Effect of platelet-derived growth factor isosforms in rat metanephric mesenchymal cells

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Ricono, Jill M., Mazen Arar, Goutam Ghosh Choudhury, and Hanna E. Abboud. Effect of platelet-derived growth factor (PDGF) B-chain or PDGF β-receptor-deficient mice lack mesangial cells. To explore potential mechanisms for failure of PDGF A-chain to rescue mesangial cell phenotype, we investigated the biological effects and signaling pathways of PDGF AA and PDGF BB in mesangial cell population of these cells potentially develop into mesangial cells. However, the failure of PDGF A-chain to rescue mesangial cell phenotype in PDGF receptor-deficient mice lack mesangial cells (16, 21). PDGF is widely expressed in a variety of mesangial cells during development. Siefert et al. (18) mapped the expression patterns of PDGF ligands and receptors in the developing and adult murine kidney using in situ hybridization (Fig. 1). During glomerular development, as the renal vesicle epithelium progresses through the comma- and S-shape stages, PDGF A-chain and B-chains are expressed in epithelial cells. PDGF A-chain is expressed earlier and is seen even at the renal vesicle stage, whereas PDGF B-chain expression occurs at later stages and, at the earliest, is seen in the S-shaped bodies of the developing glomerular structures. PDGFR-α and PDGFR-β are expressed by mesenchymal cells in the metanephric blastema. At later stages, PDGFR-α is expressed in cells surrounding the glomerulus, and PDGFR-β transcripts are present in the mesenchymal/interstitial cells that are recruited into the glomerular cleft, which will form the vascular tuft of the mature glomerulus (18). These cells express PDGF-β and PDGF B-chain transcripts at high levels during the later stages of glomerular development when microvascular (capillary) cells begin to fill the glomerular tuft. At this stage, PDGFR-α transcripts are barely detectable and PDGF A-chain transcripts are undetectable. Utilizing both immunohistochemistry and in situ hybridization, Arar et al. (2) showed similar findings in the rat, with PDGFR-β localizing to metanephric mesenchymal (MM) cells at early stages of development, cells within the cleft of the S-shaped bodies of the maturing glomerulus, and, at later stages, in the mature glomerulus. This study also demonstrated that activation of PDGFR-β by PDGF BB isoform mediates MM cell migration and DNA synthesis, providing one mechanism by which a subpopulation of these cells potentially develop into mesangial cells. However, the failure of PDGFR-α to compensate for the lack of β-receptor in the PDGFR-β-deficient mice remains unexplained, considering that the α-receptor, similar to the β-receptor, localizes to mesenchymal cells in the metanephric blastema, and

there is evidence that glomerular microvascular cells arise from metanephric mesenchyme (14). The spatial and temporal distribution of platelet-derived growth factor (PDGF) and its receptors (PDGFR) suggest a role for this growth factor in the development of mesangial cells (18) and have been conclusively demonstrated in two studies where PDGF B-chain or PDGFR-β-deficient mice lack mesangial cells (16, 21). PDGF is widely expressed in a variety of mesangial cells during development. Siefert et al. (18) mapped the expression patterns of PDGF ligands and receptors in the developing and adult murine kidney using in situ hybridization (Fig. 1). During glomerular development, as the renal vesicle epithelium progresses through the comma- and S-shape stages, PDGF A-chain and B-chains are expressed in epithelial cells. PDGF A-chain is expressed earlier and is seen even at the renal vesicle stage, whereas PDGF B-chain expression occurs at later stages and, at the earliest, is seen in the S-shaped bodies of the developing glomerular structures. PDGFR-α and PDGFR-β are expressed by mesenchymal cells in the metanephric blastema. At later stages, PDGFR-α is expressed in cells surrounding the glomerulus, and PDGFR-β transcripts are present in the mesenchymal/interstitial cells that are recruited into the glomerular cleft, which will form the vascular tuft of the mature glomerulus (18). These cells express PDGF-β and PDGF B-chain transcripts at high levels during the later stages of glomerular development when microvascular (capillary) cells begin to fill the glomerular tuft. At this stage, PDGFR-α transcripts are barely detectable and PDGF A-chain transcripts are undetectable. Utilizing both immunohistochemistry and in situ hybridization, Arar et al. (2) showed similar findings in the rat, with PDGFR-β localizing to metanephric mesenchymal (MM) cells at early stages of development, cells within the cleft of the S-shaped bodies of the maturing glomerulus, and, at later stages, in the mature glomerulus. This study also demonstrated that activation of PDGFR-β by PDGF BB isoform mediates MM cell migration and DNA synthesis, providing one mechanism by which a subpopulation of these cells potentially develop into mesangial cells. However, the failure of PDGFR-α to compensate for the lack of β-receptor in the PDGFR-β-deficient mice remains unexplained, considering that the α-receptor, similar to the β-receptor, localizes to mesenchymal cells in the metanephric blastema, and

