinal pigment epithelium (RPE), retinal glial cells, fibroblasts and inflammatory macrophages (Campochiaro, P.A., 1997). PVR remains a major obstacle to improving the long-term outcome of retinal

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detachment surgery. Aside from surgical intervention to relieve the vitreoretinal traction, there are no effective treatment options (Charteris, D.G., 1998).

There is both indirect and direct evidence supporting the idea that growth factors play a key role in PVR. Indirect evidence includes the fact that growth factors promote cellular responses that are integral to PVR such as proliferation, migration and contraction. In addition, growth factors accumulate in the vitreous of PVR patients, and the cells within the PVR membrane secrete and/or respond to these growth factors (Campochiaro, P.A. et al., 1994; Campochiaro, P.A. et al., 1989; Carrington, L. et al., 2000; Cassidy, L. et al., 1998; Chen, Y.S. et al., 1997; Choudhury, P. et al., 1997; Cui, J.Z. et al., 2007; Elner, S.G. et al., 1995; Hinton, D.R. et al., 2002; Lashkari, K. et al., 1999; Lei, H. et al., 2007; Leschey, K.H. et al., 1990; Liang, Y. et al., 2002; Robbins, S.G. et al., 1994). The direct support comes from work in animal models of PVR. Immortalized mouse embryo fibroblasts fail to effectively induce PVR unless they express receptors for platelet-derived growth factor (PDGF) (Andrews, A. et al., 1999). Systematic comparison of cells harboring different PDGF receptors (PDGFRs) revealed that cells expressing the PDGFR α subunit induced PVR, whereas PDGFR β subunit-expressing cells did not. These studies reveal that expression of PDGFR α subunit is essential for experimental PVR.

In the PDGF family, the products of four distinct genes assemble into five dimeric isoforms: PDGF-A, -AB, -B, -C, and -D (Fredriksson, L. et al., 2004a; Reigstad, L.J. et al., 2005). PDGF-A, -AB and -B, undergo intracellular processing and activation during transport in the exocytic pathway, while the novel PDGFs, PDGF-C and PDGF-D, are secreted in a latent state that requires activation by extracellular proteases. Both plasmin and tissue plasminogen activator protein (tPA) are capable of producing active PDGF-C (Fredriksson, L. et al., 2004b; Lei, H. et al., 2007; Lei, H. et al., 2008; Li, X. et al., 2000). The proteases known to process PDGF-D are plasmin and urokinase plasminogen activator (Bergsten, E. et al., 2001; Reigstad, L.J. et al., 2005; Ustach, C.V. and Kim, H.R., 2005).

A recent evaluation of PDGF isoforms that are present in the vitreous from human donors indicated that PDGF-C was the predominant isoform (Lei, H. et al., 2007). Furthermore, proteases that process PDGF-C were present, and plasmin accounted for the majority of this activity (Lei, H. et al., 2008). PDGF-C activates PDGFR α subunits (in the context of α subunit homodimers, or α/β subunit heterodimers) and PDGFR β subunits (in the context of α and β subunit heterodimers) (Fredriksson, L. et al., 2004a; Reigstad, L.J. et al., 2005). To be able to evaluate the relevance of the observation that PDGF-C was present in the vitreous of patients with PVR, we determined if PDGFRs were activated in the membranes of PVR donors. Our findings revealed that both PDGFR subunits were activated in membranes isolated from human donors. Furthermore, a greater percentage of PDGFR α subunits were activated than PDGFR β subunits, which functionally corroborates the previous studies showing that PDGF-C is the predominant PDGF isoform in the vitreous of patients with PVR.

2. Materials and methods

2.1 Patient samples

Twenty seven PVR membranes were obtained from patients undergoing vitreoretinal surgery for PVR. All cases were early stage PVR as indicated by the presence of a vitreous haze and vitreous pigment clumps, wrinkling of the inner retinal surface, rolled edges of retinal breaks, retinal stiffness and vascular tortuosity (1983 Retinal Society classification). The University of British Columbia (UBC) Clinical Research Ethics Board (CREB) approved the study protocol. The UBC CREB policies comply with the Tri Council Policy and the Good Clinical Practice Guidelines, which have their origins in the ethical principles in the Declaration of Helsinki. Written informed consent was obtained from all patients.

