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Both Platelet-Derived Growth Factor Receptor (PDGFR)-α and PDGFR-β Promote Murine Fibroblast Cell Migration

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Cell motility plays a critical role for many physiological and pathological processes including wound healing, fibrosis, angiogenesis, and tumor metastasis. Platelet-derived growth factor (PDGF) is among the most potent stimuli for mesenchymal cell migration. The PDGF B-chain homodimer PDGF BB activates both α- and β-receptor subunits (α-PDGFR and β-PDGFR), and promotes cell migration in many cell types including fibroblasts and smooth muscle cells. PDGF-A chain homodimer PDGF AA activates α-PDGFR only, and its role for cell migration is still debatable. PDGF BB, but not PDGF AA, induces smooth muscle cell migration. Interestingly, α-PDGFR was shown to antagonize β-PDGFR-induced smooth muscle cell migration. In the present study, we investigated the role of α-PDGFR and β-PDGFR in PDGF-mediated cell migration of murine fibroblasts (NIH 3T3). Unlike smooth muscle cells, both PDGF AA and PDGF BB promoted NIH 3T3 cell migration. The effect of PDGF BB activation of β-PDGFR alone for cell migration was examined using previously established NIH 3T3 clones in which α-PDGFR signaling is inhibited by a dominant-negative α-PDGFR, or an antisense construct of α-PDGFR. PDGF BB activation of β-PDGFR alone was sufficient to induce cell migration, but the efficiency was significantly lower compared to PDGF activation of both receptors. These results showed that both α- and β-PDGFRs promote fibroblast cell migration and their effects are additive. Taken together, we propose that cell-type specific α-PDGFR signaling is critical for regulation of mesenchymal cell migration in response to PDGF isoform, whereas β-PDGFR mainly promotes cell migration.

Key Words: **platelet-derived growth factor (PDGF); PDGF receptor; cell migration; fibroblasts.**

Platelet-derived growth factor (PDGF) regulates a diverse array of cellular processes including cell migration, proliferation, transformation, and apoptosis (1–4). Consequently, PDGF has been suggested to play critical roles in wound healing and also in disorders such as neoplasia and atherosclerosis (1, 5). PDGF exists in the form of homodimers or heterodimer of PDGF A and B chains. PDGF AA effectively binds only to the α-subunit of PDGF receptor (α-PDGFR), while PDGF BB binds both α- and β-subunits (α-PDGFR and β-PDGFR) (6–8). Although both PDGF AA and BB are strong mitogens, only PDGF BB induces phenotypic transformation of fibroblast cells. Studies suggest that PDGF AA and BB may also differentially regulate cell migration. While PDGF BB mediates cell migration in many cell types (9–12), PDGF AA appears to regulate cell migration in a cell type-specific fashion. Ectopic expression of wild-type and mutant α-PDGFRs in porcine aortic endothelial cells that do not naturally express α-PDGFR provided evidence that α-PDGFR has an intrinsic ability to mediate both positive and negative chemotatic signaling (13). In smooth muscle cells, inhibition of α-PDGFR using a neutralizing monoclonal antibody to α-PDGFR enhanced β-PDGFR-induced cell migration, suggesting a migration suppressing activity of α-PDGFR (12).

PDGF exerts its cellular effects by activating two structurally related cell surface receptor tyrosine kinases (α- and β-PDGFR). Dimerization and autophosphorylation of PDGFR occur upon receptor-ligand interaction. Differential binding of initial signaling molecules to phosphorylated PDGFRs is thought to mediate overlapping but distinct α- and β-PDGFRs-induced signaling pathways. Although a number of signaling molecules activated by these receptors have been identified, little is known about which signaling pathways are α- and β-PDGFR-specific, and how these signals are integrated to agonize or antagonize specific cellular processes. Using NIH3T3 cells that are highly responsive to PDGF, we have studied the differential roles of α- and β-PDGFRs in PDGF regulation of cel-