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PDGF A-chain, similar to the B-chain, is also expressed in epithelial cells of the maturing glomerulus.

Binding of PDGF dimers to the extracellular domain of the receptor increases its intrinsic tyrosine kinase activity and provides docking sites for downstream signal transduction proteins (1, 9). Many of these tyrosines interact directly or indirectly with the SH2 domains of signaling molecules and are present in homologous positions with respect to PDGFR-α and PDGFR-β. Activation of PDGFR initiates several major signal transduction cascades, which include activation of phosphoinositid 3-kinase (PI 3-K), phospholipase C (PLC)1, and Ras-Raf-mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK- [extracellular signal-activated kinase (ERK)1/2] pathways (3, 5, 8). Expression of mutant PDGFR-β, the tyrosine residues of which are replaced with phenylalanine, demonstrated an essential role for PI 3-K and PLCγ1 in proliferation and chemotaxis in different cell types (6, 20, 22, 23). Additional studies demonstrated that these responses are cell-specific (12). Because PDGF B-chain and PDGFR-β appear to be essential for mesangial cell development, and signaling through the PDGFR-α does not seem to compensate for the loss of PDGFR-β signaling, we examined the biological effects of PDGF AA and PDGF BB on MM cells and the role of ERK1/2 and PI 3-K.

**MATERIALS AND METHODS**

**Materials.** Tissue culture materials were purchased from GIBCO BRL (Rockville, MD). Recombinant PDGF-AA and PDGF-BB were obtained from R&D Systems (Minneapolis, MN). Wortmannin and PD-098059 were purchased from Alexis (San Diego, CA). Myelin basic protein (MBP), phosphatidylinositol (PI), collagenase, and collagen type I were obtained from Sigma (St. Louis, MO). Primary antibodies to PDGFR-β (A-3) and PDGFR-α (951) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies conjugated to Cy3 or FITC were obtained from Chemicon (Temecula, CA). Protein measurement and polyacrylamide gel reagents were purchased from Bio-Rad (Herceles, CA). Anti-phosphotyrosine and Erk-1 polyclonal antibodies were also purchased from Santa Cruz Biotechnology. Protein A-Sepharose was obtained from Pierce (Rockford, IL). All other reagents were high-quality analytic grade.

**Cultured cells.** Primary MM cell cultures were prepared as previously described (2, 10). Briefly, pregnant Sprague-Dawley rats were purchased at 10–11 days of gestation. At gestational day 13, mothers were anesthetized by intramuscular injection of rat mixture (60% ketamine, 40% xylazine), and embryos were collected. The age of the embryo was counted from the day of the vaginal plug (day 0). Embryos were dissected in 1× phosphate-buffered saline under a zoom model SZH Olympus stereomicroscope. Embryonic kidneys were collected, and cells were propagated in Dulbecco’s modified Eagle medium (GIBCO) including 10% fetal calf serum and grown at 37°C, 5% carbon dioxide.