2.2 Tissue preparation

Membranes were set in Optimal Cutting Temperature compound (OCT) (Tissue-Tek; Torrance, CA, USA) and stored at -80°C . Before sectioning the membranes, each block was allowed to warm to -20°C and then maintained at this temperature throughout sectioning. The membranes were sequentially cut using a Frigocut 2800 N Cryostat (Reichert-Jung; Chicago, IL, USA) into 6 micron sections, and then mounted onto slides. The sections were fixed with acetone for 5 min at room temperature and stored at -20°C until immunohistochemical processing. Membranes were put into phosphate-buffered saline (PBS, pH 7.4) during the operation and snap frozen in liquid nitrogen for mRNA extraction.

2.3 RT-PCR

PVR membranes from human donors were obtained as described above; a total of 6 membranes were analyzed by PCR. The human retinal pigment epithelial cell line ARPE-19 (19) was purchased from American Type Culture Collection (ATCC, Manassas, VA), and was engineered by stably expressing the human PDGFR α subunit subcloned into the pLHDCX² retroviral vector as previously described (Andrews, A. et al., 1999). The resulting cell line was designated 19 α ; expression of the PDGFR α subunit was confirmed by Western blot analysis (Lei, H. et al., 2007). Total RNA was extracted using the RNeasy total RNA isolation system (Qiagen, Madison, WI). The structural integrity of the RNA was confirmed by electrophoresis on a 1% agarose gel that was subsequently staining with 0.5 $\mu\text{mol/mL}$ ethidium bromide. RNA samples were treated with RNase-free DNase (Qiagen, Madison, WI) for 35 minutes at 37°C to remove traces of contaminating genomic DNA. Equal quantities of the resulting RNA were converted to DNA and subjected to 30 cycles of PCR using a OneStep RT-PCR kit (Qiagen, Valencia, CA). PCR products were resolved by on a 1% agarose gels and subsequently stained with 0.5 $\mu\text{mol/mL}$ ethidium bromide. A photograph of a representative gel is shown in Fig 1. The following primer pairs were synthesized by MWG Biotech (High Point, NC): human PDGFR α subunit (330–353, sense): GTCTGAAGAA GAGAGCTCCGATG, (1020–1000, antisense): GCACATTCGTAATCTCCACTG; human PDGFR β subunit (697–718, sense): CAGGATGGCACCTTCTCCAGC, (1492–1471, antisense): CTCGAACACTACCTGCAGTGTC; human actin- β (68–90, sense): CTCACCATGGATGATGATATCGC, (723–670, antisense): GCTTCTCCTTAATGTCACGC.

2.4 Immunohistochemical staining

Single or double immunohistochemical staining was performed with the following antibodies: pan-PDGFR α subunit, phospho-PDGFR α subunit, pan-PDGFR β subunit, and phospho-PDGFR β subunit. A list of antibodies used in this study is provided in Table 1. A detailed description of the immunohistochemical staining procedure has been recently published (Cui, J.Z. et al., 2007). Briefly, the slides were removed from -20°C and left to dry at room temperature for 20 min. Each section was circled with a Pap pen (Daido Sangyo; Tokyo, Japan), the slides were washed in a mixture of fresh PBS and 0.1% Triton X-100 for 5 min, then treated with 0.3% hydrogen peroxide (to remove any endogenous peroxidases) for 15 min. The sections were incubated with 5% normal horse serum in 0.3% Triton X-100 (TX-100; Sigma Aldrich) PBS for 20 minutes to reduce non-specific staining. Sections were incubated in a solution containing one or two primary antibodies (pan-PDGFR α subunit or phospho-PDGFR α subunit) for 1 hour. In the case of double immunohistochemistry, the first antibody was against pan-PDGFR β subunit and the second was an anti-phospho-PDGFR β subunit antibody. Antibody dilutions (into PBS) are listed in Table 1. In the negative control experiments either one, or both, primary antibodies were omitted.