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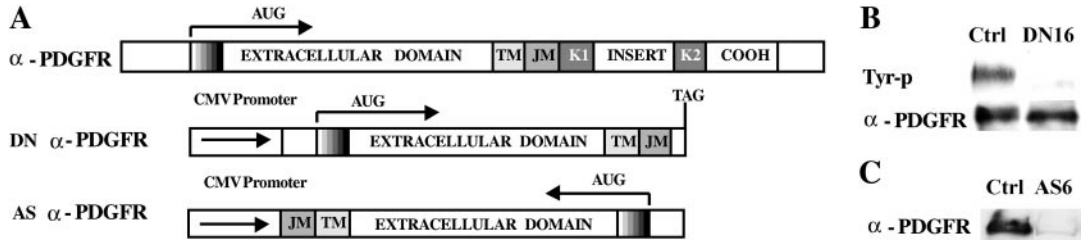

FIG. 1. Inhibition of α-PDGFR in NIH3T3 cells. (A) Functional domains of α-PDGFR are depicted; extracellular domain, transmembrane domain (TM), juxtamembrane domain (JM), kinase domains (K1 and K2), kinase insert domain (INSERT) and cytoplasmic tail (COOH). DN α-PDGFR expression vector under the control of cytomegalovirus (CMV) promoter contained a 1.9-kb fragment of α-PDGFR cDNA encoding extracellular, transmembrane and juxtamembrane domains. The translation stop codon (TAG) was provided downstream of the juxatamembrane domain (middle panel). AS α-PDGFR vector under the control of CMV promoter contained the same 1.9-kb fragment of α-PDGFR cDNA in reverse orientation (bottom panel). (B). Three millions cells grown in 100-mm culture dish were serum-starved for 48 h. After treatment with 50 ng/ml of PDGF AA for 5 min, cells were lysed in RIPA buffer. Lysates (250 μg/lane) of the control NIH3T3 and dominant negative clones (DN16) were immunoprecipitated with an anti-α-PDGFR polyclonal Ab (Ab 262) and protein G–Sepharose beads. The immunoprecipitates were resolved by reducing SDS–PAGE followed by immunoblot analysis with an anti-phosphotyrosine mAb (Oncogene) (top panel). To confirm the amount of immunoprecipitated α-PDGFR protein in each sample, the same blot was reprobed with the anti-α-PDGFR mAb (Santa Cruz Biotech) (bottom panel). (C) Three millions cells grown in 100-mm culture dish were lysed in RIPA buffer. Lysates (200 μg/lane) of the control NIH3T3 and antisense clones (AS6) were immunoprecipitated with an anti-α-PDGFR polyclonal Ab (Ab 262) and protein G–Sepharose beads. The immunoprecipitates were resolved by reducing SDS–PAGE followed by immunoblot analysis with an anti-α-PDGFR mAb.

lular processes. Recently, we reported that α-PDGFR antagonizes β-PDGFR-mediated phenotypic transformation of NIH3T3 cells through JNK-1 activation, while both α- and β-receptors effectively activate ERKs and trigger the cell cycle (14). These results revealed a striking feature of PDGF signaling: that the specificity and the strength of the PDGF-growth signal is modulated by α-PDGFR-mediated simultaneous activation of growth stimulatory and inhibitory signals, whereas β-PDGFR mainly induces a stimulatory signal.

In the present study, we investigate whether α-PDGFR also antagonizes β-PDGFR-induced fibroblast cell migration as suggested in smooth muscle cells (12). Cell migration induced by PDGF AA activation of α-PDGFR or by PDGF BB activation of both receptors was examined in control NIH 3T3 cells. The effect of PDGF BB activation of β-PDGFR alone on cell migration was examined using NIH 3T3 clones in which α-PDGFR signaling is inhibited by a dominant-negative α-PDGFR, or an anti-sense construct of α-PDGFR.

MATERIALS AND METHODS

Cell culture. NIH 3T3 cells, antisense and dominant negative clones were cultured in a humidified 5% CO_2 incubator with Dulbecco's modified Eagle medium/F12 nutrient media containing 10% bovine calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, 250 μg/ml Amphoterricin B, and 205 μg/ml of sodium deoxycholate (Life Technologies).