**MAPK assay.** MM cells were plated 7.5 × 10⁴ cells/60-mm dish, grown to confluency, and serum starved for 48 h. Re-
spective cells were pretreated with 50 μM PD-098059, an MEK inhibitor, for 45 min before being stimulated with PDGF-AA or PDGF-BB. Cells were lysed with RIPA buffer (20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Nonidet P-40, 1 mM Na3VO4, 1 mM polymethylsulfonyl fluoride, and 0.1% aprotinin) for 30 min at 4°C and centrifuged at 10,000 g for 20 min at 4°C. Protein concentrations were measured in the cell lysates. Equal amounts of protein (100 μg) were incubated with ERK-1 polyclonal antibody for 30 min on ice. Fifteen microliters of protein A-Sepharose beads (50% vol/vol slurry) were added and incubated at 4°C on a rocking platform for 2 h. The immunobeads were washed and resuspended in MAPK assay buffer (in mM: 10 HEPES, pH 7.4, 10 MgCl2, 0.5 dithiothreitol, and 0.5 Na3VO4) in the presence of 0.5 mg/ml MBP and 25 μM cold ATP plus 1 μCi [γ-32P]ATP. The mixture was incubated at 30°C for 30 min, followed by a 10-min incubation on ice. Protein-loading buffer was added, and reactions were boiled. Samples were then loaded on a 12.5% SDS-PAGE, and phosphorylated MBP was visualized by autoradiography (5).

Western blotting. Equal amounts of protein from cell lysates were separated on a 12.5% SDS-PAGE gel and electrophoretically transferred to polyvinyl membrane. The membrane was blocked with 5% nonfat milk prepared in Tris-buffered saline with 0.1% Tween 20 (TBST) buffer, washed with TBST, and incubated with ERK-1 primary antibody (1:200 dilution; Santa Cruz Biotechnology). The membrane was then washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG. The blot was developed with enhanced chemiluminesence reagent.

PI 3-K assay. MM cells were plated as above. Respective cells were pretreated with 250 nM wortmannin, a PI 3-K inhibitor, for 3 h before treatment with PDGF. Cells were lysed and protein was analyzed as previously mentioned. One hundred micrograms of protein were incubated with anti-phosphotyrosine antibody for 30 min on ice. Fifteen microliters of protein A-Sepharose beads (50% vol/vol slurry) were added and incubated at 4°C on a rocking platform for 2 h. The immunobeads were washed three times with RIPA, once with PBS, once with buffer A (0.5 mM LiCl, 0.1 M Tris·HCl, pH 7.5, and 1 mM Na3VO4), once with doubly distilled water, and once with buffer B (0.1 M NaCl, 0.5 mM EDTA, 20 mM Tris·HCl, pH 7.5). The immunobeads were then resuspended in 50 μl of PI 3-K assay buffer (20 mM Tris·HCl, pH 7.5, 0.1 M NaCl, and 0.5 mM EGTA). PI (0.5 μl of 20 mg/ml) was added and incubated at 25°C for 10 min. A cocktail of 1 μl of 1 M MgCl2 and 10 μCi [γ-32P]ATP was added and incubated at room temperature for 10 min. A mixture of chloroform, methanol, and 11.6 N HCl (150 μl, 50:100:1) was added to stop the reaction, and an additional 100 μl of chloroform were added. The organic layer is extracted and washed with methanol and 1 N HCl (1:1). The reaction was dried overnight and resuspended in 10 μl of chloroform. The samples were separated by thin-layer chromatography and developed with CHCl3/MeOH/28% NH4OH/H2O (129:114:15:21). The spots were visualized by autoradiography (5).

DNA synthesis. MM cells were plated at 7.5 × 103 cells/well dish, grown to confluency, and serum starved for 48 h. Cells were either pretreated with PD-098059 or wortmannin as previously described before stimulation with PDGF isoforms. [3H]TdR (1 μCi/25 ml) was added to each well. DNA synthesis is measured as the incorporation of [3H]thymidine into trichloroacetic acid-insoluble material (7).