The following procedure was used to determine the fraction of RPE and glial cells that expressed activated PDGFR α subunits. Sections were first stained rabbit anti-human pan-

PDGFR α subunit or rabbit anti-human phospho-PDGFR α subunit, and then either mouse anti-pan cytokeratin (Sigma-Aldrich, St Louis, MO, USA) or mouse anti-glial fibrillary acidic protein (GFAP) (Sigma-Aldrich, St Louis, MO, USA). The anti-pan cytokeratin antibody was a mixture of monoclonal anti-cytokeratin antibodies that recognize human cytokeratins 1, 4, 5, 6, 8, 10, 13, 18, and 19. This mixture recognizes a wide variety of normal, reactive and neoplastic epithelial tissues, cornifying and noncornifying squamous epithelia and pseudostratified epithelia. It does not react with nonepithelial normal human tissues.

After incubating with primary antibodies, the slides were washed three times for 15 min with fresh PBS. This was followed by a 30 min incubation with fluorescently labeled secondary antibodies, which were diluted with PBS. The secondary antibodies (used at 1:400) were anti-rabbit Alexa-488 (Molecular Probes; Eugene, OR, USA), anti-mouse Alexa-488 (Molecular Probes; Eugene, OR, USA), anti-rabbit Alexa-534 (Molecular Probes; Eugene, OR, USA), and anti-mouse Cy3 (Jackson ImmunoResearch, West Grove, PA). The slides were washed three times for 15 min with fresh PBS. To preserve the luminosity of the secondary antibodies, the slides were mounted with SlowFade (Molecular Probes; Eugene, ON), and then covered with No. 1.5 cover slips.

2.5 Data analysis

Each section was analyzed using a confocal laser-scanning microscope (Zeiss-LSM 510 META). The gain was adjusted accordingly for each wavelength to ensure that minimal cross talk. Absence of cross talk in double stained tissue was verified by analysis of negative controls in which one or both primary antibodies were absent. For each tissue three independent staining and analysis was conducted. The analysis was conducted by examination of five random fields per tissue section and the average score per field determined. Classification of staining was based on the level of observed fluorescence. Five random fields of the sections were imaged at 40X magnification. Each random field was centered over the tissue in a way that avoided the edges of the tissue specimens. The fields were scanned with laser wavelengths of 488 and 534 nm. When exposed to these wavelengths, Alexa 488 emits in the green wavelength, while Alexa 534 emits in the red wavelength. Several sections from each membrane were also scanned for transmitted images to allow bright-field visualization of the cellular content, endogenous pigment, and to compare with immunohistochemical staining. Some areas of tissue contained dense pigment deposits, which were not analyzed due to strong autofluorescence. The immunostaining was graded based on the relative intensity of immunostaining for each marker. The average of five fields per tissue section was determined.

The number of cells that expressed PDGFRs was calculated by counting the total numbers of nuclei (blue staining) in the vicinity of red staining (positive for pan PDGFR). The number of nuclei in the vicinity of green staining (positive for phospho-PDGFR) was counted to get the number of cells harboring activated PDGFR subunits. The ratio of these two numbers $\times 100$ is the percentage of PDGFR-positive cells that have activated PDGFR subunits. The same approach was used for both PDGFR subunits. Five random fields (that excluded the edges of the sample) were counted for each specimen. The data presented in Fig 4B and 5B are the mean $\pm$ standard deviation. A Student t-test; one-tail test, was used for statistical analysis of the data shown in Figs 4B and 5D.

3. Results

We previously reported that PDGF-C was the predominant PDGF isoform in the vitreous of patients with PVR (Lei, H. et al., 2007). In order to investigate if this isoform was responsible for activating PDGFRs within the PVR membrane, we tested which of the PDGFR subunits were activated. As shown in Table 2, the profile of activated PDGFR reflects the presence of PDGF isoforms, and can be used to evaluate what types of PDGF family members are present.