Immunoprecipitation and immunoblot analysis. Cells were lysed in lysis buffer [RIPA buffer: 0.1% SDS, 0.5% sodium deoxycholate acid, 0.5% Nonidet P-40, Tris 10 mM, pH 7.4, 1 mM EDTA, 2 mM sodium Vanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml of leupeptin, and 10 μg/ml aprotinin]. Protein concentrations were determined with BCA Protein Assay kit. The lysates were centrifuged for 15 min at 12,000*g* to remove debris, and immunoprecipitated using anti-α-PDGF receptor Ab, and protein G–agarose beads (Boehringer Mannheim). Immunoprecipitates were washed 5 times with RIPA buffer and resolved by reducing SDS–PAGE. Tyrosine-phosphorylated PDGFRs were detected by immunoblotting using the anti-phosphotyrosine antibody as previously described (14).

Assay for focus formation. A total of 1.5 million cells were plated in a 60-mm dish and cultured in the absence or presence of 25 ng/ml PDGF BB. The chronically stimulated confluent cells were incubated for 7 days to assess focus formation. Fresh media and PDGF BB were provided every other day.

Migration assay. The migration assay was performed in CHEMOTX plates (Neuro Probe). Filters were coated overnight with type-1 collagen (Sigma) solution at 100 μg/ml. Serum-deprived cells were trypsinized and resuspended in DMEM/12 medium at a concentration of 1×10^6/ml. Equal amounts of cell suspension (30 μl) were placed onto the filters, and the medium containing PDGF AA, PDGF BB, or bFGF in DMEM/F-12 was placed below the filter in the lower chamber and was incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air for 16 h. Cells were fixed with methanol and stained with hematoxylin for 10 min followed briefly by eosin. Cells on the upper surface of the filter were mechanically removed using cotton swabs, and the cells that migrated to the lower side of the filter were counted under microscopy at 400×. All experiments were performed at least in triplicate.

RESULTS AND DISCUSSION

To investigate the roles of β-PDGFR activation alone in PDGF-mediated cellular functions, we previously established NIH3T3 clones in which α-PDGFR activation is inhibited (14). One approach was to prevent autoactivation of α-PDGFR using a dominant-negative mutant of α-PDGFR (DN α-PDGFR) that contains the extracellular and transmembrane domains, but lacks the cytoplasmic kinase domains. The second approach was to downregulate α-PDGFR expression using an antisense construct of α-PDGFR (AS α-PDGFR). Autophosphorylation of endogenous α-PDGFR was inhib-

ited by DN α-PDGFR, and the α-PDGFR expression level was significantly downregulated by AS α-PDGFR (Fig. 1). This confirmed significant reduction of α-PDGFR activation in DN and AS clones as previously reported (14).

Although both PDGF AA and BB are strong mitogens, only PDGF BB induces phenotypic transformation of NIH3T3 cells as assessed by growth on soft agar and focus formation assay (15). When α-PDGFR signaling is inhibited, PDGF BB-mediated focus formation was significantly enhanced (Fig. 2), suggesting that α-PDGFR negatively regulate phenotypic transformation of fibroblast cells, as we previously reported (14).

We next examined the roles of α- and β-PDGFRs on cell migration. In control NIH 3T3 cells, both PDGF AA and PDGF BB effectively induced NIH 3T3 cell migration at comparable levels (Fig. 3). PDGF AA-induced cell migration in AS and DN clones was reduced by ~70% compared to the control NIH 3T3 cells, as expected from reduced α-PDGFR activation in AS and DN cells. PDGF BB activation of β-PDGFR alone (PDGF BB treatment in DN and AS clones) was sufficient to induce cell migration, but the efficiency was lower than PDGF activation of both receptors (PDGF BB treatment in the control NIH 3T3 cells). These results suggest that both α-PDGFR and β-PDGFR induce fibroblast cell migration, and their effects are additive, whereas inhibition of α-PDGFR enhanced β-PDGFR-mediated phenotypic transformation. To ensure that reduced migration efficiency following PDGF treatment in DN and AS clones compared to the control NIH 3T3 cells was not due to reduced migrative capacity of these clones, we also examined cell migration in response to basic fibroblast growth factor (bFGF), a well known chemoattractant (16–18). As shown in Fig. 3, bFGF induced cell migration in DN and AS clones as efficiently as in the control cells. This showed that reduced cell migration in DN and AS clones in response to PDGF BB resulted from inhibition of α-PDGFR signaling.