Cell migration assay. Cell migration in response to PDGF was determined using blind well chamber assays. Confluent MM cells were serum starved for 48 h and then pretreated with PD-098059, wortmannin, or LY-294002. The monolayer of cells were trypsinized and resuspended in serum-free media. The cell suspension was added to the top chamber, while the PDGF was added to the bottom chamber of the apparatus. A polycarbonate membrane filter coated with collagen I separated the chambers. After 4 h at 37°C, the cells on the upper surface of the filter were removed with a cotton-tipped applicator, and migratory cells on the lower surface of the filter were fixed in methanol and stained with Giemsa. With the use of high magnification (×450), the migration of cells was analyzed by counting the number of cells that had migrated through the polycarbonate filter (7).

Immunofluorescence. For PDGFR-α and -β double immunofluorescent staining, cells were grown to near confluency on eight-well coverslips. Cells were fixed in methanol and washed in 1× PBS with 0.1% BSA. The primary antibody (PDGFR-β) 1:20 was added, and coverslips were incubated in a humidifier for 30 min at room temperature. Cells were washed three times for 5 min each. The respective secondary antibody (donkey anti-mouse, Cy3 conjugated) 1:30 was added, and coverslips were incubated in a humidifier for an additional 30 min at room temperature. The respective secondary antibody (donkey anti-rabbit, FITC conjugated) 1:20 was added, and coverslips were incubated in a humidifier for an additional 30 min at room temperature. Cells were washed three times for 5 min each and mounted with crystal mounts. Cells were viewed with respective fluorescent filters with ultraviolet light.

RESULTS

Effect of PDGF isoforms on MM cell DNA synthesis and migration. PDGF AA and PDGF BB were examined for their ability to stimulate DNA synthesis as measured by [3H]thymidine incorporation into DNA of quiescent MM cells. When cells were treated with PDGF BB for 24 h, [3H]thymidine incorporation increased nearly fivefold above basal levels at concentrations of 10 and 100 ng/ml of PDGF BB. However, similar concentrations of PDGF AA did not increase DNA synthesis above basal levels (Fig. 2A). In addition to proliferation, migration is an important biological response during organ development. MM cells from the same passage used for the [3H]thymidine incorporation were used for the migration assays. PDGF BB induced migration of MM cells four- to fivefold above baseline, with a maximal effect seen at a dose of 10 ng/ml. PDGF AA also induced migration in the MM cells about twofold above basal levels at a dose of both 10 and 100 ng/ml; however, the response was much weaker than that of PDGF BB (Fig. 2B).

PDGF activation of MAPK in MM cells. Activated PDGFR-β is known to associate with Grb2/sos, which lies upstream of the Ras-MEK-ERK signaling pathway. In contrast, activated PDGFR-α has been reported to associate with Crk adaptor protein, which may also lie upstream of the Ras-MEK-ERK signaling pathway. To determine whether MAPK (ERK1/2) is activated in MM cells, we measured the kinase activity in ERK1/2 immunoprecipitates of PDGF-stimulated MM cells. MM cells were stimulated with PDGF AA or PDGF BB for 5, 10, and 15 min. Both PDGF AA and
PDGF BB stimulated ERK1/2 activity in MM cells (Fig. 3). Maximal activation was observed at 15 min, and therefore in all subsequent experiments cells were treated for 15 min with the PDGF isoforms. Dose-responses of PDGF-induced ERK1/2 activity showed that PDGF AA induced maximal ERK1/2 activity at 50 and 100 ng/ml, whereas PDGF BB induced maximum activity at 10 ng/ml (Fig. 4). These concentrations correspond to those required to stimulate proliferation and migration (Fig. 2, A and B). In some experiments, cells were pretreated with an MEK inhibitor, PD-098059, and assayed for ERK1/2 activity (Fig. 5). PD-098059 reduced PDGF-induced ERK1/2 activity to near basal levels, indicating that ERK1/2 activation in MM cells is mediated by the Ras-Raf-MEK-MAPK pathway. Western blot analysis of the ERK1/2 protein was performed on the same cell lysates as for loading controls.