Activation of the PDGFR involves tyrosine phosphorylation of the intracellular domain of the receptor at up to 13 tyrosine residues (Rikova, K. et al., 2007).

Phosphospecific antibodies have been raised to some of the sites and can be used to monitor the activation state of the PDGFR (Bernard, A. and Kazlauskas, A., 1999). The predominance of PDGF-C in the vitreous of patients with PVR predicts that cells will have activated PDGFR α subunits or both PDGFR α and β subunits, but never only activated PDGFR β subunits (Table 2).

The first step in determining if the PDGFRs were activated in membranes from patients with PVR was to confirm that the receptors were expressed. We used two complementary approaches to address this issue, 1) RT PCR, and 2) immunohistochemistry using antibodies specific for PDGFRs. Fig 1 shows that the mRNA for both PDGFR subunits was readily detected in all 6 membranes that were analyzed. The second approach was to evaluate protein expression of the two PDGFR subunits. Fig 2A is a representative experiment and reveals that PDGFR α subunit was readily detectable in PVR membranes. Despite the fact that immunofluorescence is often only semi-quantitative, we quantitated the data obtained from 14 membranes and thereby learned that an average of 44 $\pm$ 13% of the cells were positive for PDGFR α subunit (Fig 4B). Similarly, the PDGFR β subunit was detected (Fig 3A) and an average of 32 $\pm$ 6.5% of the cells were positive for PDGFR β subunit (Fig 4B). These findings indicate that PDGFRs were expressed in PVR membranes, and that approximately one third of the cells in PVR membrane expressed at least one of the PDGFR subunits.

Staining the PVR membranes with phosphospecific PDGFR antibodies revealed that both PDGFR subunits were activated (Fig 2B and 3B). 2.25 fold more of the PDGFR α subunit expressing cells had activated subunits as compared with the PDGFR β subunit-positive cells (Fig 4B); this difference was statistically significant ($P < 0.005$). A caveat intrinsic to these conclusions is the semi-quantitative nature of immunofluorescence. Even so, these data support the observation that PDGF-C is the predominant PDGF isoform, as this isoform would activate 3 times more α subunits than β subunits because it assembles PDGFRs that consist of α subunit homodimers and $\alpha\beta$ heterodimers.

We also evaluated which cell types expressed the activated PDGFR α subunit. Fig 5A and B show representative data from 7 membranes that were analyzed; both RPE and glial cells were positive for activated PDGFR α subunit. Quantification of the data indicated that RPE cells expressed a greater percentage of activated PDGFR α subunit as compared with glial cells (Fig 5C and D). This difference was statistically significant ($p < 0.005$).

4. Discussion

Analysis of epiretinal membranes from patients with PVR revealed that both PDGFR subunits were expressed and activated. The PDGFR α subunit was expressed more frequently than the PDGFR β subunit, and a greater fraction of the PDGFR α subunits were activated. Furthermore, both RPE and glial cells expressed activated PDGFR α subunit. These findings extend the previous report that PDGFRs were expressed in epiretinal membranes (Robbins, S.G. et al., 1994; Vinoses, S.A. et al., 1995), and corroborate the finding that the predominant vitreal isoform is PDGF-C (Lei, H. et al., 2007), a family member that is up to 3 time more likely to activate PDGFR α subunits as compared with PDGFR β subunits (Fredriksson, L. et al., 2004a; Reigstad, L.J. et al., 2003).

PDGF-C is one of the PDGF family members that is secreted in a latent form and must undergo proteolytic processing to be activated (Fredriksson, L. et al., 2004a; Lei, H. and Kazlauskas, A., 2008; Reigstad, L.J. et al., 2003). Curiously, the vast majority of PDGF-C in the vitreous of patients with PVR is in the latent form (Lei, H. et al., 2007). While the proteases that activate

PDGF-C (tissue plasminogen activator and plasmin) are also present in the vitreous (Lei, H. et al., 2007; Lei, H. and Kazlauskas, A., 2008; Lei, H. et al., 2008), the active form of PDGF-C is much less stable than the latent form (Lei, H. et al., 2007). Thus even though the majority of the vitreal PDGF-C is in the latent form, it appears to be constitutively converted to the active form, which is rapidly degraded.