Previously, it was shown that inhibition of α-PDGFR enhances PDGF BB-induced smooth muscle cell migration (12), suggesting an antagonistic activity of α-PDGFR for β-PDGFR-induced cell migration. In fibroblast, α-PDGFR has an antagonistic activity for β-PDGFR-induced phenotypic transformation [Fig. 2 and (14)], but not for β-PDGFR-induced cell migration. Taken together, we propose that cell-type specific α-PDGFR signaling is critical for regulation of mesenchymal cell migration and transformation in response to PDGF isoforms.

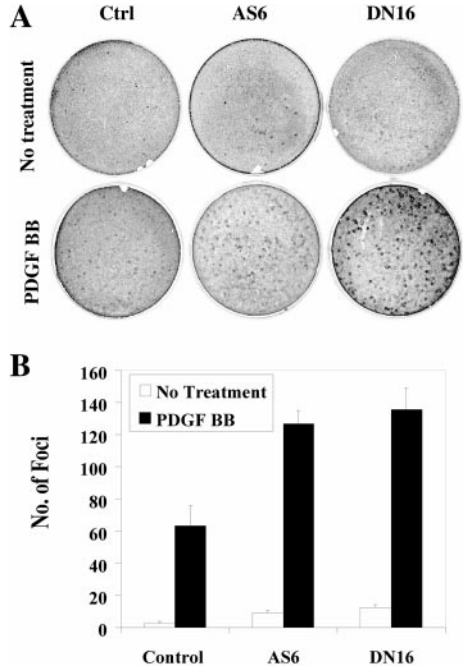

FIG. 2. Inhibition of α-PDGFR signaling enhances β-PDGFR-induced transformed phenotype. (A) Control NIH3T3, AS6 and DN16 cells in the absence or presence of 25 ng/ml PDGF BB were assayed for their ability to form foci. After 7 days, cells were stained with Giemsa (Fisher Scientific, Orangeburg, NY) and photographed. (B) Foci (>1 mm diameter) were counted, and the mean values of triplicates were plotted and the error bars represent standard deviation of the mean of triplicate.

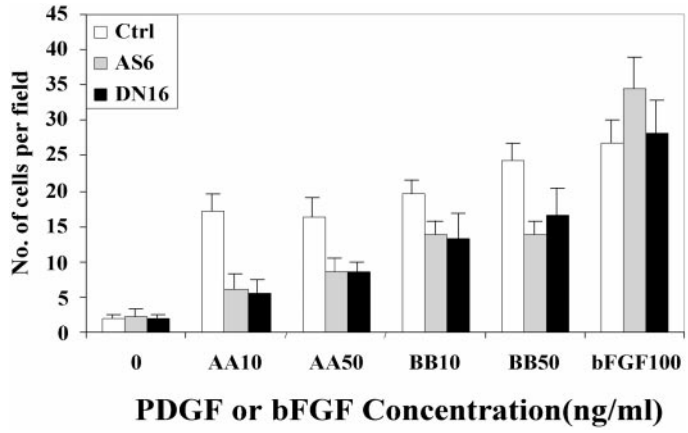

FIG. 3. Both α-PDGFR and β-PDGFR promote NIH3T3 cell migration. Control NIH 3T3, AS6 and DN16 cells were assayed for their ability to migrate through the filter in response to 10 or 50 ng/ml of PDGF AA or PDGF BB, or 100 ng/ml bFGF. Numbers of migrated cells per field were counted under 400× light microscopy. The mean values of triplicates were plotted and the error bars represent standard deviation of the mean of triplicate.

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