**Effect of MAPK inhibitor on PDGF-induced DNA synthesis and migration in MM cells.** To assess the involvement of MAPK signaling pathways in DNA synthesis and migration of the MM cells, cells were pretreated with 50 μM PD-098095 for 45 min before the addition of PDGF AA or PDGF BB. Pretreatment of cells with the MEK inhibitor abolished PDGF-induced DNA synthesis (Fig. 6A). This suggests that the Ras-Raf-MEK-MAPK pathway is a major contributor of PDGF-induced DNA synthesis in MM cells. Pretreatment of the MM cells with PD-098095 significantly...
decreased migration induced by PDGF BB (Fig. 6B). However, PD-098095 did not inhibit migration to basal levels as in PDGF-induced DNA synthesis (Fig. 6A). The same concentration of PD-098095 that completely blocked PDGF AA-induced ERK1/2 activity (Fig. 5) did not significantly reduce PDGF AA-induced migration. These data suggest that other pathways are involved in PDGF-induced migration.

**PDGF activation of PI 3-K.** PI 3-K has previously been shown to associate with tyrosine-phosphorylated PDGF receptors. However, it has not been established whether signaling by both PDGF isoforms through their respective receptors can activate PI 3-K in MM cells. PI 3-K activity was determined in anti-phospho-tyrosine immunoprecipitates of lysate from PDGF AA- or PDGF BB-stimulated cells. The immunoprecipitates were assayed for PI 3-K activity as described in MATERIALS AND METHODS. As shown in Fig. 7, both isoforms of PDGF activate PI 3-K activity. PDGF BB showed a significant effect at 10 ng/ml, whereas 100 ng/ml of PDGF AA was necessary to induce significant activation. When cells were pretreated with wortmannin, a PI 3-K inhibitor, the PDGF-associated PI 3-K activity in MM cells was significantly reduced (Fig. 8).

**Effect of PI 3-K inhibitor on PDGF-induced DNA synthesis and migration in MM cells.** We have recently shown that activation of PI 3-K is necessary for PDGF-induced DNA synthesis and migration in mesangial cells (4). To determine the importance of PI 3-K in MM cells, cells were pretreated with 250 nM wortmannin for 3 h before stimulation with PDGF isoforms. Wortmannin decreased PDGF-induced [3H]thymidine incorporation by ~20% (Fig. 9A), unlike the complete abolition of activity when cells were pretreated with PD-098059 (Fig. 6A). These data indicate that PI 3-K plays a lesser role in PDGF-induced DNA synthesis than MAPK in MM cells. Two structurally dissimilar PI 3-K inhibitors, wortmannin and LY-294002, completely blocked PDGF-induced migration in MM cells (Fig. 9, B and C), whereas the MEK inhibitor decreased PDGF BB-induced migration by ~30% and PDGF AA-induced migration by ~15% (Fig. 6B). These data suggest that PI 3-K, rather than MAPK, plays a predominant role in PDGF-induced migration.
Expression of PDGF receptors in MM cells. To study the expression of PDGFR-\(\alpha\) and -\(\beta\) in MM cells, immunofluorescence was performed using PDGFR-\(\alpha\)- and PDGFR-\(\beta\)-specific antibodies. Figure 10 shows abundant expression of both PDGFR-\(\alpha\) and PDGFR-\(\beta\) in MM cells. This suggests that these cells have the potential to respond to both PDGF isoforms.

**DISCUSSION**

There are several potential mechanisms by which PDGF, acting on MM cells, leads to the development of a subset of these cells into mature differentiated mesangial cell phenotype. Two mechanisms pertinent to development include cell migration and cell proliferation. This study explored the effects of PDGF AA and BB isoforms on DNA synthesis and migration of MM cells isolated at the earliest stage of the developing rat metanephrin blastema, embryonic day 13. Arar et al. (2) have recently demonstrated that PDGF BB stimulates DNA synthesis and migration of these cells. Stimulation of cell migration by PDGF BB was associated with activation of PI 3-K, and inhibition of PI 3-K blocked PDGF BB-induced migration. However, the role of other signal transduction pathways activated by PDGF signaling in metanephrin cells has not been fully elucidated.