A conceptual (the appreciation that tyrosine phosphorylation is intrinsic to activation of growth factor receptors) and a technical advance (the development of phosphospecific antibodies) has ushered in a functional approach to determining which PDGF family members are contributing to PVR. Examining a series of PVR membranes revealed that the PDGFR α subunit was activated 2.25 fold more often than the PDGFR β subunit. This finding supports our previous report that PDGF-C was the predominant PDGF family member. While other groups have reported additional PDGF isoforms in the vitreous of patients with PVR (Cassidy, L. et al., 1998), the level of PDGF-C was at least 300 fold higher than the other PDGF family members (Lei, H. et al., 2007). Taken together, the ligand expression level and profile of activated PDGFR subunits strongly suggest that PDGF-C is the PDGF family member responsible for PDGF-dependent activation of PDGFRs in membranes of patients with PVR. It is important to note that while our data reveal the relative amounts of the two PDGFR subunits, they do not address the capacity of the two receptors for driving PVR.

Most patients with PVR have a high level of vitreal PDGF-C, whereas PDGF-C is undetectable in the vitreous of the majority of patients with retinal issue unrelated to PVR (Lei, H. et al., 2007). While these findings establish a tight association between a high vitreal level of PDGF-C and PVR, they do not rule out the involvement of other growth factors. There are many growth factors outside of the PDGF family that are also associated with PVR. This list includes 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), fibroblast growth factors (FGFs), insulin, insulin-like growth factor-1 (IGF-1) and connective tissue growth factor (CTGF) (Campochiaro, P.A. et al., 1996; Cui, J.Z. et al., 2007; Hinton, D.R. et al., 2002; Lashkari, K. et al., 1999; Lei, H. et al., 2007). In addition, several cytokines can be found in the vitreous of PVR patients and/or in the epiretinal membrane, and these include IL-1, IL-6, monocyte chemotactic protein, macrophage-colony stimulating factor (Campochiaro, P.A. et al., 1996; Choudhury, P. et al., 1997; Elner, S.G. et al., 1995). Determining the relative contribution of the various vitreal growth factors remains a pressing question in PVR research because those growth factors/cytokines that induce PVR are ideal therapeutic targets.

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Figure 1. RT PCR analysis of PVR membranes

Total RNA was extracted and subjected to RT-PCR as described in Materials and Methods. Each lane of the gel was loaded with the PCR products from a single membrane. All 6 of the PVR membranes were positive for both PDGFR subunits; the β -actin control indicates that the same amount DNA was generated in the reverse transcriptase step of the procedure. "H₂O" was a negative control in which distilled water was added instead of RNA. "19 α " was the positive control for these experiments and consisted of RNA isolated from ARPE-19 cells that had been engineered to express both PDGFR subunits. Similar results were observed in 3 independent experiments.

Figure 2. Expression of the PDGFR α subunit in PVR membranes

A representative immunohistochemical analysis of PVR membranes stained with an anti-pan PDGFR α subunit antibody (A) or a phospho-PDGFR α subunit antibody (B). The sections are serial; the primary antibodies were raised in the same species and hence it was not possible to stain the same section with both antibodies as was done in Fig 3. Fourteen membranes were analyzed; all were strongly positive for pan-PDGFR α subunit seen in red (A) and moderately positive for phospho-PDGFR α subunit staining seen in green (B).

Figure 3. Expression of PDGFR β subunits in PVR membranes

A representative immunohistochemical analysis of PVR membranes stained with an anti-pan PDGFR β subunit antibody (A) or a phospho-PDGFR β subunit antibody (B). Panel C is a merge of panels A and B, and shows colocalization of the pan- and phospho-PDGFR β subunit signal (arrows). Fourteen membranes were analyzed; all were strongly positive for pan-PDGFR β subunit seen in red (A) and moderately positive for phospho-PDGFR β subunit staining seen in green (B).

	α subunit	β subunit
% of cells that express PDGFRs	44 +/- 13%	32 +/- 6.5%
% of cells with activated PDGFRs	36 +/- 10%	16 +/- 5.5%

Ratio of activated α subunit/activated β subunit is 2.25:1

Figure 4. Summary of PDGFR subunit expression and activation in early stage PVR membranes
 The data from all PVR membranes analyzed were pooled and graphed (A) or presented in tabular form (B). The data show that more of the cells in the epiretinal membranes expressed the PDGFR α subunit, and a greater percentage of these receptors were activated. *Statistical analysis (Student's t-test; one tail test) indicated that the difference in percentage of cells expressing an activated PDGFR α subunit versus activated PDGFR β subunit was statistically significant ($p < 0.005$).

	RPE cells	Glial cells
% that express pan-PDGFR α subunit	51 +/- 8.3%	29 +/- 6.2%
% that express phospho-PDGFR α subunit	45 +/- 9.2%	22 +/- 5.4%

Ratio of activated PDGFR α subunit in RPE/activated PDGFR α subunit in glial cells is 2:1

Figure 5. Expression of activated PDGFR α subunit in RPE and glial cells

A representative immunohistochemical analysis of PVR membranes co-stained with an anti-phospho PDGFR α subunit antibody (green) and an anti-cytokeratin (A) or an anti-GFAP (B) antibody (red); the nuclei are blue. Yellow indicates co-expression. Seven membranes were analyzed as shown in panels A and B, and also stained with a pan-PDGFR α subunit antibody. Arrows indicate colocalization, whereas as arrowheads mark cells stained with only one of the two antibodies. The resulting data were pooled and are shown in a graph and table (C and D, respectively). The results show that both RPE and glial cells expressed PDGFR α subunits, and that there is a two-fold greater percentage of activated PDGFR α subunits in RPE cells. This difference was statistically significant ($p < 0.005$).

Table 1

Antibodies used for immunohistochemistry

Primary Antibody	Concentration	Supplier
Pan-PDGFR α subunit, Cat# 3164 Detects total PDGFR α subunit; rabbit polyclonal antibody.	1:100	Cell Signaling
Phospho-PDGFR α subunit, Cat# sc-12910R Detects PDGFR α subunit when phosphorylated at tyr 720; rabbit polyclonal antibody.	1:100	Santa Cruz Inc.
Pan-PDGFR β subunit Detects total PDGFR β subunit; rabbit polyclonal antibody.	1:100	Kazlauskas lab (Kazlauskas, A. and Cooper, J.A., 1990)
Phospho-PDGFR β subunit, Cat# 3166 Detects PDGFR β subunit when phosphorylated at tyr 751; mouse monoclonal antibody.	1:100	Cell Signaling
A mixture of monoclonal Pan-Cytokeratin, Cat# C2562	1:200	Sigma
Monoclonal GFAP, Clone G-A-5, ascites fluid, Cat# G3893	1:200	Sigma

Table 2

Preference of PDGF isoforms for activating PDGFR subunits

PDGF isoform	PDGFR subunits that are activated
PDGF-A	α only
PDGF-AB or C	α and β ; α alone or together with β ; never β alone
PDGF-B	both subunits, all possible combinations
PDGF-D	β only; one report indicates the PDGF-D also activates α in the context of an $\alpha\beta$ heterodimer (LaRochelle, W.J. et al., 2001)

Because PDGF-AB and C have the same preference for PDGFR subunits, these two isoforms will induce the same pattern of activation. Note that the presence of multiple PDGF isoforms (especially PDGF-B) precludes extrapolating from the PDGFR subunit activation pattern to the PDGF isoforms that are present.