**Fig. 6.** Effect of MEK inhibitor on PDGF-induced DNA synthesis and migration. Quiescent MM cells were pretreated with 50 \(\mu\)M PD-098059 for 45 min before treatment with PDGF AA or PDGF BB. 

A: \(^{3}H\)thymidine incorporation was measured as an index of DNA synthesis in response to treatment of 10 or 100 ng/ml of PDGF AA or BB in quiescent MM cells. Values are means ± SE of 4 independent experiments. \(**P < 0.01\) vs. untreated and treated with PD-098059. 

B: serum-deprived quiescent MM cells were used in cell migration assay in the presence of 10 or 100 ng/ml of PDGF AA or BB, as described in MATERIALS AND METHODS. Values are means ± SE of 3 independent experiments. \(**P < 0.01\) vs. untreated and treated with PD-098059.

**Fig. 7.** Activation of phosphatidylinositol 3-kinase (PI 3-K) in PDGF-treated MM cells. A: serum-deprived MM cells were treated with 10 or 100 ng/ml PDGF AA or PDGF BB for 15 min and cell lysates were immunoprecipitated with anti-phosphotyrosine antibody. The immunoprecipitates were then assayed for PI 3-K activity in the presence of phosphatidyl inositol (PI) and [\(\gamma\)-\(^{32}\)P]ATP as described in MATERIALS AND METHODS. The arrow indicates the position of PI 3-P spot. B: each barogram represents the radioactivity incorporated into PI 3-P by PhosphorImager analysis. Values are means ± SE of 3 independent experiments and are expressed as the percentage of control, where the untreated cells were defined as 100%. \(**P < 0.01\) vs. untreated control.
PDGF BB was not explored. More importantly, the role of the PDGF A-chain in activating MM cells is not known. This information is pertinent because PDGFR-α/H9251 does not appear to compensate for PDGFR-β/H9252 in rescuing mesangial cell phenotype in PDGFR-β deficit.

Moreover, during early stages of development and maturation of the glomerular capillary bed, the PDGF A-chain and PDGFR-α have a spatial and temporal distribution similar to that of the PDGF B-chain and PDGFR-β, respectively (18).
PDGF BB, as reported previously (2), stimulates DNA synthesis and robust migration of MM cells. We now demonstrate that PDGF AA, even at a concentration as high as 100 ng/ml, was not mitogenic for these cells and had a modest effect on cell migration. Therefore, the lack of a mitogenic effect of the PDGF A-chain and its inability to stimulate robust migration are potential mechanisms for failure of PDGFR-α to compensate for PDGFR-β in rescuing mesangial cell phenotype. Mouse chimeras composed of PDGFR-β−/− and PDGFR-β+/+ cells demonstrated that PDGFR-β−/− cells fail to populate the glomerular mesangium, whereas PDGFR-β+/+ cells do, suggesting a direct permissive role of PDGF BB in mesangial cell development and maturation (17). Studies utilizing bromodeoxyuridine labeling demonstrated active proliferation of mesangial cell progenitors in cup-shaped and S-shaped glomeruli of wild-type, but not mutant, mice. These studies suggested that proliferation of mesangial cell progenitors is a critical step for mesangial cell development (17). Our finding of the failure of PDGF AA to induce proliferation of MM cells supports this contention. We next examined the activation of ERK1/2 by PDGF AA or PDGF BB and its involvement in PDGF-induced DNA synthesis and migration of MM cells. PDGF AA and PDGF BB increased ERK1/2 activity in a dose- and time-dependent manner (Figs. 3 and 4), with PDGF BB being slightly more potent than PDGF AA. Pretreatment of MM cells with the MEK inhibitor PD-098059 at a concentration that abolished MAPK activity resulted in complete inhibition of DNA synthesis. However, the MEK inhibitor only partially blocked PDGF BB-induced cell migration and exerted a small but insignificant effect on PDGF AA-induced cell migration. These data indicate that the Ras-Raf-MEK-MAPK pathway is essential for PDGF BB-induced DNA synthesis in MM cells.

Cells expressing a PDGFR-β mutant devoid of the binding sites for PI 3-K, i.e., lacking Tyr740 and Tyr751, show no chemotactic responses to PDGF (15, 24), suggesting a role for PI 3-K in cell migration. However, PI 3-K regulates growth factor-induced migration in a cell type-specific manner. For example, there is evidence that PI 3-K does not mediate cell migration in smooth muscle cells activated by PDGF BB (12). PDGFR-α-mediated migration also appears to be cell type specific. In lung fibroblasts, Swiss 3T3 and hematopoietic 32D cells, activation of PDGFR-α induces migration (13, 19, 25). However, in other cell types, such as aortic endothelial cells and vascular smooth muscle cells, PDGF AA inhibits the chemotactic response. PDGF BB as well as PDGF AA induce PI 3-K activity in MM cells, with the AA isoform resulting in somewhat lesser induction of enzyme activity. Wortmannin, at concentrations that decreased PI 3-K enzymatic activity, markedly inhibited PDGF-induced migration of MM cells. In contrast to its potent effect on cell migration, pretreatment of MM cells with wortmannin reduced PDGF BB-induced DNA synthesis by ~20%. The data indicate that migration of MM cells in response to both PDGF isoforms is mediated via PI 3-K. It is very unlikely that the differential effect of PDGF isoforms is due to differential expression of PDGFR-α and -β in the cells, because both receptors were homogenously distributed in MM cells. The data also demonstrate that the lack of biological response to PDGF AA is not due to a low number of PDGFR-α or poor coupling of the AA ligand with the receptor, because PDGF AA was almost as potent as PDGF BB in activating ERK1/2 at a wide range of concentrations. Furthermore, the PDGF AA isoform potently activated PI 3-K to a degree almost similar to that for the PDGF BB isoform. However, activation of these pathways, PI 3-K and MAPK, by PDGF AA is not sufficient to induce DNA synthesis or robust migration in these cells. These data, taken together with the mesangial cell phenotype in the PDGFR-β-deficient mouse, suggest that, in the absence of significant DNA synthesis, activation of PI 3-K and subsequent migration is insufficient for PDGFR-α, which can be activated by PDGF AA or PDGF BB, to compensate for the loss of PDGFR-β. Of interest is the recent observation that mice with a PDGFR-β mutant for PI 3-K binding sites develop normally and do not exhibit an overt phenotype in the mesangium (11), suggesting that β-receptor-mediated signaling through activated PI 3-K is only of minor importance during mesangial cell development. Alternatively, other signaling molecules activated by the mutant β-receptor are able to compensate for the loss of PI 3-K signaling.

In conclusion, in this study we have shown that PDGF AA and PDGF BB activate PI 3-K and MAPK enzymatic signaling pathways in MM cells. We have also shown that PDGF BB induces DNA synthesis primarily through the MAPK pathway and migration.

Fig. 10. Expression of PDGFR-α and -β in MM cells. A: immunofluorescent localization of PDGFR-α. B: immunofluorescent localization of PDGFR-β. All MM cells stained for both PDGFR-α and -β. Magnification: ×40.
through the PI 3-K pathway. The finding that PDGF AA had no effect on DNA synthesis, whereas it stimulated modest migration of the cells, suggests that the failure of PDGFR-α to compensate for loss of PDGFR-β may be due to its inability to mediate these fundamental biological responses of MM cells. It is tempting to speculate that mesangial cell progenitors that may be stimulated to migrate eventually undergo apoptosis in the absence of the PDGF B-chain or PDGFR-β or fail to sustain their proliferation or even their survival. A more comprehensive examination of molecules activated by PDGFR-β is required to understand the mechanism by which PDGF BB and PDGFR-β activation result in mesangial cell development and maturation.

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